SOIL BIOGEOCHEMICAL PROCESSES AND FINE ROOT DYNAMICS OF VEGETATION IN LFH MINERAL SOIL MIX AND PEAT MINERAL SOIL MIX ORGANIC CAPPING MATERIALS USED FOR OIL SANDS RECLAMATION

by

Ghulam Murtaza Jamro

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Department of Renewable Resources
University of Alberta

ABSTRACT

Peat mineral soil mix (PMM) and LFH, identifiable litter (L), fragmented litter (F) and humus (H), mineral soil (MS) mix are organic capping materials commonly used over overburden (OB) and tailings sand (TS) substrate materials in oil sands reclamation. These organic capping materials have different biological properties and nutrient availabilities due to differences in the carbon to nitrogen (C to N) ratio, exogenous organic input and organic to MS ratio. Substrate materials inherently possess high pH, electrical conductivity (EC) and soil compaction. The main goal of this research was to evaluate biogeochemical and fine root processes affected by required reclamation practices for oil sands reclamation.

Soil sampling was conducted from 0 to 10 and 10 to 20 cm soil depth from June to October in 2011 and 2012 and N availability and enzyme activities were analyzed. Organic substrate type and diversity effects on microbial processes including carbon dioxide (CO_2) emission, enzyme activities, available N and community level physiological profiles (CLPPs) were evaluated in a laboratory experiment using three organic substrates (glucose, acetic acid, alanine). The organic substrates were applied singly and in a mixture of two or three in an LFH-MS and a PMM. Effects of organic to MS ratio on biogeochemical processes were evaluated in a laboratory experiment using five ratios of LFH or peat to MS at 0:100, 30:70, 50:50, 70:30 and 100:0. Fine root properties such as root length density, surface area, total root biomass and rates of root production, turnover and decomposition of lodgepole pine and white spruce planted on the PMM placed over TS and OB substrates, respectively and were assessed from May to October in 2011 and 2012.

The N availability and N-acetyl glucosaminidase, arylamidase and protease activities were greater in LFH-MS than in PMM, decreased along the soil depth and were influenced by the time of sampling. These differences were attributed to the lower C to N ratio in LFH-MS than in PMM. The addition of fresh labile C through root exudates and litter fall likely induced the N availability and enzyme activities in fall rather than in summer.

The addition of organic substrates (laboratory study) significantly segregated CLPPs from the control (no substrate) in LFH-MS and PMM. The significant increase in enzyme activities, available N through increasing the organic substrate diversity, was likely associated with changes in CLPPs and a reduction in C to N ratio with a substrate addition in LFH-MS and PMM. The mixing of organic single substrates enhanced the CO_2 emission rate and NO_3^--N concentration only in LFH-MS and PMM. The laboratory study results revealed that the β-glucosidase, cellobiohydrolase, phenol oxidase and leucine aminopeptidase activities, CO_2 emission rates and available N were increased along the increasing organic to MS ratio regardless of LFH and peat. The increase in soil processes was due to changes in C to N ratios and pH along with increase in organic to MS ratios.

Fine root length density, fine root production and turnover rates were increased along the low < medium < high productivity level in pine stands, and were positively correlated with tree height and diameter at the breast height. Fine root surface area was the only parameter that was increased along the productivity gradient in spruce. These differences were attributed to negative relationships of EC and soil compaction with root properties in pine and spruce, respectively. The root decomposition did not change along the productivity level of both pine and spruce species but was affected by the time of incubation, due to differences in species and OB and TS properties.

The LFH-MS is a better soil quality organic capping material than PMM due to N availability and enzyme activities; however, the availability of LFH-MS is limited for reclamation. Hence, the ratios of organic to MS for optimization, particularly LFH, help to take advantage of available LFH material. Alternatively, augmenting LFH-MS with PMM can overcome the limitations of both materials, as indicated from organic substrate diversity and organic to MS ratios in this research. An evaluation of the effects of substrate properties on fine root properties can help improve current reclamation practices.

PREFACE

This dissertation is an original work conducted by Ghulam Murtaza Jamro. Versions of Chapter 1 and 4 of this thesis have been published as Jamro, G.M., Chang, S.X., Naeth, M.A. "Organic capping type affected nitrogen availability and associated enzyme activities in reconstructed oil sands soils in Alberta, Canada," Ecological Engineering, 73, 92-101 (2014); and Jamro, G.M., Chang, S.X., Naeth, M.A., Duan, M., House, J. "Fine root dynamics in lodgepole pine and white spruce stands along productivity gradients in reclaimed oil sands sites," Ecology & Evolution, 5, 4655-4670 (2015). Versions of chapters 2 and 3 are being prepared for submission to peer reviewed journals. I was responsible for data collection, data analysis and manuscript writing. Kwak, J.H., Duan, M., House, J. and Pokharel, P. assisted with data collection from the field and laboratory. Chang, S.X. and Naeth, M.A. were the supervisory authors and involved in research concept formation and edited manuscripts.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ACRONYMS

AOSR: Athabasca oil sands region

AlaAP: alanyl aminopeptidase

ARA: arylamidase

AWCD: average well color development

Bglu: beta glucosidase

C: carbon

$CaCl_2$: calcium chloride

CEC: cation exchange capacity

C to N ratio: carbon to nitrogen ratio

CLPPs: community level physiological profile

Co: cobalt

DBH: diameter at breast height

DOC: dissolved organic carbon

DON: dissolved organic nitrogen

EC: electrical conductivity

ECN: enzyme classification number

FIA: flow injection analysis

FRB: fine root biomass

FRLD: fine root length density

FRP: fine root production

HCl: hydrochloride

k: Decomposition rate constant

KCl: calcium chloride

K_2SO_4: potassium sulphate

LAP: leucine aminopeptidase

L-DOPA: L-dihydroxyphenylalanine

LFH: identifiable litter (L), fragmented and partially decomposed litter (F), and highly decomposed humus (H) material

MBC: microbial biomass carbon

MBN: microbial biomass nitrogen

MRT: mean residence time

MS: mineral soil

MUB: 4-methylumbelliferyl

m:v: mass to volume ratio

N: nitrogen

NA: not analyzed

NaCl: sodium cholride

NAGase: β-1, 4-N-acetylglucosaminidase

Na_2CO_3: sodium carbonate

NaOH: sodium hydroxide

ND: not detected

NH_4^+: ammonium

NO_3^-: nitrate

OB: overburden

OM: organic matter

PMM: peat mineral soil mix

PRS^{TM}: Plant root simulator probes

PRT: protease

POX: phenol oxidase

RSA: root surface area

SAR: sodium adsorption ratio

SWC: soil water content

TC: total carbon

TCA: tricloracetic acid

TN: total nitrogen

TOC: total organic carbon

TON: total organic nitrogen

TOR: Turnover rates

TS: tailings sand

UR: urease

WHC: water holding capacity

XRD: X-ray diffraction

CHAPTER 1 GENERAL INTRODUCTION

1. Research background

1.1 Oil sands development and mining

Canada's boreal forests are home to one of the world's largest oil deposits, the Alberta oil sands located in the Athabasca River, Cold Lake and Peace River regions. The Alberta oil sands underlie an area of 142,200 km^2 in northeastern Alberta, representing an estimated one trillion barrels of oil reserves (Alberta Environment, 2006). The Athabasca oil sands region (AOSR) near Fort McMurray, Alberta, is known as the single largest oil reserve in the world (Alberta Government, 2015). Surface mining is the predominant technique used in the AOSR (Fung and Macyk, 2000) to recover oil deposits; since approximately 60% of bitumen is extracted by this method. During surface mining all ecological layers including plant cover, topsoil, subsoil and overburden (OB) are removed to expose the oil sands. All the soil layers and OB, a geological material found between the surface soil and oil sands, are usually stored for later reclamation purposes. As a result of the removal of all ecological layers, the ecosystem must be reconstructed when mining is completed (MacKenzie and Naeth, 2007). According to the Alberta Government (2015), about 813 km^2 of the boreal region had been disturbed by surface mining as of December 2013 and further destruction of forests in this region is expected. Mining companies are required by the Alberta Provincial Government environmental regulations to reclaim the disturbed area to a land capability equivalent to that which existed predisturbance (Cumulative Environmental Management Association, 2006).

1.2 Oil sands reclamation process and practices

After cessation of mining activities, the oil sands reclamation process follows different phases: surveying natural soils and selection of suitable reclamation materials; on-site salvaging, storing and placing reclamation materials; restoration of soil and suppressing of soil erosion by planting grasses/legume; re-vegetation; monitoring; and certification (Fung and Macyk, 2000). The key phase of oil sands reclamation process is the re-establishment of a functioning ecosystem (Figure 1-1; Macdonald et al., 2012) through genesis of a novel soil profile with salvaged mineral soil and organic capping materials. Various organic capping materials such as peat mineral soil mix (hereafter PMM) and LFH-mineral soil (MS) mix (hereafter LFH), which

is identifiable litter (L), fragmented litter (F) and humus (H) (MacKenzie and Naeth, 2007; Naeth et al., 2013), are used as capping materials for land reclamation in the AOSR. These materials are applied in different organic matter (OM) to MS ratios (either peat or LFH) during the reclamation process (Naeth et al., 2013). The peat to MS ratios (vol:vol) vary from 1:3 (Moskal et al., 2001) to 1:1 (Fung and Macyk, 2000; Hemstock et al., 2010) and the LFH to MS ratio (vol:vol) ranged from 1:1 to 1:2 for shallow salvage and from 1:3 to 1:5 for deeper salvage (Alberta Environment and Water, 2012). Oil sands reclamation also uses some inorganic materials, such as OB, tailings sand (TS), lean oil sands, coke, consolidated tailings and subsoil, below the organic capping materials. However, OB and TS are commonly used as substrate materials. Current oil sands reclamation involves several prescriptions of these materials for the genesis of a soil profile. Generally, 20 to 50 cm of organic capping material is applied over 100 cm of poor quality substrate materials of either OB or TS. In some cases subsoil (B and C horizon) is also applied between organic capping and substrate materials. The variations in prescription depend on the type and availability of material (Rowland et al., 2009).

Organic capping materials are applied to provide sufficient nutrients, to establish a community of vegetation until natural nutrient cycling may function (Barbour et al., 2007), and to provide a medium for rooting (Jung et al., 2014). Organic capping materials vary in physical, chemical and biological properties and nutrient availability (Hahn and Quideau, 2013; Jamro et al., 2014; Béasse et al., 2015; Kwak et al., 2015a) as summarized in Tables 1-1 and 1-2. The PMM is commonly used as an OM source due to its availability in mining areas (Mackenzie and Naeth, 2010; Naeth et al., 2013). PMM has been less supportive to native vegetation (Brown and Naeth, 2014; Forsch, 2014). In contrast, LFH-MS is a rich source of native seed bank propagules (Mackenzie and Naeth, 2010) and fosters microbial community (McMillan et al., 2007; Béasse et al., 2015; Kwak et al. 2015a). Relative to PMM, the microbial community in LFH-MS more closely resembled that of a natural forest (Hahn and Quideau, 2013) and had greater nitrogen (N) availability (Jamro et al., 2014). However, the availability of LFH-MS is limited, and its effects on biogeochemical processes are not well documented (Noah et al., 2014; Kwak, 2015).

The substrate materials possess some inherent properties such as high soil pH and electrical conductivity (Jung et al., 2014; Duan et al., 2015) that affect the reclaimed ecosystem (Table 1-2). The OB is a highly compacted natural material with poor drainage (Jamro et al., 2015), and TS has low nutrient and water holding capacity (Duan et al., 2015; House, 2015). These factors

may negatively affect vegetation growth (Fung and Macyk, 2000; Duan et al., 2015) and fine root performance (Jung et al., 2014; Jamro et al., 2015), and raise concerns about sustainability of the reconstructed ecosystem.

To assess the success of reclamation, it is crucial to know the soil mechanisms and processes that are affected by standard reclamation practices (EPEA, 2009). Understanding soil biogeochemical processes (Sorenson et al., 2011; Noah et al., 2014; Kwak, 2015) and fine root growth and their dynamics (Lazorko and Van Rees, 2012; Jung et al., 2014) in reclaimed oil sands is a key to successful reclamation, as they are related to both belowground ecosystem functions and to aboveground productivity.

1.2.1 Soil biogeochemical processes

The soil microbial community, a key indicator for the assessment of reclamation success (Bentham et al., 1992; Hahn and Quideau, 2013), is involved in nutrient cycling processes and OM accumulation which supports plant growth (Mummey et al., 2002a) and ecosystem establishment (Archibald, 2014). The soil microbial community in reclaimed land is affected by abiotic factors such as soil pH and water content (Will et al., 2010), vegetation composition (Schaaf et al., 2011; Hahn and Quideau, 2013), year of reclamation (Hahn and Quideau, 2013; Zahraei, 2015) and type of OM used in reclamation (Béasse et al., 2015).

Soil enzymes are proteins in nature, produced by microorganisms (bacteria and fungi), plant roots, residues and dead soil animals (Dick, 1994). Soil enzymes mainly exist in viable cells (intracellular) which help in the evaluation of the activity of biological life in soil. Some enzymes, such as extracellular, remain in soil solution and form complexes with the soil matrix; the evaluation of these enzymes may reflect soil management practices effects on soil biology (Dick, 1988). Soil enzymes catalyze key biochemical reactions involved in OM decomposition (Sinsabaugh et al., 1991), nutrient cycling processes and stabilization of soil structure (Dick, 1988). They are often regarded as sensitive indicators of soil quality because they help in the measurement of soil microbial reactions occurring in soil, they may immediately respond to environmental changes caused by natural and anthropogenic factors and their measurement can be done easily (Gianfreda and Bollag, 199).

Soil enzymes are also known as highly sensitive indicators of environmental changes and may respond even before any other soil quality indicators during land reclamation (Pascual et al.,

2000; Baldrian et al., 2008; Dimitriu et al., 2010; Burns et al., 2013). Soil enzymes are typically regulated by substrate quality and quantity (Degens, 1998; Hernandez and Hobbie, 2010), vegetation composition (Zak et al., 2003) and types of reclamation materials (Dimitriu et al., 2010). Other factors are substrate availability (Will et al., 2010; Jamro et al., 2014), mineral composition (Shindo and Huang, 1985; Marx et al., 2005) and the presence of mycorrhizal fungi (Smith and Read, 2008; Burke et al., 2011). Soil enzymes may be affected by environmental factors such as soil water content, temperature, oxygen (O_2) concentration and pH (Eilers et al., 2012). Among these factors, pH is particularly influential for soil enzyme activity, as each enzyme has different optima that may either inhibit or accelerate its activity (Baldrian et al., 2008; Burns et al., 2013). Location of enzymes in the soil matrix, either free in soil solution or adsorbed on the soil surface (Turner, 2010), may indicate their degree of stability (Allison, 2006; Totsche et al., 2010).

Understanding nutrient cycling processes, especially N cycles, is important for successful land reclamation, as these cycles are linked to aboveground and belowground productivity (Yan et al., 2012; Duan et al., 2015). Nitrogen mineralization and nitrification rates are mainly affected by environmental changes, such as disturbance, OM quality differences (Mummey et al., 2002a; McMillan et al., 2007; Jamro et al., 2014), carbon to nitrogen (C to N) ratio (Mohanty et al., 2013) and soil pH (Myrold, 2005).

1.2.2 Fine root dynamics

Fine root dynamics is another key component of nutrient cycles. Fine roots are involved in capturing resources from soils (Brassard et al., 2009) and alter soil biogeochemistry by increasing substrate availability in the form of root detritus and exudates (Hutsch et al., 2002; Schaaf et al., 2011). It is well known that fine root processes, including production and decomposition, contribute more to the soil nutrient pool than aboveground litter fall (Aerts et al., 1992). These processes are affected by soil properties such as salinity (Lazorko and Van Rees, 2012) and compaction (Kozlowski, 1999; Jamro et al., 2015) and environmental factors (Espeleta and Donovan, 2002; Brassard et al., 2009). Each tree species has a unique root system to support its plant structure and to access, store and transport resources such as water and nutrients from the soil (Lazorko and Van Rees, 2012), resources that are required for the establishment of trees in reclaimed oil sands (Duan et al., 2015). Most tree species in the boreal

forest have mycorrhizae (Smith and Read, 2008) which may help to improve biogeochemical processes (Rillig, 2004; Burke et al., 2011). Knowledge of fine root dynamics is essential to build a sustainable ecosystem in oil sands reclamation (Bradshaw, 1984).

1.3 Literature review

1.3.1 Effects of capping materials on enzyme activity and N availability

The PMM and LFH-MS are two commonly used organic capping materials in oil sands reclamation (Naeth et al., 2013). These materials have contrasting biological properties and nutrient availabilities, including differences in C to N ratio, pH, microbial community composition (Table 1-2 and 1-3) and vegetation cover (Brown and Naeth, 2014), mainly due to their different sources. Peat is salvaged from wetlands, while LFH is collected from upland boreal forests. Various studies have shown (Table 1-2) that PMM contained more total carbon (TC) and total nitrogen (TN) than LFH-MS (McMillan et al., 2007; Béasse et al., 2015; Kwak et al., 2015a). Stockpiling of reclamation materials (Béasse et al., 2015) and application depth of capping materials (Mackenzie and Naeth, 2010) both affect the TC and TN concentrations.

Some studies found LFH-MS contains more decomposed OM than PMM, corresponding to a lower C to N ratio in LFH-MS than in PMM (Kong et al., 1980; McMillan et al., 2007; Mackenzie and Naeth, 2010; Jamro et al., 2014) due to differences in vegetation types (Forch, 2014). However, other studies showed a lower C to N ratio in PMM than in LFH-MS (MacKenzie and Quideau, 2012; Quideau et al., 2013; Forsch, 2014; Kwak et al., 2015a). The difference is likely associated with the peat source, which is either fens or bogs (Hemstock et al., 2010; Alberta Environment and Water, 2012), whether the material was fresh or stockpiled (Béasse et al., 2015), and the time after reclamation (Brown and Naeth, 2014; Forsch, 2014). Some researchers found that PMM had a higher pH than LFH-MS (MacKenzie and Quideau, 2012; Hahn and Quideau, 2013; Quideau et al., 2013; Brown and Naeth, 2014; Kwak et al., 2015b) due to the alkaline nature of the salvaged mineral material (Fung and Macyk, 2000) and the fen sourced peat material (Forsch, 2014). This is likely due to the fen type peatlands being fed by the mineral rich surface waters having neutral or alkaline pH (Charlton and Hilts, 1989).

The quality of organic capping materials plays a key role in various biogeochemical processes (Table 1-3) such as microbial community changes (Hahn and Quideau, 2013; Béasse et al., 2015; Kwak et al., 2015a), microbial biomass carbon (MBC) and nitrogen (MBN) (McMillan

et al., 2007; Jamro et al., 2014; Zahraei, 2015), enzyme activity (Dimitriu et al., 2010; Quideau et al., 2013; Jamro et al., 2014; Zahraei, 2015), N cycle processes and availability (McMillan et al. 2007; MacKenzie and Quideau, 2010; Jamro et al., 2014; Kwak et al., 2015b) and CO_2 emission (Zahraei, 2015; Kwak et al., 2016). The greater MBC and MBN observed in LFH-MS than in PMM was found to correspond to the higher level of decomposed OM in LFH-MS (McMillan et al., 2007; Jamro et al., 2014). As a result, LFH-MS also had greater enzyme activity than PMM (Quideau et al., 2013; Jamro et al., 2014). Enzyme activity in oil sands reclaimed soils was also found to depend on the type of OM (Kwak et al., 2015a) and the substrate type below the capping materials, such as TS or OB (Dimitriu et al., 2010). Greater enzyme activity in LFH-MS was attributed to the presence of more decomposed OM (Jamro et al., 2014), higher vegetation cover (Forsch, 2014) and greater mycorrhizal biomass (Brown and Naeth, 2014). Lower activity in PMM was due to the higher pH of PMM (Dimitriu et al., 2010; Jamro et al., 2014) since each enzyme has a different pH optima (Baldrian et al., 2008) and low levels of decomposed OM (Jamro et al., 2014; Kwak et al., 2015a), resulting in less substrate availability for the enzymes. In addition, soil enzyme activity itself is an indicator of decomposition of OM as shown in previous studies that the greater β- glucosidase and phenol oxidase activities in PMM than in LFH-MS (Quideau et al., 2013) suggest that PMM has less decomposed OM.

Researchers found that the greater N availability in LFH-MS than in PMM was mainly due to the former's greater mineralization rates (McMillan et al., 2007; MacKenzie and Quideau, 2012; Yan et al., 2012) and lower C to N ratio (Mackenzie and Naeth, 2010; Jamro et al., 2014; Kwak et al., 2015a). The greater N availability in LFH-MS can also be attributed to the greater abundance of genes linked to N transformation processes in LFH than in PMM (Zahraei, 2015) and with positive relationships with enzyme activity (Jamro et al., 2014). The availability of N decreased with depth of the capping materials with greater N availability found at 0-10 cm than 10-20 cm due to an increase in C to N ratio and microbial biomass (Jamro et al., 2014). Researchers found that most available N was in the form of nitrate rather than ammonium, as N availability in oil sands reclamation was controlled by nitrification (McMillan et al., 2007; Hemstock et al., 2010). Although several studies have tested N availability and enzyme activity in the soil of reclaimed oil sands, no field scale comparisons have been conducted on both LFH-MS and PMM for N availability and associated enzyme activity in oil sands reclamation (Naeth et al., 2013).

1.3.2 Organic substrate type and diversity effects on microbial processes

Microorganisms play an important role in nutrient cycling processes (Schulz et al., 2013) and architecture of the ecosystem (Mummey et al., 2002b; Rajendhran and Gunasekaran, 2008). Microbial activities are typically affected by substrate availability (Baldrian et al., 2008; Jamro et al., 2014) and composition (Orwin et al., 2006). Plants add diverse materials to soils in the form of leaf litter and root biomass and exudates (Orwin et al., 2006), providing organic substrates for various chemical and biological processes (Schutter and Dick, 2001) such as decomposition of OM (Kuzyakov et al., 2000) and enzyme activities (Hernandez and Hobbie, 2010).

Microbial community composition is associated with OM inputs (Noah et al., 2014; Béasse et al., 2015) and stand type; for example, aspen dominated and spruce dominated forests have distinct microbial communities (Hannam et al., 2004). Thus, plant species composition can be a proxy for altering substrate availability (Jamro et al., 2014) and quality and quantity of substrate inputs as litter fall and root exudates (Orwin et al., 2006; Hernandez and Hobbie, 2010) and reestablishment of plant-soil relationships (Sorenson et al., 2011). In earlier oil sands reclamation studies, LFH-MS supported a more diverse microbial community and greater microbial biomass than PMM (Hahn and Quideau, 2013; Béasse et al., 2015; Kwak et al., 2015a), as it contained more decomposed OM and a greater diversity of plant species and vegetation cover (Naeth et al., 2013; Brown and Naeth, 2014). Other studies found greater plant species diversity and vegetation cover in LFH-MS increased the priming effect on enzyme activity, N availability (Jamro et al., 2014; Kwak et al., 2015b), functional gene abundance (Zahraei, 2015), soil respiration (Kwak et al., 2016) and fungi to bacteria ratio (MacKenzie and Quideau, 2010). Understanding the effects of organic substrate type and diversity on microbial processes is essential to improve current reclamation strategies; as such research has not been conducted in oil sands reclamation.

1.3.3 Effect of organic to mineral soil mixing ratio on biogeochemical processes

The soil consists of different inorganic and organic components that form a complex network, a biogeochemical interface (Huang et al., 2005). Transformative processes such as decomposition of OM, nutrient cycles and soil enzymes activity (Allison, 2006; Totsche et al., 2010) are regulated and stabilized through organo-mineral interactions (Kögel-Knabner et al., 2008). However, the regulation of biogeochemical processes by organo-mineral interaction

varies with the catalytic potential of clay minerals and oxide minerals (Shindo and Huang, 1985; Marx et al., 2005). Oil sands reclamation currently involves materials with many different ratios of OM to MS (Naeth et al., 2013) such as peat to MS ratios (vol:vol) which vary widely, from 1:3 (Moskal et al., 2001) to 1:1 (Fung and Macyk, 2000; Hemstock et al., 2010) to 3:1 (Moskal et al., 2001). The LFH to MS ratio (vol:vol) ranges from 1:1 to 1:2 for shallow salvage and from 1:3 to 1:5 for deeper salvage (Alberta Environment and Water, 2012). Mixing ratio also varies with the type of MS, such as 1:3 for fine textured MS and 1:5 for coarse textured MS (Alberta Environment and Water, 2012).

The quality of OM is discussed in previous sections; however, the effects of organic to MS ratios on the biogeochemical processes in soil have been less studied. The amount of mineral soil mixed into organic capping materials may affect soil biogeochemistry through changes in pH, cation exchange capacity (CEC), surface area and charge, sodium adsorption ratio (SAR) and nutrient retention (Fisher and Binkley, 2000; Haider and Schäffer, 2009). In the AOSR, mineral soils are of two categories: fine textured (clay) soils and coarse textured (sandy) soils; in some places loam type soils are also found (Hahn and Quideau, 2013). Fine textured soils have greater CEC and OM content than coarse textured soils (Bauhus et al., 1998; Alberta Environment and Water, 2012). However, coarse textured mineral soils are predominantly mixed with both types of organic capping material used in the AOSR (Moskal et al., 2001; Naeth et al., 2013). Mixing mineral soils with different horizons and textures along with organic materials may change the soil properties, such as pH (Fung and Macyk, 2000, Mackenzie and Quideau, 2012), enzyme activities (Quideau et al., 2013), TC concentration (Hahn and Quideau, 2013) and CO_2 emission (Norris et al., 2013) in the AOSR. Mixing mineral soils of either kaolinite or loamy sand with litter material could decrease OM decomposition (Skene et al., 1996) and consequently decrease soil enzyme activity (Allison, 2006) and nutrient availability (Silver et al., 2000). However, recent studies indicated that clay particles either did not affect or increased decomposition of OM (Fissore et al., 2008) due to increased microbial biomass carbon (MBC) (Wei et al., 2004). Soil pH also plays a key role in the biogeochemical processes that shift microbial community composition in sandy soils, which could ultimately affect other soil processes. Fungi are found in sandy soils in much greater abundance than in clayey soils. Thus it is necessary to understand how changes in the organic to mineral soil ratio can affect soil biogeochemical processes in reclaimed oil sands soils.

1.3.4 Reclamation practice effects on fine root dynamics

Placing PMM over TS or OB substrates is a common prescription for oil sands reclamation. The inherent properties of these substrates include high soluble salt concentration, poor drainage and heavy compaction (Jung et al., 2014; Duan et al., 2015; Jamro et al., 2015), which can reduce tree growth and stand productivity (Jung et al., 2014; Duan et al., 2015) and consequently fine root performance (Strand et al., 2008). Jung et al. (2014) indicated that TS and OB may differ in soil texture and pore size, with TS having more macropores and OB having more micropores. The differences in pore size may affect nutrient leaching and infiltration and drainage of water, which could affect fine root growth and associated processes. Other researchers noted that lack of micropores in TS prevented capillary rise of water to the capping material (Li et al., 2013) and reduced water holding capacity (Khasa et al., 2005), limiting fine root growth of lodgepole pine (Jamro et al., 2015). Fine root production and turnover rates were also associated with water and nutrient limitations in TS (Jung et al., 2014). Soil salinity of substrate materials altered the morphology of fine roots (Lazorko and Van Rees, 2012) and reduced fine root biomass (Jung et al., 2014). Soil salinity also reduced the turnover and decomposition rates of fine roots (Zhang et al., 2009). Compaction caused by heavy equipment can increase bulk density of OB, causing a reduction in fine root growth of white spruce (Jung et al., 2014; Jamro et al., 2015). This compaction also reduces aeration and microbial activity (Lazorko and Van Rees, 2012) and slows decomposition of white spruce fine roots (Jamro et al., 2015). The high temperature of TS, meanwhile, may speed decomposition of fine roots of lodgepole pine (Naeth et al., 2011)

A few studies have examined the growth and distribution of fine roots of boreal forest species in oil sands reclamation (Lazorko and Van Rees, 2012; Jung et al., 2014). To date, however, no study has reported whether fine root dynamics is related to stand productivity in oil sands reclamation.

2. Thesis Structure

The overall objective of this research is to evaluate soil biogeochemical and fine root processes affected by current reclamation practices of oil sands. The findings of this study will help to improve the current reclamation practices used in oil sands reclamation. Two field and

two laboratory incubation experiments were conducted to test the following specific hypotheses.

- The LFH-MS will have greater N availability and greater soil enzyme activities than PMM due to more decomposed OM, microbial biomass and vegetation cover in LFH than in PMM.
- Increased organic substrates diversity through addition of litter fall and root exudates in reclaimed soils may create new niches for microbial growth which may support a more diverse microbial community.Thus, microbial processes would be affected by the type and diversity of organic substrates due to changes in the microbial community in materials used in oil sands reclamation.
- The effect of the type and diversity of organic substrates on microbial processes depends on the type of organic matter used for oil sands reclamation due to differences in biotic origin, vegetation input and C to N ratios.
- Changes in the organic to mineral soil ratio would alter the biogeochemical processes in oil sands reclamation due to changes in OM content and organo-mineral interaction. Thus, greater enzyme activities and N availability will be expected with a greater organic to mineral soil ratio regardless of organic capping material type used.
- Fine root growth would have a positive relationship with aboveground tree growth along forest productivity gradients of different tree species planted in reclaimed oil sands sites. The different fine root dynamics would likely be associated to inherited characteristics of tailings sand and overburden materials used in oil sands reclamation.

This thesis consists of six chapters. Figure 1-2 provides a synthesis of the studies. Each of chapters 2 to 5 constitutes a manuscript that is currently published or will be submitted for publication shortly.

- Chapter 1 provides background information and a review of reclamation practices on biogeochemistry and fine root dynamics.
- Chapter 2 focuses on organic capping type effects on nitrogen availability and associated enzyme activities in reconstructed oil sands soils in Alberta, Canada. A manuscript entitled Organic capping type affected nitrogen availability and associated enzyme activities in reconstructed oil sands soils in Alberta, Canada was published in Ecological Engineering 73, 92-101 (2014).
- Chapter 3 focuses on effects of substrate type and diversity on microbial processes in materials used in oil sands reclamation.

- Chapter 4 focuses on effects of organic to mineral soil ratios on biochemical processes of organic capping materials used in oil sands reclamation.
- Chapter 5 focuses on fine root dynamics in lodgepole pine and white spruce stands along productivity gradients in reclaimed oil sands sites. A manuscript entitled Fine root dynamics in lodgepole pine and white spruce stands along productivity gradients in reclaimed oil sands sites is published in Ecology and Evolution 5, 4655-4670 (2015).
- Chapter 6 provides a summary of research findings and suggested future research.

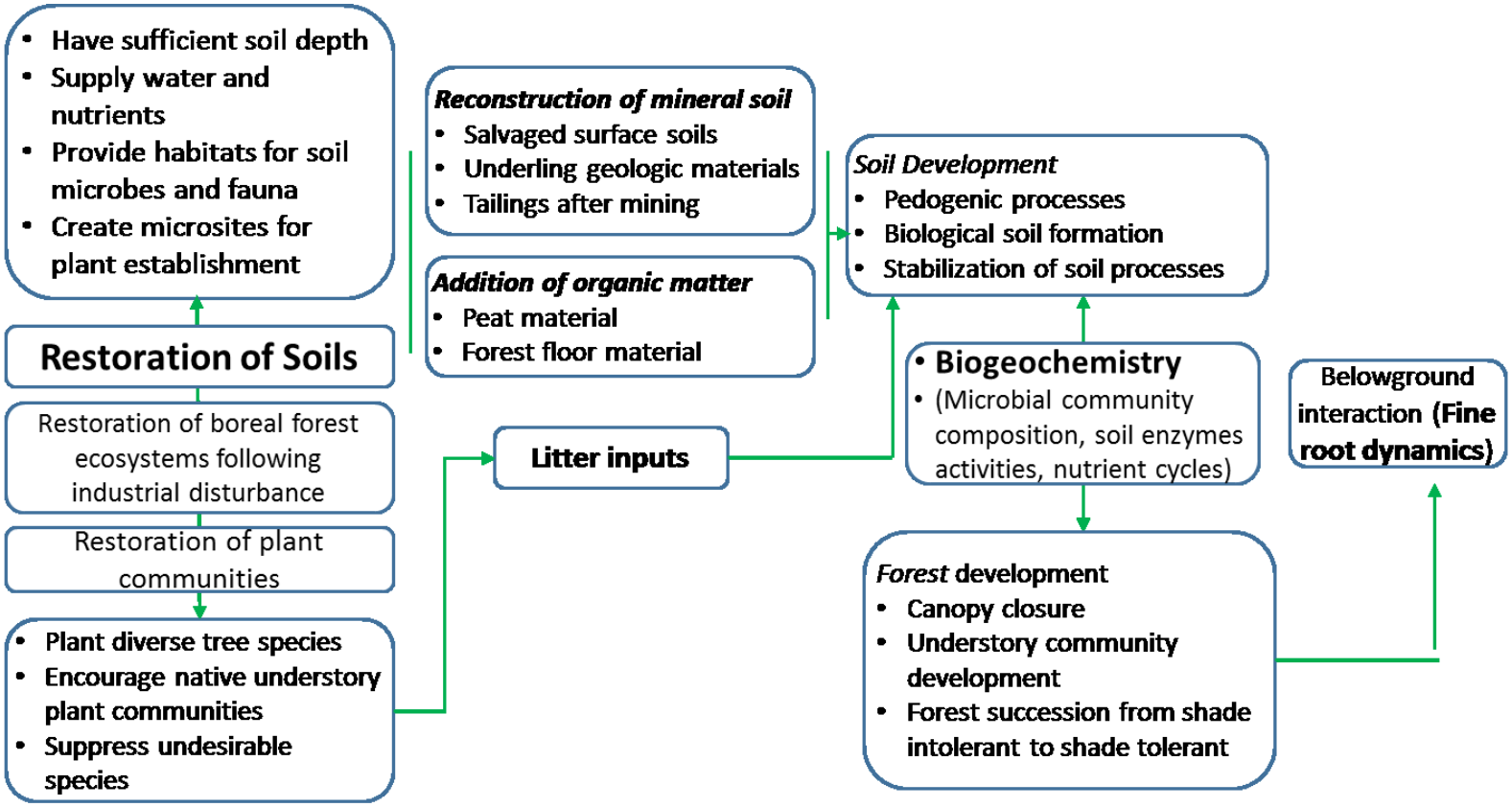

Figure 1-1 Important processes for oil sands reclamation adopted from Macdonald et al., 2012.

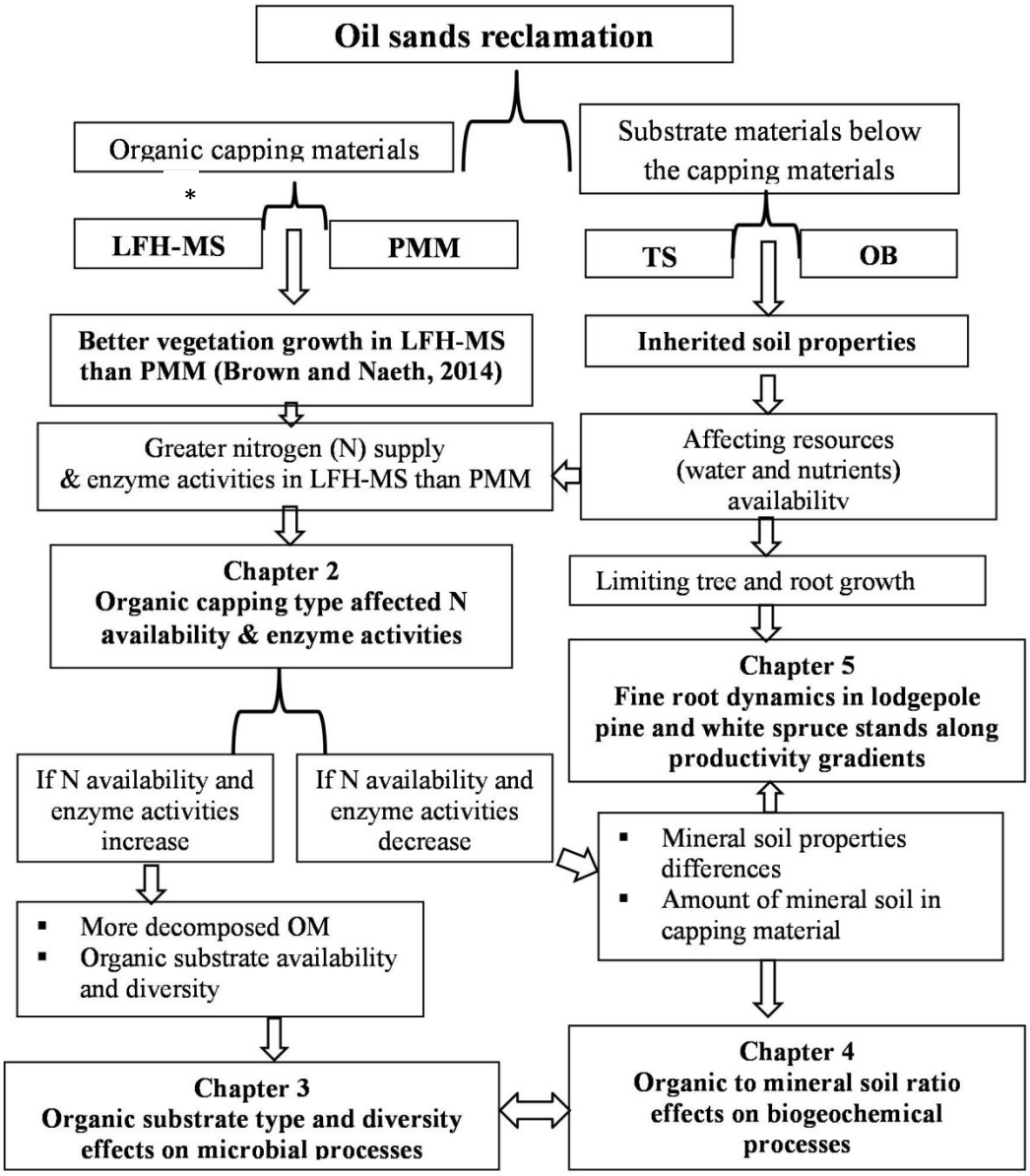

Figure 1-2 Flow chart showing the synthesis of research studies

*Abbreviations: LFH-MS= LFH-mineral soil, PMM= peat mineral soil mix, OB= overburden, TS= tailings sand, OM= organic matter

Table 1-1 Organic capping materials and their properties used in oil sands reclamation.

Amendment type	Physico-chemical properties
Peat mineral soil mix	2% - 17% organic C, near neutral pH
LFH mineral soil mix	Combination of LFH overlying mineral horizon (Naeth et al., 2013). Organic horizon containing > 17% organic C, developed primarily from accumulation of leaves, twigs and woody materials and is normally associated with upland forested soils
Overburden	Fine or coarse textured, slightly alkaline (Ph 8.0+), low to no organic C
Subsoil	Non-saline, pH 5.0 - 8.0, low organic C (<2%), may be mixed with peat or LFH during salvage operation
Lean oil sand	< 6% bitumen by weight , pH 5.5 – 6.0
Clearwater shales	Fine textured, saline-sodic; EC = 10-20 dS m^{-1}, sodium adsorption ratio (SAR) > 20, pH 8.0+
Coarse woody debris	Includes dead trees, logs, branches, twigs, dead coarse roots (Pyle and Brown, 1998), used as supplemental amendment over capping materials for developing microsites and consequently have affected soil properties (Brown and Naeth, 2014; Kwak et al., 2015a)

Source: Adapted from Rowland, 2008

Table 1-2 Chemical properties of peat mineral soil mix (PMM) and LFH mineral soil mix (LFH-MS) organic capping materials used in oil sands reclamation

Soil property[a]	Unit	PMM	LFH	Reference	Comments for consideration
TOC	g kg^{-1}	92.2	50.5	McMillan et al., 2007	PMM had 25 to 50 % (by volume) mineral soil
TOC	g kg^{-1}	164[1]-428[2]	101[1]-243[2]	Beasse et al., 2015	Fresh material[2] had greater than aged[1] material
TOC	g kg^{-1}	68.4-72.2	39.7-40.8	Kwak et al., 2015a	Affected by coarse woody debris (CWD) and was greater in PMM than LFH-MS
TOC	%	3.3-5.5	8.9-9.2	Mackenzie and Naeth, 2007	Lower in surface (10 cm) than sub surface (20 cm)
TOC	%	5.52-5.93	7.39-6.35	Brown and Naeth, 2014	Slightly increased during measurement year
TOC	%	6.62	7.34	Forsch, 2014	
TON	g kg^{-1}	4.5	2.4	McMillan et al., 2007	Slightly greater in PMM than FFM
TON	g kg^{-1}	5.5[1]-9.3[2]	9.3[2]-9.2[1]	Beasse et al., 2015	Fresh[1] material has greater than aged[2] material
TON	%	0.16-0.29	0.33	Mackenzie and Naeth, 2007	Lower in surface (10 cm) than sub surface (20 cm)
TON	%	0.23	0.24	Forsch, 2014	
C to N		20-80[1]	20-25[2]	[1]Kong et al., 1980, [2]Mackenzie, 2006	
C to N		21.5	21.3	McMillan et al., 2007	
C to N		19.6-24.4	28.9-29.7	Mackenzie and Naeth, 2007	Lower in surface (10 cm) than surface (20 cm)
C to N		21.9	28.5	MacKenzie and Quideau, 2012	Organic and mineral soil material 1:1 (by volume)
C to N		25	31	Quideau et al., 2013	
C to N		31.3	33.1	Forsch, 2014	
C to N		18-78	23-24	Beasse et al., 2015	Fresh[2] material greater than stockpiled[1] material
C to N		28.6-31.3	30.1-32.8	Kwak et al., 2015a	Affected by CWD and greater in PMM than LFH
pH		3.7-6.6[1]	5.5-6.1[2]	Kong et al., 1980, Mackenzie, 2006	
pH		6.2-6.4	6.4-6.8	Mackenzie and Naeth, 2007	Variable with capping depth
pH		5.51	5.95	McMillan et al., 2007	Significantly lower in PMM than FFM
pH		6.3	5.3	MacKenzie and Quideau, 2012	Organic and mineral soil material 1:1 (by volume)
pH		5.9-6.2	5.0-5.4	Hahn and Quideau, 2013	Materials were different in soil textures
pH		7.1	6.6	Quideau et al., 2013	
pH		6.4	7.4	Forsch, 2014	Higher in PMM than in LFH-MS
pH		3.6	5.9	Beasse et al., 2015	
pH		7.1-7.2	5.9-6.1	Kwak et al., 2015a	Greater in PMM than in LFH-MS

[a]Abbreviations: TOC: total organic carbon, TON: total organic nitrogen, C to N: carbon to nitrogen ratio

Table 1-3 Biogeochemical differences in peat mineral soil mix (PMM) and LFH mineral soil mix (LFH-MS) in oil sands reclamation

Soil process[a]	Unit	PMM	LFH	Reference	Comment for consideration
Respiration	nmol CO_2 g^{-1} hr^{-1}	49.16	54.59	Zahraei, 2015	
Respiration	mg CO_2 m^{-2} hr^{-1}	293 - 677	461 - 1148	Kwak et al., 2016	CWD affected by time of sampling
MBC	mg kg^{-1}	11.1	47.1	Brown and Naeth, 2014	
MBC	mg kg^{-1}	302	413.8	McMillan et al., 2007	
MBC	mg kg^{-1}	0.28	0.445	Zahraei, 2015	Differences greater in September than June
MBN	mg kg^{-1}	11.1	24.7	McMillan et al., 2007	
MBN	mg kg^{-1}	0.023	0.036	Zahraei, 2015	Differences greater in September than June
DOC	mg kg^{-1}	717	569.4	McMillan et al., 2007	
DON	mg kg^{-1}	9.3	8.6	McMillan et al., 2007	
BGlu	µmol g^{-1} hr^{-1}	5.0-9.4		Dimitriu et al., 2010	Mineral soil vs organic layer affected by depth
BGlu	µmol g^{-1} hr^{-1}	0.068	0.118	Zahraei, 2015	Greater in LFH than PMM increased with time greater in September than June
NAGase	µmol g^{-1} hr^{-1}	1.6-4.2	NA	Dimitriu et al., 2010	Mineral soil vs organic layer affected by depth
NAGase	µmol g^{-1} hr^{-1}	0.055	0.078	Zahraei, 2015	Greater in LFH than PMM increased with time greater in September than June
POX	µmol g^{-1} hr^{-1}	6.5-8.5	NA	Dimitriu et al., 2010	Mineral soil vs organic layer affected by depth
POX	µmol g^{-1} hr^{-1}	194	139	Quideau et al., 2013	PMM had greater than FFM
URE	µg NH_4^+-N g^{-1} hr^{-1}	0.5-2.4 mg	NA	Dimitriu et al., 2010	Mineral soil vs organic layer affected by depth
Nitrate N	mg kg^{-1}	6.1	4.5	Brown and Naeth, 2014	Decreased with time
Nitrate N	mg kg^{-1}	3.34-3.79	3.89-4.20	Mackenzie and Naeth, 2007	Depth of caps 10 vs 20 cm
Ammonium N	mg kg^{-1}	<0.3	1	Brown and Naeth, 2014	
Ammonium N	mg kg^{-1}	4.26-5.50	4.80-5.47	Mackenzie and Naeth, 2007	
Root gluco	mg g^{-1}	1.4	47.1	Brown and Naeth, 2014	
Fungi:bacteria		0.24	0.27	Quideau et al., 2013	Slightly greater in FFM than in PMM

[a]Abbreviations: MBC: microbial biomass carbon, MBN: microbial biomass nitrogen, DOC: dissolved organic carbon, DON: dissolved organic nitrogen, Bglu: beta glucosidase, NAGase: N-acetyl-B-D- glucosaminidase, POX: phenol oxidase, URE: urease, Root gluco : root glucosamine, NA: not analyzed

CHAPTER 2 ORGANIC CAPPING TYPE AFFECTED NITROGEN AVAILABILITY AND ASSOCIATED ENZYME ACTIVITIES IN RECONSTRUCTED OIL SANDS SOILSIN ALBERTA, CANADA

1. Introduction

Mining of oil sands in the Athabasca oil sands region in northern Alberta is one of the largest anthropogenic disturbances of terrestrial ecosystems in the world (Alberta Government, 2010). Surface mining activities in this region have greatly impacted approximately 767 km^2 of land, representing 0.2% of the boreal forest (Alberta Government, 2013). Surface oil sands mining involves the removal of several ecological layers including the vegetation, soil and geological material (Giesy et al., 2010). Current reclamation regulations require that oil sands companies reclaim disturbed areas to equivalent land capability after cessation of surface mining operations (Cumulative Environmental Management Association, 2006), but given the magnitude of the disturbance, much research is needed to help restore disturbed ecosystems to pre-disturbance conditions.

Successful reclamation of disturbed oil sands areas requires a broad understanding of nutrient cycling and ecosystem development. Nitrogen (N) is often a limiting nutrient in boreal forest soils in the oil sands region (Cheng et al., 2011), particularly in newly reconstructed ecosystems in the oil sands, where native N inputs are often lacking (Bradshaw, 1987). Availability of N in reclaimed ecosystems is strongly regulated by organic matter (OM) decomposition, which is greatly influenced by soil enzyme activities (Sinsabaugh et al., 1991). Soil enzymes associated with N cycling include β-1, 4-N-acetylglucosaminidase (NAGase), which is involved in the degradation of chitin, a component of fungal cell walls (Ueno et al., 1991), protease, which catalyses protein hydrolysis to peptides and amino acids and may also supply a large part of the bioavailable N (Ladd and Butler, 1972), and arylamidase, which is involved in hydrolysis of N-terminal amino acid from peptides and amides in soils (Acosta-Martinez and Tabatabai, 2000). In contrast to that, urease plays a major role in the hydrolytic reaction of urea to form ammonium and carbon dioxide in the soil. The soil enzymes are highly sensitive to environmental changes and can therefore be used as indicators of functional processes related to vegetation establishment and soil quality. Thus, the measurement of the soil enzyme activities may provide an estimate of N cycling intensity (Dick et al., 1988).

A critical component of oil sands reclamation involves re-building the soil organic layer and accelerating soil profile development, often by large scale applications of organic matter. Two types of OM commonly used as organic capping materials include peat mineral soil mix (PMM) and LFH mineral soil mix (LFH-MS), with the LFH including identifiable litter (L), fragmented and partially decomposed litter (F), and highly decomposed humus (H) material (MacKenzie and Naeth, 2007). The PMM is generally salvaged from lowlands within the mining footprint. The LFH-MS is salvaged from upland boreal forest sites and typically includes Ae horizons from Luvisolic soils (Soil Classification Working Group, 1998) and fine roots and tree stumps (MacKenzie and Naeth, 2007). The organic capping materials have differing nutrient availabilities. The PMM has a high total N and low available N due to more recalcitrant organic carbon (C) which mineralizes slowly and results in high C to N ratios (Hemstock et al., 2010), a widely accepted indicator of N release and organic substrate availability (Mohanty et al., 2013). The LFH-MS has a lower C to N ratio and provides a rich source of labile C and nutrients (MacKenzie and Naeth, 2010). Despite high nutrient availability in LFH-MS and potential use of LFH-MS as a reclamation material, its role in oil sands reclamation has not been thoroughly investigated. Despite past research in the oil sands region, more is needed to help understand how PMM and LFH-MS will perform as organic capping materials in reclamation. Although some small scale preliminary studies have been conducted on N cycling and enzyme activities on PMM and LFH-MS materials (McMillan et al., 2007; Dimitriu et al., 2010; Mackenzie and Quideau, 2012), no rigorous large scale, field based investigations comparing LFH-MS and PMM as organic capping material for oil sands reclamation have been conducted (Naeth et al., 2013). Due to contrasting biological properties and nutrient availabilities with more decomposed OM in LFH-MS, we hypothesized that N availability and associated enzyme activities will be greater in LFH-MS than PMM in reconstructed sites. Findings from this study will help determine reclamation materials for soil quality improvement in large scale reclamation.

2. Materials and Methods

2.1 Research site

The research was conducted on Suncor Energy Inc., Lease 86/17, located 24 km north of Fort McMurray, Alberta (56°39′ N,111° 390′ W) in the central mixedwood natural subregion of

the boreal forest region (Fung and Macyk, 2000). The area is characterized by long cold winters and short warm summers with a mean annual temperature of 0.7 °C from 1971 to 2000. Mean annual precipitation is 455 mm, which mostly 342 mm falls as rain during summer (Environment Canada, 2003). Dominant tree species in natural forests in the region are trembling aspen (*Populus tremuloides* L.) and white spruce (*Picea glauca* L.) that exist in pure or mixed wood stands (Fung and Macyk, 2000). The majority of the soils have developed on glacial and glacial fluvial deposits. Gray Luvisolic soils (based on the Canadian system of soil classification, same below) are associated with till and lacustrine deposits, while Dystric Brunisols are associated with glaciofluvial outwash and eolian sands (Turchenek and Lindsay, 1982).

Air temperature and total precipitation data for the study period indicated that 2012 was slightly warmer and wetter than 2011. The mean average temperature was 14.6 °C in 2011 and 15.2 °C in 2012 during the sampling period (data not shown). The study site received 101 mm of precipitation in 2011 and 324 mm in 2012 during the sampling period (June - October) (Figure 2-1) (O'Kane Consultants Inc.)

2.2 Experimental design and plot establishment

Research plots were established at Southeast Dump (56° 58′ N 111° 220′ W) at Suncor Energy Inc., between November 2007 and February 2008 (Brown and Naeth, 2014). Two rows of plots were arranged along a slightly east-facing slope in a completely randomized block design. The plot size was 10 m × 30 m. The plots were separated by a 5 m buffer. Half of the plots received freshly salvaged LFH-MS and the other half received PMM following standard reclamation prescriptions. The LFH-MS was applied at a depth of 20 cm, over 30 cm of mixed B and C horizon subsoil and 100 cm of clean overburden. The PMM was applied at a depth of 30 cm over 100 cm of clean overburden. The LFH-MS plots had a greater vegetation cover (65%) of plant groups including forbs, grasses, sedges and woody species than the PMM plots (33%) in the second growing season in 2009 (Brown and Naeth, 2014). During the third (2010) and fourth (2011) growing seasons the density of woody species in LFH-MS was 2.9 and 12.1 plants m^{-2} and in PMM was 1.3 and 7.8 plants m^{-2}, respectively. In LFH-MS, total vegetation cover (%) for the third and fourth growing seasons were 62.7 and 64.7, respectively. Whereas, in PMM it was 69.7 and 48.8, respectively (Forsch and Naeth, unpublished; Naeth et al., 2013; Archibald, 2014). This study was carried out in 2011-2012 on three-year old established plots from another

research program using a 2 × 2 (two organic capping types and two sampling depth intervals of each organic capping type) completely randomized factorial design with six replications. Four 1 m × 1 m quadrats were randomly established in each plot of organic capping (LFH or PMM) for monthly soil sampling from two depths (0-10 and 10-20 cm) that represent a major part of the main rooting zone.

2.3 Soil sample collection and analyses

Soils were sampled monthly from the 0 - 10 and 10- 20 cm layers from June to August (summer) and September to October (fall) in 2011 and 2012 from each organic capping type. Four soil samples were randomly collected from each quadrat and bulked to form a composite sample of each organic material for each layer, with a total of 24 samples collected at each sampling. Soil samples were transported to the laboratory in cooler containing ice packs. Fresh soil samples were homogenized then sieved (2 mm) and stored at 4°C until the analyses were complete in 7 days. A sub-sample of each sample was used for analysis of enzyme activities, microbial biomass C (MBC) and N (MBN) and available N (NH_4^+-N and NO_3^- - N). The remainder of each sample was air-dried at room temperature, ground, and used for measuring pH and electrical conductivity (EC). A portion of the air-dried soil sample was ground with a ball mill (Mixer Mill MM 200, Thomas Scientific, Swedesboro NJ) for 30 s and used for measurement of total C (TC) and total N (TN) as described below.

2.4 Analyses of soil physical and chemical properties

Gravimetric water content in the fresh soil samples was determined by drying the soil in an oven at 105 °C for a 24 h period (Kalra and Maynard, 1991). The soil pH was measured in a 1:2 (m:v) soil:0.01 mol L^{-1} $CaCl_2$ solution using a pH meter (Kalra and Maynard, 1991). Electrical conductivity was measured using an electrical conductivity meter following a 1:5 soil:water extraction (m:v) (Kalra and Maynard, 1991). For the NH_4^+-N and NO_3^- - N analyses, soil samples were extracted using 2 mol L^{-1} KCl (Mulvaney, 1996). The extract was analyzed colorimetrically by the indophenol blue method for NH_4^+-N (Keeny and Nelson, 1982) and by the vanadium oxidation method for NO_3^- - N (Miranda et al., 2001). The TC and TN were analyzed by the dry combustion method using an automated elemental analyser (NA-1500 series, Carlo Erba, Milan, Italy).

2.5 Soil microbial biomass measurement

Soil MBC and MBN were measured using the chloroform fumigation-extraction method (Vance et al., 1987). All extractions were done within a week of sample collection. Fresh soil samples were fumigated with ethanol free chloroform for 24 h in an evacuated desiccator. A 0.5 mol $L^{-1}K_2SO_4$ solution was used to extract C and N from fumigated and unfumigated samples at 1:5 (m:v) soil to solution ratio. After shaking for one hour on a shaker (Eberbach Corp., Michigan, U.S.A), extracts were filtered using Fisher Q2 filter papers. Extractable C and N were analyzed using a TOC-VCSN analyzer (Shimadzu, Kyoto, Japan). The MBC and MBN were calculated by dividing the difference in extractable C or N between fumigated and unfumigated samples by a conversion factor of 0.45 for MBC and 0.54 for MBN (Joergensen, 1996).

2.6 Soil enzyme assays

Four extracellular enzymes involved in N cycling were measured, including N-acetyl-β-D-glucosaminidase (Enzyme classification number (ECN), ECN 3.2.1.14), urease (ECN 3.5.1.5), protease (ECN 3.4) and arylamidase (ECN 3.4.11.2). For NAGase activity, soil sample suspensions were prepared by placing one gram of fresh soil in a 125 mL nalgene bottle. A 125 mL of sodium acetate buffer (50 mol L^{-1}, pH 5) was added to the bottle, and the suspension was homogenized using a magnetic stir plate until suspensions were transferred into black 96 well plates for determining NAGase activity (Sinsabaugh et al., 2003). A 200 mL soil suspension, and 50 mL of 200 mol L^{-1} of substrate were pipetted onto the plate. Reference standards and quench controls were then added to each plate. The plates were placed in an incubator for three hours at 20°C in the dark. A 20 mL of 0.5 mol L^{-1} NaOH was added to the plates using an auto dispenser to stop the enzymatic reaction. Fluorescence was measured at 360 nm excitation and 460 nm emissions using a Synergy HT multi-detection microplate reader (Bio-Tek Instruments, Winooski, VT) and NAGase activity (nmol of substrate g^{-1} h^{-1}) was calculated on an oven-dry mass basis. Urease (amidohydrolase) activity was measured in clear 96 well plates (Sinsabaugh et al., 2000). Soil assay wells received 200 mL of soil suspension and 10 mL of 400 mmol L^{-1} urea substrate. A 200 mL of soil suspension and 10 mL of Milli-Q (Millipore, Bedford, MA) deionized water were pipetted into the negative control wells. Substrate control wells contained 200 mL of acetate buffer and 10 mL of urea substrate. Microplates were incubated for 18 h at 20 °C. After incubation, ammonium concentration was quantified using colorimetric reagent packets

including salicylate and cyanuarate from Hach (Loveland, CO 80,539, U.S.A). Urease activity was calculated as nmol of ammonium released per gram of soil per hour (nmol NH_4 g^{-1} h^{-1}). Protease activity was measured using a modified method of Ladd and Butler (1972). One gram of fresh soil was mixed with 2.5 mL of sodium caseinate (10 g mL^{-1}) in 0.1 mol L^{-1} of tris–sodium borate buffer at pH 8.1. The mixture was incubated at 37°C for 1 h. The reaction was stopped with 2 mL of 17.5% tricloracetic acid (TCA) and centrifuged. After centrifugation, 2 mL of the supernatant was mixed with 3 mL of 1.4 mol L^{-} Na_2CO_3 and 1 mL of Folin-Ciocalteu reagent. Absorbance was recorded at 700 nm using a UV-spectrophotometer (Genesys 10-S, Rochester, NY). Arylamidase activity was assayed according to Acosta-Martinez and Tabatabai (2000). One gram of fresh soil was incubated with 3 mL of 0.1 m mol L^{-1} tris (hydroxyl methyl) amino methane (THAM) buffer (pH 8.0) and 1 mL of 8.0 m mol L^{-1} l-leucine β-naphthylamide hydrochloride at 37 °C for 1 h. The supernants were converted to an azo-compound by reacting with p-dimethylaminocinnamaldehyde. The absorbance was measured colorimetrically at 540 nm UV-spectrophotometer (Genesys 10-S, Rochester, NY).

2.7 In-situ N availability measurement using plant root simulator probes

Plant root simulator (PRS^{TM}, Western Ag Innovations Inc., Saskatoon, SK, Canada) probes were used to measure soil N supply rates. PRS^{TM} probes have an ion exchange membrane encapsulated in a plastic casing. The membrane captures cations and anions from soil solution and integrates the temperature and water effects on nutrient fluxes during incubation. Each probe has 10 cm^2 area of membrane with an adsorbing surface area of 17. 5 cm^2 (both sides). Four pairs of probes (cation and anion) were inserted in established quadrats of each amendment four times from June 2011 to July 2013 (June to September 2011, October 2011 to May 2012, June 2012 to October 2012, October 2012 to July 2013). At each sampling, probes were retrieved, washed with deionized water and sent to Western Ag Innovations Inc. Elution was conducted with 0.5 mol L^{-1} HCl solution and NO_3^--Nand NH_4^+-N were analyzed colorimetrically using an automated flow injection analysis (FIA) system (PRS^{TM} -Probe Operations Manual, 2009).

2.8 Soil temperature and soil water content measurement

HOBO micro station data loggers (Model H21-002; Onset Computer Corporation, Bourne, MA, U.S.A) connected with volumetric smart soil water sensors (Decagon Devices Inc.,

Pullman, WA, U.S.A.) and 12-bit smart temperature sensors (Onset Computer Corporation) were installed. Two sensors were installed at 5 cm depth in both amendment plots on relatively level ground in the bottom row of plots of each amendment (Brown and Naeth, 2014). The mean of sampling day data of soil temperature for each month of the sampling period of 2011 and 2012 were used for correlation analysis with N availability and soil enzyme activities.

2.9 Statistical analyses

A repeated measures analysis of variance (ANOVA) was used to assess capping material type and sampling depth effects over time on N availability, soil enzyme activities and MBC and MBN using the PROC MIXED model. The month of each sampling was considered the repeated measured variable for determining seasonal variation in 2011 and 2012. In this analysis, the output statistics passed tests for compound symmetry. Tukey's HSD test was used to determine if there were significant differences between the capping treatments, depths, month of sampling and their interactions. A repeated measures ANOVA was also used to assess the capping type effects on N supply rate at different times of incubation of PRS^{TM} probes. One- way ANOVA was used to determine the differences in the basic characteristics of caps. Correlation analysis was conducted to determine the relationship between N availability, soil enzyme activities, MBC, MBN, soil pH, soil temperature and soil water in each capping material separately. Linear regression was used to determine which enzyme activity had a strong relationship with N availability in each of the caps. All statistical analyses were performed using the SAS 9.2 (SAS Institute Inc., USA) software.

3. Results

3.1 Basic characteristics of capping materials

There were strong differences in soil properties between the capping material types. The TC was approximately two-fold greater in PMM than in LFH-MS (Table 2-1). The TN ranged from 3.0 to 3.7 g kg^{-1} in LFH-MS and from 2.8 to 3.4 g kg^{-1} in PMM, with no significant differences between the two capping materials. The C to N ratio of LFH-MS and PMM organic caps did not differ significantly due to large variability in the dataset. The pH was higher in PMM than in LFH-MS and higher in the 0-10 than in the 10-20 cm layer (Table 2-1). The EC was generally low in both LFH-MS and PMM, indicating the non-saline nature of the LFH-MS

and PMM (Table 2-1). Between 2011 and 2012, soil water content (SWC) was similar in PMM and LFH-MS. The SWC was greater in the PMM than in the LFH-MS plots and ranged from 16.2 to 23.4% in the LFH-MS and 28.8 to 29.5% in the PMM plots when the 0-10 and 10-20 cm layers were considered together (data not shown).

3.2 Microbial biomass C and N

Soil MBC was consistently the greatest in the 0–10 cm layer in LFH-MS and the lowest in the 10-20 cm layer in PMM (Table 2-2). Soil MBC was significantly affected by capping material type, sampling depth and time, and their interactions (Table 2-3); the effect of time of sampling was greater in 2012 than in 2011 (Table 2-2). The MBC was consistently greater in the 0-10 cm than in the 10-20 cm layer. Soil MBN response to the treatments was similar to that of MBC, but did not show as much variation among sampling months. The greatest MBN was found in the 0-10 cm layer in LFH-MS in 2011 and 2012 (4.4 mg N kg^{-1}, on average, same below), followed by the 10-20 cm layer in LFH-MS (2.9 mg N kg^{-1}) and 0-10 cm layer in PMM (2.7 mg N kg^{-1}), and was consistently lowest in the 10-20 cm layer in PMM (2.2 mg N kg^{-1}).

3.3 Available N

Available N (NH_4^+-N and NO_3^--N) was significantly influenced by the organic capping material type, and the sampling depth and time (Tables 2-2 and 2-3). Concentrations of NH_4^+-N and NO_3^--N were generally the highest in the 0-10 cm layer of LFH-MS and the lowest in the 10-20 cm layer of PMM (Table 2-2). Mean NH_4^+-N availability was 4.2 mg N kg^{-1} in 2011 and 4.8 mg N kg^{-1} in 2012. Mean NO_3^--N increased from 3.6 in 2011 to 6.3 mg N kg^{-1} in 2012. Changes in NO_3^--N of each organic capping material from 2011 to 2012 followed a pattern similar to that of the NH_4^+-N. The magnitude of these changes, however, was greater for the NO_3^--N than for the NH_4^+-N (Table 2-2). Both of the NH_4^+-N and NO_3^--N concentrations were greater in the fall than in the summer.

The N supply measured using the PRS probes was approximately 50% greater in LFH-MS than in PMM over the entire study (Table 2-4). The dominant form of N supply was NO_3^--N in both capping materials. During incubation from September 2011 to May 2012, the greatest NO_3^--N supply occurred in LFH-MS. However, NH_4^+-N supply was below detection limit during incubation from September 2011 to May 2012.

3.4 Soil enzyme activities

Interactions between organic capping material types, sampling depths and sampling times significantly affected activities of NAGase, arylamidase, protease and urease in both 2011 and 2012 (Table 2-3). The NAGase activity was significantly greater in the 0-10 cm layer of LFH-MS than in the other treatments. However, in most sampling times, differences between the 10-20 cm layer of LFH-MS and both layers of PMM were non-significant. NAGase activity varied from 2011 to 2012 by 46, 29, 9 and 8% for LFH-MS 0-10, LFH-MS 10-20, PMM 0-10 and PMM 10-20 cm, respectively (Figure 2-2a). Arylamidase and protease activities followed a similar trend to that of NAGase activity (Figure 2-2b and c). Both arylamidase and protease activities were greater in the fall than in the summer for all capping material type by depth combinations, particularly in 2012. The increase in arylamidase and protease activities from summer to fall in 2012 was twice as high in LFH-MS as that in PMM. Urease activity declined from 2011 to 2012, with the greatest reduction in the 10-20 cm layer of LFH-MS (17.8%) and 0-10 cm layer of PMM (16.3%) (Figure 2-2d).

3.5 Linkages among soil properties

Many of the strong correlations were observed among SWC, pH, NH_4^+-N, NO_3^--N, activities of NAGase, protease, arylamidase and urease, MBC, and MBN (Table 2-5). The SWC, pH and the soil enzyme activities in LFH-MS were significantly correlated with response variables representing N availability. Similarly, NH_4^+-N and NO_3^--N were significantly correlated with soil enzyme activities in PMM. The NH_4^+-N and NO_3^--N were significantly correlated with SWC in LFH-MS, but not in PMM. Regression analysis indicated that available N was positively related with NAGase, arylamidase, protease and urease activities in both organic capping materials (Figure 2-3).

4. Discussion

A key finding of our study is that four and five years after incorporation the two most common organic capping materials used for oil sands reclamation in northern Alberta had contrasting soil properties (e.g., MBC, MBN, activities of NAGase, arylamidase, protease and urease, and N availability), consistent with previous findings in reclaimed soils in the area (McMillan et al., 2007; MacKenzie and Quideau, 2010). The differences in soil properties

affected N availability and as such could influence early ecosystem development. The two capping materials differed in total C, MBC and MBN and, therefore, likely had different decomposition potentials. The narrower C to N ratio in LFH-MS indicated more decomposed OM and a greater potential of N availability through OM mineralization relative to PMM. Greater microbial activity would result in greater OM decomposition and the lower organic matter C to N ratio in LFH-MS supported higher MBC and MBN in LFH-MS as compared with PMM (Hahn and Quideau, 2013).

Soil enzyme activities are considered soil quality indicators in the reclamation of disturbed ecosystems in the oil sands region (Dimitriu et al., 2010). The greater NAGase, arylamidase and protease activities in LFH-MS than in PMM in the 0–10 cm layer in our study is consistent with the greater N availability in LFH-MS than in PMM. The lower enzyme activities, and MBC and MBN in the 10–20 cm than in the 0–10 cm layer in both organic capping materials may be explained by the changes in nutrient levels, soil pH, SWC and O_2 concentrations with depth (Ekenler and Tabatabai, 2004; Eilers et al., 2012). Among them, soil pH likely was the most influential factor (Table 2-1). Soil pH strongly affects abiotic and biotic factors such as C and nutrient cycling processes (Kemmit et al., 2006) and microbial population composition (Will et al., 2010). Acidic soil pH favours fungal growth, whereas neutral or alkaline soil pH promotes bacterial growth (Will et al., 2010). Hence, changes in microbial population composition caused by soil pH may also influence soil enzyme activities since many enzymes exhibit pH optima (Baldrian et al., 2008). Thus, soil enzyme activities are affected by pH and composition of the soil microbial community (e.g., enzymes originating from different organisms have different pH optima) and location of the enzyme in the soil matrix (e.g., intracellular, free in solution, or adsorbed on solid surfaces) (Turner, 2010). Thus, soil enzyme activities in our study were different between 0-10 and 10-20 cm soil layers. However, it is difficult to pinpoint whether these changes were associated with soil pH alone or other confounding factors since we have not determined the partition in fungal and bacterial biomass in this study (Rousk et al., 2009). Different soil enzyme activities between 0 and 10 and 10 and 20 cm layers in this study might also be related to the higher aeration (Ellis and Atherton, 2003) and the greater amount of soluble C compounds added by plant roots in surface than in deeper soil layers (Will et al., 2010).

Another important indicator for potential reclamation success in the oil sands region is N availability. We observed greater available N (NH_4^+-N and NO_3^--N) in LFH-MS than in PMM.

The greater N availability indicates greater N mineralization in LFH-MS than in PMM that was linked with the narrower C to N ratio in LFH-MS (Brown and Naeth, 2014; Yan et al., 2012). The greater NO_3^--N than NH_4^+-N in both LFH-MS and PMM indicated that in those highly disturbed reclaimed soils nitrification activities were high (McMillan et al., 2007). This is in contrast with the low nitrification activities in natural forest soils in the vicinity of the study site (Yan et al., 2012). The low nitrification activities and the resultant low soil NO_3^--N concentrations in natural forests are typically affected by the low soil pH (Fisher and Binkley, 2000). For example, in the 21 stands studied in Yan et al. (2012), with the exception of two stands that had pH of 5.94 and 6.02, the other 19 stands had pH ranging between 3.67 and 4.95 (Chang et al., unpublished data). Because the LFH-MS and PMM were mixed with mineral soil and the mixing with mineral soils increased the pH of those reclamation material (pH ranged from 5.70 to 7.50, Table 2-1), those higher pH and the physical disturbance likely encouraged soil nitrification activities (Kaur et al., 2010). Between the two organic capping types, NO_3^--N was more predominant in PMM than in LFH-MS, again likely attributable to the higher pH in PMM than in LFH-MS. The higher pH in PMM than in LFH-MS is consistent with earlier studies on reclaimed oil sands soils (Mackenzie and Naeth, 2010; MacKenzie and Quideau, 2012). The nitrification process is known to be more pH dependent than the ammonification process and nitrification activity can be inhibited at low pH (Myrold, 2005). The strong positive correlations among soil enzyme activities, NH_4^+-N, NO_3^--N, SWC, pH, MBC, and MBN suggest that enzyme activities and N availability are affected by both biotic and abiotic factors (Tan et al., 2008). The stronger relationships between N availability, NAGase, arylamidase and protease in LFH-MS than in PMM were similar to previous studies where it was shown that activities of NAGase (Andersson et al., 2004), arylamidase (Muruganandam et al., 2009) and protease (Lucas and Casper, 2008) have been associated with soil fungal biomass. Hence, we suggest that enzyme activities in this study may be dependent on mycorrhizal (part of the total fungal community) biomass, as indicated in Brown and Naeth (2014).

Mycorrhizae fungi often dominate the microbial biomass in forest soils and litter and LFH material and many mycorrhizal fungi produce extracellular enzymes that catalyze C, N and phosphorus mineralization from OM and litter material (Smith and Read, 2008). Mycorrhizae fungi are also capable of utilizing some organic forms of N such as amino acids (Burke et al., 2011). The very low urease activity in our study is similar to the oil sands study of Dimitriu et al.

(2010) and this might have been linked to the low availability of urease specific substrate in the studied soils. Different soil chemical properties such as soil pH of organic capping material may have influenced soil enzyme activities (Table 2-5). The lower enzyme activity in PMM than in LFH might be linked to the pH of PMM. Seasonal changes in soil temperature, water content and pH typically affect organic substrate availability and soil microbial activities and processes (Baldrian et al., 2008). The seasonal changes may facilitate production of organic substrates for microbes, which in turn affect microbial processes. Thus, seasonality may affect microbial substrate availability, which is considered one of the main limiting factors for microbial activity and decomposition of OM (Corre et al., 2002).

Fresh litter input to soils that mainly occur in the fall provides an important substrate and energy source for soil microorganisms that enhance microbial activities and SOM decomposition, in the form of positive priming effects (Kuzyakov et al., 2000). The higher MBC, MBN, soil enzyme activities and available N in the fall in this study were likely a result of the addition of labile C from fresh litter input, because soil temperature had no relationship with (and were thus not limiting) those studied parameters. Similarly, Baldrian et al. (2008) found that peak soil enzyme activities and MBC in October was associated with input of fresh litter in their study. The activities of NAGase (Burke et al., 2011), protease (Werdin-Pfisterer et al., 2012), arylamidase (Acosta-Martinez and Tabatabai, 2000) and urease (Cochran et al., 1989) can increase due to fresh litter input. Burke et al. (2011) found that decomposition of litter on the soil surface in late summer could have liberated and transported organic and inorganic compounds from litter into the soil which altered ectomycorrhizal and microbial activities, increasing N availability. The vegetation cover and soil water content in our study also changed from summer to fall and contributed to a greater change in organic substrate availability in LFH-MS than in PMM. Changes in the composition of vegetation and SWC between the study periods and sites with different organic capping materials could change the priming effect (Criquet et al., 2002; Schaaf et al., 2011). For example, in a study conducted on our site (Brown and Naeth, 2014) vegetation cover and plant species richness were greater in LFH-MS than in PMM plots.

Therefore, greater vegetation cover in LFH-MS than in PMM would mean greater priming effect in the former than in the latter. Greater cover and species richness would contribute to greater and diverse litterfall that might have caused the greater effect in LFH-MS than in PMM plots. However, it is not possible to directly quantify effects of litter fall on N availability and

associated enzyme activities in this study, since we have not measured litter fall during the study period. In general, annually litter fall from herbaceous plants can comprise as much as 16% of forest litter fall (Gilliam, 2007). Waldrop and Firestone (2006) indicated that both seasonal soil water and C substrate differences were caused by differences in aboveground vegetation.

Soil pH and EC in both organic capping treatments were within the acceptable range according to Alberta Tier 1 guidelines (Alberta Environmental Protection, 1994) for the oil sands region. The higher pH and EC in PMM may be due to the alkaline nature of salvaged mineral material in the oil sands region (Fung and Macyk, 2000). In general, we considered LFH-MS as a better soil material than PMM because most of the studied soil properties for LFH-MS were better suited (such as having higher N availabilities) for vegetation development than those of PMM. The LFH-MS material may also provide more native seeds and propagules for revegetation than PMM (Mackenzie and Naeth, 2007) that can help to expedite ecosystem development in oil sands reclamation (Naeth et al., 2013). However, the availability of LFH-MS for reclamation is generally more limited (Naeth et al., 2013). In addition, the long-term effect of LFH-MS and PMM application for land reclamation is not very well understood and should be further studied in the future.

5. Conclusions

The two organic capping materials commonly used for land reclamation in the oil sands region had contrasting properties. The LFH-MS was a capping material that had a better quality than the PMM for land reclamation in the oil sands region in Alberta, based on its greater N availability and its ability to support greater enzyme activities. The N availability and enzyme activities were highly related and greater N availability in the capping material will reduce the risk of N deficiencies and minimize the need for N fertilization in developing ecosystems after land reclamation. This calls for reclamation policies that promote careful planning for salvaging LFH-MS materials and using them for land reclamation in the oil sands region, to take advantage of the existence of LFH-MS materials that are considered superior than peat materials.

Table 2-1 Selected properties as determined 4 years after oil sands reclamation of LFH mineral soil mix (LFH-MS) and peat mineral soil mix (PMM) organic capping materials.

Organic capping type and depth (cm)	pH	EC[a] (dS m^{-1})	TC[b] (g kg^{-1})	TN[c] (g kg^{-1})	C to N[d]
LFH-MS 0- 10	6.55b[†]	0.02a	56.8b	3.7a	17.0a
LFH-MS 10-20	5.70c	0.03a	54.2b	3.0a	21.5a
PMM 0-10	7.50a	0.06a	102.0a	2.8a	37.7a
PMM 10-20	6.60b	0.04a	107.0a	3.4a	46.5a
Two-way ANOVA					
Organic capping	***	ns	**	ns	ns
Depth	***	ns	ns	ns	ns
capping* depth	ns	ns	ns	ns	ns

[†]Means with different lowercase letters indicate significant difference between organic capping and their depths in each column $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; and ns, not significant
Abbreviations: [a]EC = electrical conductivity, [b]TC = total C, [c]TN = total N, and [d]C to N = C to N ratio

Table 2-2 Effects of organic capping types on selected soil properties in organic caps used for oil sands reclamation.

Soil process [a]	Capping type and depth (cm)	2011					2012				
		Jun.	Jul.	Aug.	Sept.	Oct.	Jun.	Jul.	Aug.	Sept.	Oct.
MBC (mg C kg^{-1})	LFH-MS 0-10	19.7a[†]	15.8a	20.0a	12.5a	18.3a	33.7a	24.3a	9.6a	31.1a	35.2a
	LFH-MS 10-20	8.4b	6.3b	10.3b	7.9ab	8.7b	9.9b	16.2b	6.2bc	18.6b	20.2b
	PMM 0-10	7.7b	8.6b	9.6b	6.7ab	11.9ab	27.0a	13.6bc	9.0ab	15.3b	17.1b
	PMM 10-20	5.5b	4.3b	4.1b	4.1b	4.0b	6.1b	6.3c	4.3c	7.1c	7.9c
MBN (mg N kg^{-1})	LFH-MS 0-10	4.6a	4.0a	4.9a	3.1a	4.3a	5.7a	4.5a	1.9a	5.0a	4.5a
	LFH-MS 10-20	2.7ab	2.3b	3.8ab	2.4ab	2.2b	3.0ab	3.2a	1.2ab	3.8ab	3.8ab
	PMM 0-10	3.0ab	2.6b	3.0b	2.3ab	3.9a	4.2ab	3.7a	1.5ab	3.1ab	1.5b
	PMM 10-20	2.4b	1.9b	2.7b	1.3b	2.8ab	2.2b	3.0a	1.0ab	2.2b	2.0ab
NH_4^+-N (mg N kg^{-1})	LFH-MS 0-10	2.6a	3.9a	7.5a	3.6a	7.6a	4.3a	6.1a	6.5a	6.6a	7.6a
	LFH-MS 10-20	1.9b	2.5b	6.4b	2.8b	5.7ab	2.4ab	5.4a	6.4a	6.5a	6.4ab
	PMM 0-10	2.4ab	3.2b	7.3ab	2.6b	6.4a	2.8ab	4.1a	5.4ab	5.2ab	5.9ab
	PMM 10-20	1.9b	2.6b	5.0c	3.1a	4.2b	1.7b	2.6b	2.8b	3.9b	4.0b
NO_3^--N (mg N kg^{-1})	LFH-MS 0-10	3.0a	4.9a	5.4a	6.2a	8.0a	5.5a	6.4a	6.7ab	9.8ab	10.8ab
	LFH-MS 10-20	1.3c	1.6c	3.4b	3.5b	4.8bc	2.8b	3.4b	4.8b	5.7b	6.0b
	PMM 0-10	2.1b	2.9b	3.6b	3.8b	6.5ab	4.9a	7.5a	8.8a	10.5a	11.2a
	PMM 10-20	1.2c	1.7c	1.9c	2.5c	4.4c	2.2b	3.7b	5.1b	5.6b	5.6b

[†]Means with different lowercase letters indicate significant difference between organic capping and their depths in each column

[a] soil property: MBC: microbial biomass C, MBN: microbial biomass N, NH_4^+-N: 2 mol L^{-1} KCl extracted ammonium, NO_3^--N: 2 mol L^{-1} KCl extracted nitrate

Table 2-3 Effects of organic capping type, depth, time and their interactions on soil processes in oil sands reclamation.

Soil processes [a]	Capping type		Depth		Capping type × Depth		Time		Capping type × Time		Depth × Time		Capping type × Depth × Time	
	F value	*p* value	F value	*p* value	F value	*p* value	F value	*p* value	F value	*p* value	F value	*p* value	F value	*p* value
2011														
MBC	83.4	<0.001	104.8	<0.001	32.8	<0.001	23.4	<0.001	7.8	<0.001	1.6	0.188	1.4	0.238
MBN	28.4	<0.001	66.8	<0.001	7.0	<0.015	15.2	<0.001	3.6	0.002	8.7	<0.001	1.3	0.290
NH_4-N	18.8	<0.001	32.5	<0.001	0.1	0.739	74.1	<0.001	2.1	0.019	1.4	0.249	2.4	0.057
NO_3-N	84.8	<0.001	169.2	<0.001	12.9	0.001	82.1	<0.001	1.9	0.044	2.3	0.068	1.1	0.382
NAGase	60.8	<0.001	28.8	<0.001	14.3	0.001	18.4	<0.001	3.7	<0.001	0.7	0.594	0.5	0.715
UR	31.7	<0.001	66.4	<0.001	2.7	0.110	114.5	<0.001	2.3	0.013	4.6	0.002	0.3	0.881
PRT	165.0	<0.001	125	<0.001	2.9	0.104	21.3	<0.001	7.1	<0.001	9.0	<0.001	4.4	0.003
ARA	345.7	<0.001	296.3	<0.001	14.3	0.001	53.9	<0.001	6.8	<0.001	18.9	<0.001	0.3	0.877
2012														
MBC	107.7	<0.001	118.7	<0.001	1.5	0.241	32.8	<0.001	8.4	<0.001	17.1	<0.001	0.7	0.583
MBN	16.4	<0.001	12.8	0.001	0.9	0.362	14.2	<0.001	1.8	0.055	3.3	0.014	0.3	0.850
NH_4-N	13.6	<0.001	21.2	0.002	0.8	0.371	21.3	<0.001	0.6	0.799	0.5	0.741	0.3	0.860
NO_3-N	39.6	<0.001	60.6	<0.001	2.4	0.139	32.3	<0.001	2.0	0.028	5.3	0.001	0.2	0.913
NAGase	121.1	<0.001	38.1	<0.001	25.6	<0.001	36.9	<0.001	10.0	<0.001	6.6	0.001	3.6	0.061
UR	140.4	<0.001	231.9	<0.001	16.7	0.006	185.7	<0.001	10.0	<0.001	24.4	<0.001	9.7	0.081
PRT	112.0	<0.001	89.7	<0.001	9.3	0.01	16.6	<0.001	4.1	<0.001	8.5	<0.001	1.9	0.125
ARA	394.1	<0.001	346.5	<0.001	19.1	<0.001	126.6	<0.001	27.1	<0.001	46.9	<0.001	9.4	0.058

[a] Soil property: MBC = microbial biomass C, MBN = microbial biomass N, NAGase = β-1, 4-N-acetylglucosaminidase, UR = Urease, PRT = Protease, ARA = Arylamidase

Table 2-4 Soil N supply (µg N per 10 cm^2), measured using Plant Root Simulator (PRS ^{TM}) probes, in LFH-MS and PMM organic capping materials used for oil sands reclamation.

Incubation period	NO_3^--N		NH_4^+-N		Mineral N	
	LFH-MS	PMM	LFH-MS	PMM	LFH-MS	PMM
Jun. to Sept. 2011	4.4b	0.6b	1.7bc	0.4c	6.1abc	1.0c
Sept. 2011 to May 2012	10.9a	3.9b	ND	ND	11.0a	4.0bc
May to Oct. 2012	2.9b	2.4b	1.4bc	1.1bc	4.3bc	3.4bc
Oct. 2012 to Jul. 2013	3.7b	4.2b	3.4a	2.1b	7.0ab	6.2abc
Total	22.1a	11.1b	6.4a	3.5b	28.5a	14.6a
Repeated Measures ANOVA						
Capping type	**		**		**	
Time	**		**		**	
Capping type* Time	*		ns		*	

$p < 0.05$; **, $p < 0.01$; ***, and ns; not significant

[†]Means with different lowercase letters indicate significant difference between organic capping materials [a] Abbreviations: ND = not detected

Table 2-5 Pearson correlation coefficient (*r* -value) and significance[+] among soil variables in LFH and PMM organic caps used for oil sands reclamation (n=120).

Variable[a]	SWC	MBC	MBN	NH_4^+	NO_3^-	AN	NAGase	UR	PRT	ARA	Stemp	pH
	LFH-MS											
MBC	0.48**											
MBN	0.35**	0.52**										
NH_4^+-N	0.31**	0.49**	0.15									
NO_3^--N	0.31**	0.58**	0.21*	0.72**								
AN	0.33**	0.57**	0.19*	0.94**	0.91**							
NAGase	0.50**	0.45**	0.21*	0.58**	0.71**	0.69**						
UR	0.34**	0.23**	0.07	0.52**	0.63**	0.61**	0.71**					
PRT	0.45**	0.59**	0.26**	0.63**	0.75**	0.74**	0.75**	0.73**				
ARA	0.49**	0.61**	0.27**	0.75**	0.84**	0.86**	0.81**	0.71**	0.88**			
Stemp	-0.29	-0.13	0.05	-0.43	-0.39	-0.45	-0.52	-0.46	-0.35	-0.39		
pH	0.25**	0.51**	0.40**	0.24**	0.43**	0.35**	0.39**	0.44**	0.60**	0.48**	0.02	
EC	-0.07	-0.06	-0.01	-0.01	0.10	-0.01	0.04	0.02	-0.02	0.05	-0.01	-0.08
	PMM											
MBC	0.19*											
MBN	0.05	0.41**										
NH_4^+-N	0.10	0.35	0.01									
NO_3^--N	0.09	0.46**	0.02	0.60**								
AN	0.11	0.46**	-0.01	0.85**	0.93**							
NAGase	0.04	0.25**	0.01	0.32**	0.42**	0.43**						
UR	0.20*	0.17	0.05	0.49**	0.53**	0.57**	0.42**					
PRT	0.16	0.49**	0.12	0.43**	0.65**	0.62**	0.38**	0.53**				
ARA	0.08	0.42**	0.08	0.51**	0.71**	0.67**	0.45**	0.63**	0.88**			
Stemp	-0.36	-0.27	-0.04	-0.29	-0.37	-0.37	-0.36	-0.46	-0.31	-0.28		
pH	-0.02	0.47	0.23**	0.27**	0.36**	0.34**	0.17	0.28**	0.69**	0.66**	0.03	
EC	-0.21	0.07	0.06	-0.01	0.04	0.03	0.04	0.11	0.21	0.10	-0.02	0.22

[+*]Significant at the $p < 0.05$ level ** Significant at the $p < 0.01$ level

[a] Variables: SWC: soil water content, MBC: microbial biomass C, MBN: microbial biomass N, NH_4^+: ammonium, NO_3^-: nitrate, AN: available N, NAGase: β- 1, 4-N- acetylglucosaminidase, UR: urease, PRT: protease, ARA: arylamidase, Stemp: soil temperature EC: electrical conductivity, pH

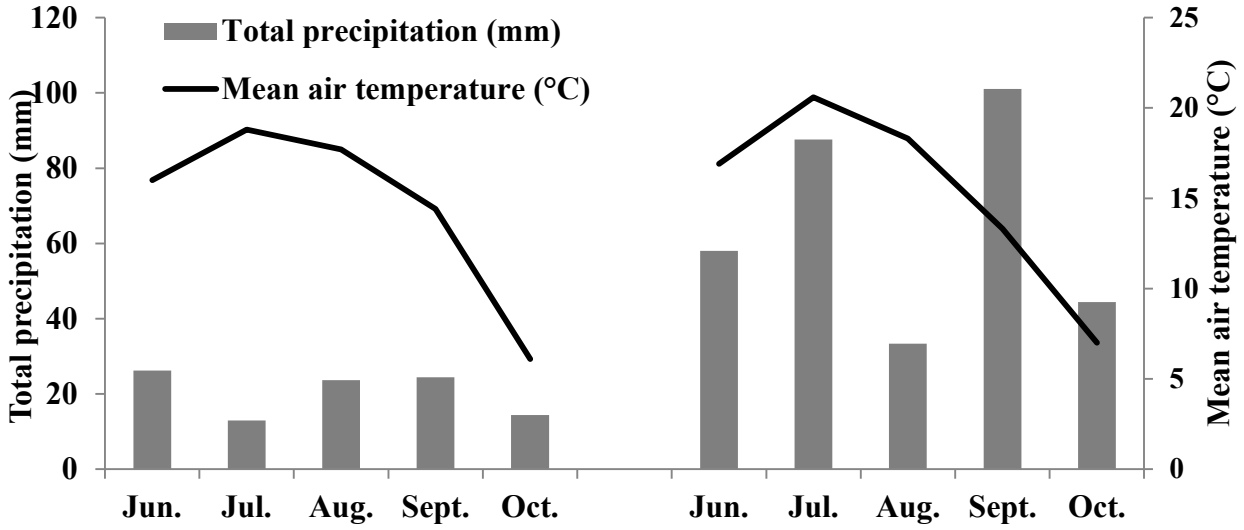

Figure 2-1 Monthly precipitation (bars) and mean monthly air temperature (line) during sampling periods.

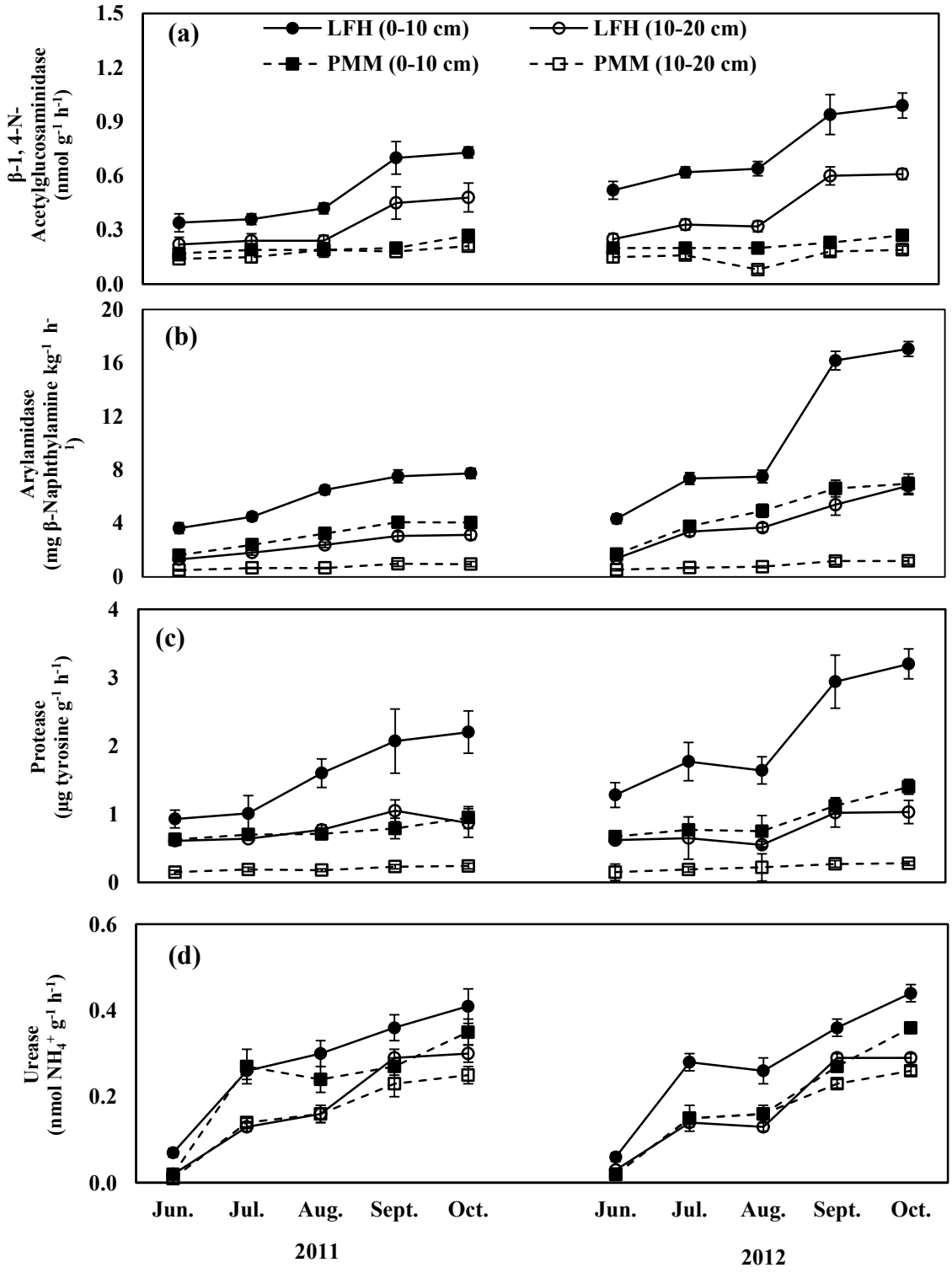

Figure 2-2 Changes in (a) β-1, 4-N-acetylglucosaminidase, (b) arylamidase, (c) protease and (d) urease activities in LFH-MS and PMM from June to September in both 2011 and 2012 in oil sands reclamation. Error bars indicate standard errors (n=6).

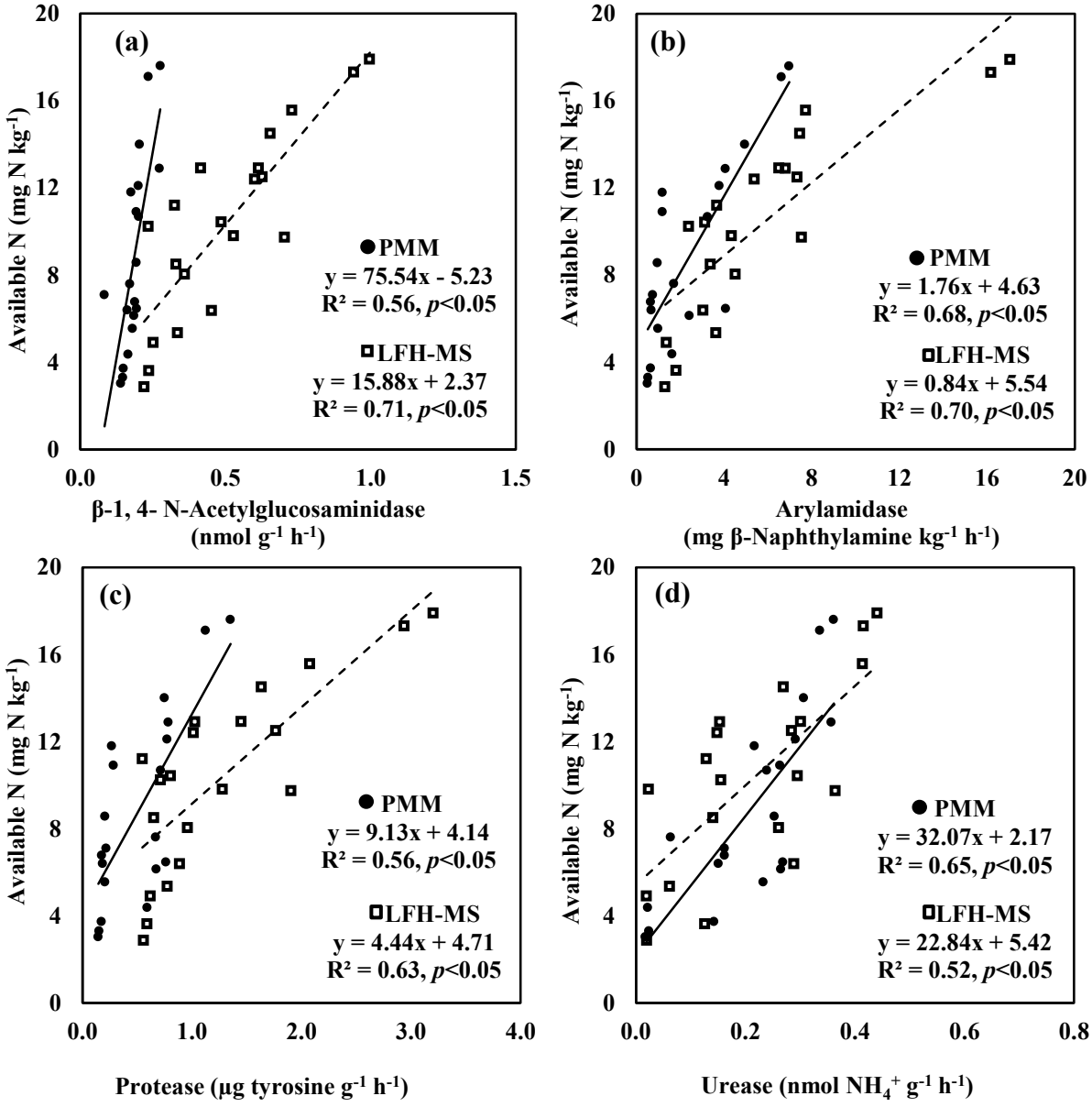

Figure 2-3 Relationships between N availability and (a) β-1, 4-N-acetylglucosaminidase (b) arylamidase, (c) protease, and (d) urease activities in LFH-MS and PMM.

CHAPTER 3 ORGANIC SUBSTRATE TYPE AND DIVERSITY ALTERED MICROBIAL PROCESSES IN ORGANIC CAPPING MATERIALS USED IN OIL SANDS RECLAMATION

1. Introduction

Microorganisms are involved in various ecosystem processes such as nutrient mineralization, decomposition and removal of toxic materials (Schimel and Bennett, 2004). These processes are affected by availability (Baldrian et al., 2008; Jamro et al., 2014) and nature (Orwin et al., 2006) of the substrates supporting microbial populations. Most processes are associated with conversion of complex substrates into easily decomposable compounds that microorganisms then assimilate for growth and metabolism (Nilsson et al., 2008). In all terrestrial ecosystems, plants add diverse organic materials to the soil in various forms such as leaf litter and root exudates which may create a variety of niches to support a diverse microbial community (Zak et al., 2003). The diverse microbial community can substantially influence soil processes (Hooper and Vitousek, 1997; Dehlin et al., 2006), soil chemistry (Orwin et al., 2006), soil enzyme activities (Hernandez and Hobbie, 2010), decomposition of OM and nitrogen (N) cycling processes (Loreau, 2001).

Two of the mechanisms that may affect the microbial composition and their processes are lability of substrates that affects availability of easily accessible energy for the microorganisms (Blagodatskaya and Kuzyakov, 2008) and the diversity of substrates which may influence the microbial diversity and the microbial exploitation of their functional niches (Hamer and Marschner, 2005). Availability of diverse substrates enhances the ability of soil microorganisms to decompose complex substrates (Fontaine et al., 2003; Hamer and Marschner, 2005) and increases the soil enzyme activities since the enzymes are required for decomposition of complex organic substrates (Hamer and Marschner, 2005). Thus, the type and diversity of organic substrates could alter microbial niches, and multi-trophic interactions in disturbed and reconstructed ecosystems (Zhang et al., 2011; Saleem and Moe, 2014) such as those in the Athabasca oil sands region (AOSR), one of the largest disturbed ecosystems in the world (Alberta Government, 2015). Understanding the effect of organic substrate type and diversity on specific soil processes will help to design optimal reclamation practices using these types of capping materials.

Oil sands reclamation predominantly involves use of peat-mineral soil mix (PMM) as an organic capping material to establish ecosystems in a dramatically altered landscape due to large amounts of peat available in the mining foot print (Fung and Macyk, 2000). However, wider carbon to nitrogen ratio (C to N) (Jamro et al., 2014), inactive microbial community (Beasse et al., 2015) and nutrient limitation (Duan et al., 2015) are considered key factors affecting microbial processes and raised concern for sustainability of ecosystem re-establishment in oil the sands region. Another commonly used source of organic capping material, LFH (litter (L), fragmented and partially decomposed litter (F) and highly decomposed humus (H) material) mineral soil mix (LFH-MS) (Mackenzie and Naeth, 2007), is considered a better reclamation material to expedite re-establishment of a functioning ecosystem (Naeth et al., 2013; Brown and Naeth, 2014) due to its narrower C to N ratio, higher soil enzyme activities, nitrogen (N) availability (Jamro et al., 2014; Kwak et al., 2015a) and more diverse microbial community composition (Beasse et al., 2015) than PMM.

Studies on organic substrate diversity effects on microbial processes have used plant species composition and their diversity as a proxy for changes in organic substrate inputs (Orwin et al., 2006; Hernandez and Hobbie, 2010) in natural ecosystems. Few have studied organic substrate diversity effects on microbial processes in reclaimed ecosystems such as in the AOSR, where microbial processes and function largely depend on exogenous organic input (Noah et al., 2014) or organic capping type (Kwak et al., 2016) and the type of vegetation that re-establishes on the disturbed sites (Dimitriu et al., 2010). The LFH-MS supports a more diverse microbial community (Hahn and Quideau, 2013) and has a greater priming effect on microbial processes (Jamro et al., 2014) than PMM due to the greater vegetation diversity and cover on sites with LFH-MS as an organic capping material (Brown and Naeth, 2014).

Increasing organic substrate diversity could be a potential strategy to establish a diverse soil microbial community for enhancing early establishment of a new ecosystem in oil sands reclamation. We hypothesized that 1) microbial processes would be affected by type and diversity of organic substrates available in the soil; and 2) the effect of type and diversity of organic substrates on microbial processes depends on the organic capping material used for oil sands reclamation due to differences in quality (Jamro et al., 2014) and botanical origin (Turcotte et al., 2009). This study will provide a better mechanistic understanding of the impact of organic substrate and organic capping type on ecosystem functioning and aid in oil sands reclamation.

2. Materials and Methods

2.1 Site description

A detailed description of the research site used for collecting the LFH-MS and PMM capping materials is provided in a previous study by Brown and Naeth (2014). The research site is located on an oil sands lease about 24 km north of Fort McMurray in the AOSR, Alberta, Canada. The area is characterized by a continental boreal climate where winters are long and cold and summers are short and warm with a mean annual temperature of 1.0 °C from 1981 to 2010 (Environment Canada, 2015). Mean annual precipitation is 419 mm, which mostly falls as rain (316. mm) during the summer (Environment Canada, 2015).

A total of 36 randomly located 10 × 30 m^2 plots were established between November 2007 and February 2008. Half of the 120 plots were covered with LFH-MS and the other half with PMM. The LFH-MS was salvaged from the LFH-MS of a mesic aspen white spruce mixed forest and applied to a depth of 20 cm, over 30 cm of mixed B and C horizon subsoil. The PMM was applied to a depth of 30 cm over 100 cm clean overburden. In the first year of plot establishment, the LFH-MS plots were covered with 65% of diverse plant species including forbs, grasses, sedges and woody species, while PMM plots were covered with 33% of vegetation cover mainly with grasses (Brown and Naeth, 2014). The vegetation cover was increased from the first year to fifth year after reclamation (Forsch, 2014). The litter cover was increased from 7.3 to 82.8 % in LFH-MS and from 4.2 to 58.4 % in PMM over three years after reclamation (Forsch, 2014).

2.2 Experimental design, sampling and processing of soil

The study involves two organic capping materials, LFH-MS and PMM, and three organic substrates, one each of glucose, alanine and acetic acid, representing three main groups of organic compounds such as carbohydrates, amino and carboxylic acids. These compounds are commonly available in litter fall and root exudates and differ in their chemical composition (Lynch and Whipps, 1990). Glucose is the most common example of labile carbon and simple carbohydrate which initiates the microbial activity immediately after the addition in soil (Van Hees et al., 2005). Alanine is a simple amino acid containing very low N as compared to other amino acids (Hernández and Hobbie, 2010) and contributed 9-15% of total amino acid

composition of litterfall and root exudates from boreal forest (Werdin-Pfisterer et al., 2012). Acetic acid is a simple organic acid and aliphatic hydrocarbon compound with low complexing capacity with mineral soil (Keiluweit et al., 2015). The substrate treatments (number of treatments in brackets) comprised one treatment of each substrate (3), a pair of each substrate (3), mixture of all three substrates (1) and the control (no substrate applied and only water added), with each treatment replicated three times for each capping material. The LFH-MS and PMM samples were collected at 0-10 cm depth from the established quadrats using a soil auger (Jamro et al., 2014). The LFH-MS and PMM samples were transported to the laboratory in a cooler containing ice packs. Plant roots and other plant debris were carefully removed by hand followed by soil homogenization by passing the soil through a 2 mm sieve. The soil was stored at 4 °C in a refrigerator until used for the laboratory incubation experiment.

2.3 Incubation experiment procedures

Two sets (glass jars for microbial properties measurement and conical flask for CO_2 measurement) of each treatment were prepared and covered with aluminum foil with three tiny holes to allow air exchange. Twenty five grams (oven dry basis) of fresh soil was placed in a 250 mL conical flask; 250 g of soil was placed in 500 mL jars. The water content was adjusted to 60% water holding capacity (WHC) by weighing the jar or flask once every 5 days and adding deionized water when necessary. This WHC is considered within the optimum range of 50-70% for laboratory incubation of soils (Paul et al., 2001). The jars and flasks were placed in an incubator at 25 °C in the dark for one month prior to starting the experiment for maximizing the response of microbial processes to substrate addition, allowing most of the native labile C to respire (Orwin et al., 2006; Hernandez and Hobbie, 2010) and allowing the microorganisms to acclimatize to the laboratory incubation condition (Zeng et al., 2010).

Each substrate, with an equivalent application rate of 6 mg carbon (C) g^{-1} soil, was applied to each jar and flask and dissolved in 1 mL Milli-Q (Millipore, Bedford, MA) deionized water. The respective amount of each substrate treatment was repeatedly applied every six days until the end of experiment using a 10 mL syringe with a needle tip on the basis of stable microbial activity measured by carbon dioxide (CO_2) emission, where no further decline in microbial activity was observed in a preliminary study (data not shown). Only Milli-Q deionized water was added to the control treatment. Care was taken to ensure even distribution of substrate or

deionized water without saturating the soil, which could restrict CO_2 evolution from the sample. For soil property measurement, 50 g of soil sample was taken from each jar. Half of the soil was kept at 4 °C until analyses of soil enzymes, dissolved organic carbon (DOC), dissolved organic nitrogen (DON), soil community level physiological profiles (CLPPs), available nitrogen (N) (ammonium (NH_4^+-N) and available nitrate (NO_3^--N). The other half was air dried and used for the measurement of total C, total N and pH as described below.

2.4 Laboratory measurement

2.4.1 Soil properties

Total C and total N concentrations were determined at initiation (0 day of incubation) and termination (after 60 days of incubation) of the experiment by dry combustion method using an automated elemental analyzer (NA-1500 series, Carlo Erba, Milan, Italy). The pH was measured using a digital DMP-2 mV/pH meter (Thermo Fisher Scientific Inc., Waltham, MA) in a 1:2 (m:v) soil:0.01 mol L^{-1} $CaCl_2$ (Kalra and Maynard, 1991). Fresh soil samples were extracted with 2 mol L^{-1} KCl for NH_4^+-N and NO_3^--N analyses (Mulvaney, 1996). The extract was analyzed calorimetrically by the indophenol blue method for NH_4^+-N (Keeny and Nelson 1982) and NO_3^--N by the vanadium oxidation method (Miranda et al., 2001). The NH_4^+-N, NO_3^--N and pH were measured at 0, 1, 15, 30 and 60 days of incubation.

The fresh soil samples were extracted with a 0.5 mol L^{-1} K_2SO_4 solution at 1:10 (w:v) soil to solution ratio for DOC and DON concentrations. After shaking for one hour on a shaker (Eberbach Corp., Michigan, U.S.A), the extracts were filtered using Fisher Q2 filter papers. The C and N concentrations in the extractant were measured using a TOC-V_{CSN} analyzer (Shimadzu, Kyoto, Japan).

2.4.2 CO_2 emission rate

The CO_2 emission was measured at 0, 1, 3, 7, 10, 30 and 60 days after incubation. For each sampling time, flasks were capped to collect gas samples. Gas samples were taken at time 0 and 1 h after capping at each sampling time using a 20 mL gas tight syringe (BD Luer-Lok™ Tip) and were injected into evacuated 10 mL glass vials (Isomass Exetainers) equipped with a septa. The gas samples were analyzed using a gas chromatograph (Varian GC-3800, Mississauga,

Canada) equipped with a thermal conductivity detector. The rate of CO_2 emission (mg CO_2-C g^{-1} soil hr^{-1}) was calculated from the changes in concentration between time 0 and 1 h samplings.

2.4.3 Soil enzyme activities

The activities of five extracellular enzymes involved in C and N cycling, including N-acetyl-*β*-D-glucosaminidase (Enzyme classificationnumber (ECN), ECN 3.2.1.14), *β*-glucosidase (ECN 3.2.1.21), esterase (ECN 3.1.1.1), phenol oxidase (ECN 1.10.3.2) and alanyl aminopeptidase (AlaAP) (EC 3.4.11.2) were measured at 0, 10, 30 and 60 days of incubation.

For NAGase, β-glucosidase, esterase, phenol oxidase and AlaAP measurement, soil sample suspensions were prepared by placing one gram of fresh soil in a 250 mL nalgene bottle. A 125 mL of sodium acetate buffer (50 mmol L^{-1}, pH 5) was added to the bottle, and the suspension was homogenized using a magnetic stir plate until suspensions were transferred into black 96 well plates for determining NAGase, β-glucosidase, and esterase activities (Sinsabaugh et al., 2003). A 200 µL soil suspension and 50 µL 200 µmol L^{-1} substrate were pipetted onto each plate. Soil background wells, quench controls and reference standards were then added to each plate. The plates were placed in an incubator for three hours at 20 °C in the dark. A 20 µL of 0.5 mol L^{-1} NaOH was added to each plate using an auto dispenser to stop the enzymatic reaction. After the incubation, fluorescence was measured at 360 nm excitation and 460 nm emissions using a multi-detection microplate reader (Bio-Tek Instruments, Synergy HT, Winooski, VT).

Phenol oxidase and AlaAP activities were measured using the colorimetric method (Stursova et al., 2006). Phenol oxidase was measured in clear microplates containing 50 µL of 25 mM L^{-1} dihydroxyphenylalanine (L- DOPA); color development was measured at 460 nm after incubation at 20 °C for 24 hours. For AlaAP activity 7-amino-4-methylcourmarin-linked substrate was used and incubation was done at 20 °C in the dark for 24 hours. After incubation, absorbance was measured at 460 nm using the multi-detection microplate reader. All enzyme activities (nmol of substrate g^{-1} h^{-1}) were calculated on soil mass on an oven dry mass basis.

2.4.4 Soil microbial community level physiological profile

Soil bacterial CLPPs were determined at termination of the laboratory incubation experiment using Biolog Ecoplate (Biolog Inc., California, USA) containing 31 C substrates. Five grams of each soil sample were placed into a sterile test tube with 45 mL of 0.87%

sterilized sodium chloride (NaCl) solution. The soil suspension was shaken for 30 min and diluted for 1×10^4 times. A 125 µL aliquot from each suspension was inoculated into each well of an Ecoplate. Each Ecoplate was incubated at 25 °C in the dark. Optical density of each well was read at 750 nm every 24 h for a total of 168 h using a microplate reader (Molecular Devices, Sunnyvale, California). The final color development in each well was calculated as optical density by subtracting the values at 420 nm.

Two sets of CLPP data average well color development (AWCD) and area under the curve (*A*), were assessed with optical density values. The AWCD data were calculated as follows from the equation (Garland and Mills, 1991): AWCD = Σ (C-R)/n, where *C* is color production within each well, *R* is the absorbance value of the control well and *n* represents the number of C sources used in an Ecoplate.

2.5 Statistical analyses

The effect of substrate type and diversity on OM quality measured as total C, total N, C to N ratio and pH was analyzed using a one-way analysis of variance (ANOVA) for each capping material. A principal component analysis (PCA) was used to determine the effect of different substrate treatments on CLPPs. A repeated measures ANOVA was used to assess the effect of substrate diversity and identity and sampling time on soil enzyme activities, N availability, DOC, DON and CO_2 emission rate. Time of sampling during incubation was used as the repeated measures variable. Tukey's honestly significant difference (HSD) test was used to determine the significance of the effect of substrate diversity on microbial processes in each capping material. The data were checked for normality and homogeneity of variance using a Shapiro-Wilk test and Bartlett test, respectively, before performing the ANOVA. To confirm the effects of mixture of substrates on microbial processes, expected values were calculated on the basis of values obtained when the substrate was applied singly and in combination in each substrate, and subtracted from values in the mixture. The difference of each parameter was assessed for significance using a paired *t* test. Pearson correlation analysis was conducted to determine the relationship between microbial processes and soil properties. An α value of 0.05 was used to indicate significant differences in all analyses. All analyses were performed using the Statistix 8.1 (Analytical software, Tallahassee, FL) software except the PCA which was performed using the Vegan Package in the R software.

3. Results

3.1 Characteristics of the organic capping materials

Initial properties of the organic capping materials prior to the substrate treatments application were significantly different between LFH-MS and PMM (Table 3-1). Total C, total N and pH were significantly higher in PMM than in LFH-MS, while C to N ratio did not differ between the two (p>0.05). Type and diversity of the organic substrate employed significantly altered total C, total N, C to N ratio and pH for both LFH-MS and the PMM (Table 3-1). In LFH-MS, lowest total C was in the acetic acid treatment (8.7 g kg^{-1}) and highest was in the alanine treatment (47.5 g kg^{-1}) (Table 3-1).

On average (data not shown), when organic substrates were applied singly, the greatest decrease in total C was in the acetic acid treatment (approximately −78%) relative to the control, followed by the glucose treatment (−60%); the alanine treatment increased (26%) total C. High organic substrate diversity markedly decreased total C relative to the control for LFH-MS and PMM. The decrease (% not shown) in total C was −48% (mixture of all substrates) to −12% (acetic acid + alanine) in LFH-MS and −92% (glucose + acetic acid) to −34% (glucose) relative to the control in PMM. Application of alanine increased total N in LFH-MS when applied either singly or in a mixture of organic substrates (glucose + acetic acid), while application of glucose and acetic acid significantly decreased total N relative to the control (Table 3-1). In PMM, total N significantly decreased following all organic substrate treatments relative to the control. Total N was 0.80 g kg^{-1} (mixture of all organic substrates) to 1.67 g kg^{-1} (glucose), while that of the control (2.05 g kg^{-1}) was highest in PMM. The C to N ratio was 9.2 (acetic acid) to 31.1 (control). In general, applying a mixture of two or three substrates resulted in lower C to N ratios relative to the control in LFH-MS. In PMM, the C to N ratio was 9.1 (glucose+acetic acid) to 38.2 (alanine). Application of glucose and acetic acid decreased the C to N ratio in most cases relative to the control and alanine (Table 3-1).

The pH was more affected by substrate type than by organic substrate diversity in LFH-MS and PMM capping materials. Acetic acid application consistently reduced the pH, whereas glucose and alanine applications increased it relative to the control regardless of capping material. In LFH-MS, the pH was 4.68 (acetic acid + alanine) to 6.79 (glucose), while in PMM, it was 5.72 (acetic acid) to 7.21 (glucose).

3.2 Community-level physiological profiles (CLPPs)

The CLPPs were significantly altered by organic substrate type and diversity (Figure 3-1A and B). Analysis of co-variance (data not shown) showed mean CLPPs were separated into three groups in LFH-MS and two groups in PMM along substrate treatments, although these groups did not differ statistically (Figure 3-1A). The PCA revealed that organic substrate type and diversity contributed 24.6% (PC1) and 19.1% (PC2) of the variations in LFH-MS (Figure 3-1C), and 28.5% (PC1) and 19.9% (PC2) of the variations in CLPPs in PMM (Figure 3-1D). In LFH-MS, CLPPs in most organic substrate treatments were distinctly different from the control; treatments with high organic substrate diversity and acetic acid + alanine addition showed similar trends (Figure 3-1C). Treatments with glucose and alanine had different trends than the mixture of organic substrates and acetic acid treatments and were plotted in a separate quadrant (Figure 3-1C). The CLPPs of most organic substrate treatments differed from the control in PMM (Figure 3-1D). The CLPPs in glucose and alanine were separated from the mixture of substrates. Mixtures of organic substrates, glucose + acetic acid were located in similar quadrants. Similarly, glucose and alanine occupied the same quadrant (Figure 3-1D).

3.3 Effects of organic substrate diversity on microbial processes

Most measured parameters were significantly affected by organic substrate addition and time of measurement, with CO_2 emissions, DOC and DON affected by interactions of organic substrate and time in LFH-MS and PMM (Table 3-2). For both capping materials, CO_2 emissions (mg CO_2-C g^{-1} hr^{-1}) were significantly increased from 0 to 60 days after incubation, with the highest rates at the first day of incubation, and higher rates with organic substrate treatments compared to control in most sampling dates (Figure 3-2A and B). The CO_2 emission rate differences in organic substrate types and their diversity (data not shown) were 16 (control) to 35 (glucose + alanine) in LFH-MS (Figure 3-2A). In PMM, CO_2 emissions were 15 (control) to 88 CO_2-C (mg CO_2-C g^{-1} hr^{-1}) in the mixture of all organic substrate treatment (Figure 3-2B).

The DOC concentration (mg C kg^{-1}) increased from day 0 to 60, from 192 (control) to 329 (mixture of all substrates) and from 469 (control) to 593 (glucose + alanine) in LFH-MS and PMM at day 60, respectively (Figure 3-2C and D). The DOC concentrations changed more dramatically on day 1 than day 0 for all treatments (Figure 3-2C and D). Application of alanine significantly increased DON concentration in both capping materials relative to other organic

substrate treatments (Figure 3-2E and F). This is clear for DON changes from 0 to 60 days; alanine either singly or in mixture increased DON concentrations with greater increases (92 vs 395 mg N kg^{-1} in alanine treatment) in LFH-MS (Figure 3-2E). In PMM most organic substrate treatments increased DON from day 0 to 60 with greater increases when alanine was applied singly (Figure 3-2F).

The β-glucosidase activity was not affected by substrate diversity in LFH-MS (Figure 3-3A) or PMM (Figure 3-4A). It significantly increased from day 0 to day 60 (5.5 to 13.6 nmol of substrate g^{-1} hr^{-1}) in LFH-MS (Figure 3-3B) and did not differ between day 0 to day 60 in PMM (Figure 3-4B). The esterase activity was significantly affected by organic substrate addition in LFH-MS and PMM (Table 3-2) with greater increases in the mixture of all treatments in LFH-MS (Figure 3-3C) and PMM (Figure 3-4C). Esterase activity was suppressed in LFH-MS (Figure 3-3D) and increased in PMM (Figure 3-4D) after organic substrate addition. The difference in the amount of organic substrate (nmol of substrate g^{-1} hr^{-1}) from day 0 to day 60 (values not shown) were −0.32 (control) to 3.4 (mixture of all organic substrates) and from 7.32 (glucose) to 12.7 (alanine) for LFH-MS (Figure 3-3D) and PMM (Figure 3-4D), respectively. The phenol oxidase activity was significantly reduced in LFH-MS following most organic substrate treatments (Figure 3-3E) and increased over time during incubation (Figure 3-3F), with the exception of LFH-MS exposed to a mixture of all treatments. In PMM, phenol oxidase activity increased by organic substrate treatments (Figure 3-4E) from day 0 to 60 (Figure 3-4F). The AlaAP activity followed an inconsistent trend after organic substrate addition throughout the experiments for LFH-MS (Figure 3-3G and H) and PMM (Figure 3-4G and H). The greatest activity was in the mixture of all the substrates in LFH-MS, and in alanine in the PMM. The NAGase activity consistently increased from day 0 to 60 in the LFH-MS (Figure 3-3I and J) and PMM (Figure 3-4I and J), with the largest increase generally linked to treatment with high diverse organic substrates.

The NH_4^+-N and NO_3^--N concentrations showed no consistent pattern from day 0 to 60 in LFH-MS (Figure 3-5A, B, E and F) and PMM (Figure 3-5C, D, G and H); although increased concentrations were linked to higher organic substrate diversity. Overall, a greater response was shown by highly diverse organic substrates on day 1 (Figure 3-5) for NH_4^+-N than for NO_3^--N. The differences in NH_4^+-N concentration (mg N kg^{-1}) between days 0 and 60 were 1.88 (acetic acid) to 5.04 (mixture of all organic substrates) and 0.77 (acetic acid) to 6.38 (mixture of all

organic substrates) in LFH-MS and PMM, respectively. In general, acetic acid suppressed the amount of available N, whereas alanine increased N availability for both capping materials.

Paired *t* test data (Table 3-3) indicated that observed values of CO_2 emission for the capping materials did not alter (p>0.05) in most mixture treatments in LFH-MS and PMM, except application of all organic substrate mixtures generally resulted in significantly higher CO_2 (Table 3-3). In most cases, differences between observed and expected activities of β-glucosidase, esterase and AlaAP were not significant in response to application of organic substrate mixtures in LFH-MS and PMM (Table 3-3). The NAGase activity was significantly greater in observed than expected values in response to the mixture of all organic substrate treatments (high organic substrate diversity) in LFH-MS and PMM. Phenol oxidase activity was not affected in LFH-MS, but was suppressed in PMM by high diversity. Concentrations of NH_4^+-N and NO_3^--N were significantly different for observed and expected values in response to high organic substrate diversity in LFH-MS and PMM (Table 3-3).

3.4 Linkages among soil properties

In LFH-MS, the CLPPs were significantly correlated with pH, DOC and AlaAP activity (Table 3-4). The NAGase and AlaAP activities were positively correlated with NH_4^+-N and DOC. The phenol oxidase activity was negatively correlated with DOC to DON ratio and positively correlated with NH_4^+-N and NO_3^--N. The NO_3^--N was negatively correlated with DOC to DON ratio and pH, and positively correlated with CO_2 emissions. In PMM, the CLPPs were positively correlated with NAGase and esterase activities, NH_4^+-N , NO_3^--N, CO_2 emission and DOC (Table 3-4). The NAGase, esterase activities were positively correlated with CO_2 emission and the DOC. The AlaAP activity was positively correlated with DON, NH_4^+-N and NO_3^--N (Table 3-4)

4. Discussion

This study suggests that microbial processes were affected by the type and diversity of substrate in the capping soils. Other studies have shown that microbial activity is directly associated with the chemical property of the organic substrates added to the soil (Orwin et al., 2006; Hernandez and Hobbie, 2010), quality of the reclamation material (Quideau et al., 2013; Kwak et al., 2015b) and indirectly affected by changes in microbial community composition (Zak

et al. 2003) and vegetation (Hahn and Quideau, 2013). The increase in DOC, DON and CO_2 emissions by single substrates in this study suggests that labile organic substrates are crucial to decomposition of organic matter (Orwin et al., 2006). There are two important mechanisms involved: labile nutrients have a patchy distribution in the soil and can develop large concentration gradients at the μm to mm scale (Jones et al., 2009); and bacteria and archaea possess limited capability to capitalize on a new non-labile resource pool (Resat et al., 2012). Consequently, microorganisms react rapidly to availability of labile nutrients (Farrell et al., 2014), decomposing them faster than less labile substrates. The greater CO_2 emissions in LFH-MS and PMM after addition of acetic acid likely result from mineralization of organic acids that took place rapidly, with half-lives from 1 to 5 hours in organic top soil and 5 to 12 hours in subsoils (Jones et al., 2003).

The higher rates of CO_2 emissions in response to addition of alanine in LFH-MS suggest the CO_2 emissions were not likely associated with change in CLPPs (Wild et al., 2014) directly as indicated in this study. In N limited systems such as oil sands reclamation (Duan et al. 2015), N addition can provide extra energy to fungi, producing greater enzymes (Allison et al., 2009) and altering the C to N ratio which affected CO_2 emission in LFH-MS and PMM. This is likely associated with change in fungal population from NAGase activity change from day 0 to day 60 in this study, since NAGase activity is typical with fungal population (Burk et al., 2011). The greater change in CLPPs induced by acetic acid in LFH-MS and PMM suggests potential changes in the fungal population (Graaff et al., 2010). Under high C substrate availability, microbial demand for N would increase and subsequently reduce N mineralization or create incomplete mineralization, as shown by the DON increase with addition of a single organic substrate (glucose, acetic acid or alanine) by 72, 88 and 76%, respectively, in LFH-MS and 85, 89 and 90%, respectively, in PMM, relative to the control. Since soil enzymes remained unaffected by a single organic substrate addition, most of these enzymes likely require complex organic substrates for their activities (Hernandez and Hobbie, 2010). Thus there is a greater microbial demand for N and microorganisms may efficiently use amino acids (Orwin et al., 2006; Hernandez and Hobbie, 2010).

Substrate diversity affected CLPPs and decreased C to N ratio in LFH-MS and PMM. The greater CLPPs change observed in PMM was likely associated with a shift in composition of the fungal population (Graaff et al., 2010) as the quality of peat changed (Artz, 2009). Since CLPPs

in LFH-MS were less affected by the organic substrate diversity they were likely associated with the increase in DOC, which provided substrate for microbial activity (Jackson et al. 2002). Another driving factor was soil pH changes in PMM that likely affected the fungal population, as fungi generally grow better in acidic environments than bacteria (Will et al., 2010). The greater change in CLPPs observed with higher substrate diversity in LFH-MS and PMM would have involved a greater variety of enzymes, which may have required a more functionally diverse microbial community (Orwin et al., 2006), and CO_2 emissions may have been higher than with a single organic substrate addition. However, the lack of a direct relationship between CLPPs and CO_2 emission in LFH-MS in this study did not correspond to a shift in microbial community structure (Eilers et al., 2010). The increase in CLPPs might increase the microbial community's ability to rapidly metabolize more or less structurally complex organic substrates, which were demonstrated by higher than expected values of DOC and DON with diverse organic substrates in LFH-MS and PMM. The capacity of a microbial community to mineralize specific organic C substrates likely reflects differences in the type, abundance and bioavailability of C substrates in the in situ OM pool, through a priming effect (Hamer and Marschner, 2005) or through altered physiological demands for nutrients such as N (Orwin et al., 2006).

Thus, our first hypothesis supported by findings of this study that addition of a mixture of organic substrates changed CLPPs because the diversity of substrates allows exploitation of more functional niches and reduced the C to N ratio. However, we are unable to conclude how changes in fungi and bacteria populations were caused by substrate diversity in LFH-MS and PMM since fungi and bacteria populations were not quantified in this study. We must consider the drawbacks of CLPP measurements obtained using Biolog EcoPlate, known to exert bias towards fast growing bacteria and fungi and therefore may not represent the true diversity of the soil (Garland and Mills, 1991).

It has been proposed that addition of a mixture of organic substrates could produce a wider variety of enzymes, which in turn may enhance the ability of soil microorganisms to decompose other organic substrates in the soil (Fontaine et al., 2003; Hamer and Marschner, 2005). However, in this study, the activities of most enzymes remained unaffected by organic substrate mixtures, indicating that this mechanism does not play a part. It is clear from the correlations that enzyme activities, associated with either CLPPs or organic substrate availability (DOC, DON,

available N) and their limitations (Hernandez and Hobbie, 2010) are probably lacking in suitable organic substrates for enzyme activities in LFH-MS and PMM (Zhang et al., 2011).

The greater changes in the NH_4^+-N and NO_3^--N concentrations linked with organic substrate diversity likely resulted from changes in microbial community composition, as indicated from CLPP and changes in N availability. Microbial populations can be stimulated by diverse organic substrates (Bardgett and Shine, 1999), which likely stimulate decomposition of OM. The richness of plant species correlates with a substantial increase in N mineralization (Cong et al., 2015). The greater content of NO_3^--N than NH_4^+-N in diverse organic substrates was also due to changes in soil pH. In diverse systems (Setala and McLean, 2004) and in those with diverse vegetation (Zak et al., 2003), pH was higher than in simple systems with less diverse vegetation. The high pH could induce microbial changes and alter N cycling processes in oil sands reclamation (Kwak et al., 2015b). This was demonstrated by the negative relationship between soil pH and NO_3-N since nitrifying bacteria are sensitive to acidity (Myrold, 2005). N availability could have been affected by DOC to DON ratio, a highly regarded indicator of OM quality (McDowell et al., 2004). A higher ratio of DOC to DON may block breakdown of phenolic compounds and inhibit enzymes involved in N mineralization (Jones and Kielland, 2012). This was confirmed by the negative relationship between phenol oxidase activity and N availability in our study.

Organic substrate diversity affected a range of microbial processes in LFH-MS and PMM capping materials. A few consistent trends showing additive effects, priming effects and, in a few cases, non-additive effects were observed for different microbial processes. This is consistent with the literature showing chemical diversity of organic substrates is positively correlated with microbial community composition and respiration rates (Hernandez and Hobbie, 2010; Meier et al., 2010); such a relationship might be associated with complementary effects of the different substrates. The effect of organic substrate diversity on microbial processes explains that plant diversity can affect bacterial to fungal ratios (Hahn and Quideau, 2013) and mycorrhizal biomass (Brown and Naeth, 2014).

The enhanced activity of esterase, NAGase, phenol oxidase and AlaAP in LFH-MS and PMM by increased organic substrate availability (Jamro et al., 2014; Kwak et al., 2015a) suggests that the resource limitation model could be applied to these activities (Hernandez and Hobbie, 2010) in the studied capping soils. Enzyme activity changes in response to organic

substrate addition are likely to be a function of the rates of enzyme production and degradation (Geisseler et al., 2011). Higher phenol oxidase activity is likely to be associated with decomposition potential of PMM rather than LFH-MS. Thus, enzyme production by soil microorganisms is regulated by demand and organic substrate availability (Burns et al., 2013; Jamro et al., 2014; Kwak et al., 2015a). Overall, our results for soil enzyme activities support the general understanding that decomposition is seldom limited by a single factor. French (1988) proposed that enzyme activity is constrained directly by a combination of organic substrate properties (e.g., quantity and quality of C), environmental conditions and physical access of the substrate to the enzyme (Vance and Chapin, 2001). The greater N availability associated with organic substrate diversity is governed by two mechanisms. The higher diversity indirectly increases decomposition rate of OM through positive effects on the microbial community (Zak et al., 2003). The greater number of plant species enhances microbial release of NH_4^+ from OM causing greater gross N mineralization rates (Fornara et al., 2009) that, surprisingly, are not significantly associated with the presence of particular plant functional groups but might be affected by size of the soil organic matter pool (Booth et al., 2005).

Increased CO_2 emissions after addition of organic substrates (day 1 in this study) likely caused an increase in overall microbial catabolic activity and a phenomenon known as the priming effect. The mechanisms depend on soil type and involve an apparent priming effect caused by pool substitution (Wu et al., 1993), a real priming effect due to increased enzyme production (Blagodatskaya and Kuzyakov, 2008) and a real priming effect due to an increase in microbial biomass of K-strategists (Fontaine et al., 2003). The changes in CO_2 emissions induced by substrate diversity in this study were associated with alterations in CLPP and DOC concentrations in LFH-MS and PMM (Table 3-5). Previous studies found use of sugars, organic acids and amino acids to understand the priming processes in soil were either positive, negative or no priming effect (Hamer and Marschner, 2005; Blagodatskaya and Kuzyakov, 2008) depending on soil type. The priming effects in organic capping materials differed between LFH-MS and PMM (Jamro et al., 2014). The greater priming effect of a diverse organic substrate in PMM might be associated with increased CLPPs than with LFH, which enhanced decomposition rates by altering the C to N ratio. In summary, the causes and mechanisms of priming are complex (Blagodatskaya and Kuzyakov, 2008) and are closely linked to organic substrate type

(Jagadamma, 2014), soil characteristics and quality (Jamro et al., 2014), and/or microbial functions (Orwin et al., 2006).

5 Conclusions

Organic substrate type and diversity affected microbial processes with greater enzyme activities and N availability in the presence of more diverse organic substrates than the single or mixture of two substrates. The greater response of the organic substrate diversity observed in PMM than in LFH-MS could be associated with differences in CLPPs and changes in OM quality. This study increases our understanding of the soil-plant feedback mechanism in oil sands reclamation. Extending the research to field conditions should be considered for the next step. Addition of organic acid and amino acid has shown different trends for decomposition of organic matter in LFH-MS and PMM and the response of the ecosystem to the addition of an organic substrate would improve our understanding of in-situ priming effects and the dynamics of rhizo deposits in the field conditions.

Table 3-1 Organic matter quality of organic capping materials used in oil sands reclamation at initiation (0 day of incubation before addition of substrate) and termination (60 days after incubation) of the experiment

Organic capping type	Substrate type*	Total carbon (g kg^{-1})	Total nitrogen (g kg^{-1})	Carbon to nitrogen ratio	pH
Initiation of experiment					
LFH-MS	-	40.7 (0.1)**	1.30 (0.09)	31.8 (2.3)	5.38 (0.03)
PMM	-	67.8 (0.6)	2.27 (0.10)	30.1 (1.8)	6.84 (0.05)
Termination of experiment					
LFH-MS	Cont	37.5 (0.1) b***	1.20 (<0.01) d	31.3 (2.0) a	5.38 (0.03) d
	Glu	14.8 (0.3) e	0.75 (0.02) ef	19.8 (0.2) c	6.79 (0.02) a
	Acet	8.7 (0.2) f	0.96 (0.01) e	9.2 (0.3) e	5.15 (0.05) e
	Ala	47.5 (1.1) a	2.09 (0.04) a	22.7 (0.5) b	6.22 (0.03) b
	Glu+Acet	21.9 (0.7) cd	0.73 (0.01) ef	30.1 (0.5) a	5.18 (0.03) e
	Glu+Ala	25.1 (0.7) c	1.72 (0.05) b	14.6 (0.3) de	6.10 (0.03) b
	Acet+Ala	33.0 (1.3) ab	1.93 (0.04) b	17.0 (1.0) c	4.68 (0.01) f
	Glu+Acet+Ala	19.3 (0.6) d	1.55 (0.02) c	12.5 (0.2) d	5.87 (0.04) c
PMM	Cont	60.8 (0.2) d	2.05 (<0.01) a	29.7 (0.7) b	6.92 (0.01) c
	Glu	40.2 (0.7) a	1.67 (0.01) b	24.1 (0.3) cd	7.21 (0.01) a
	Acet	21.4 (0.4) c	0.95 (0.01) cd	22.4 (0.6) cd	5.72 (0.02) e
	Ala	30.8 (0.7) b	0.81 (0.02) d	38.2 (0.2) a	6.76 (0.02) d
	Glu+Acet	7.8 (0.5) d	0.96 (<0.01) cd	9.1 (0.3) e	7.16 (0.02) a
	Glu+Ala	30.4 (0.4) b	1.60 (0.01) b	19.0 (0.6) d	6.94 (0.03) bc
	Acet+Ala	39.4 (0.3) a	1.03 (0.02) c	37.9 (0.3) ab	6.96 (0.04) bc
	Glu+Acet+Ala	22.0 (0.7) c	0.80 (0.02) d	27.6 (1.0) bc	6.98 (<0.01) b
	One-Way ANOVA				
LFH-MS	F value	215.4	209.8	167.4	360.4
	p value	<0.001	<0.001	<0.001	<0.001
PMM	F value	420.8	61.8	40.8	1628.5
	p value	<0.001	<0.001	<0.001	<0.001

*Abbreviations: Cont: control, Glu: glucose, Acet: acetic acid, Ala: alanine, Glu + Acet: glucose + acetic acid, Glu + Ala: glucose + alanine, Acet + Ala: acetic acid + alanine, Glu + Acet + Ala: glucose + acetic acid + alanine

** Values shown in brackets indicate standard errors of the mean (n=3)

***Means with different lowercase letters indicate significant difference among substrate type and diversity within each organic capping type in each column

Table 3-2 Analysis of variance (*F* and *P* values) of the effects of organic substrate type and diversity and their interaction with sampling time on soil processes used for oil sands reclamation

[a]Soil processes	LFH-MS						PMM					
	Substrate		sampling time		Substrate*sampling time		Substrate		sampling time		Substrate*sampling time	
	F value	*p* value	F value	*p* value	F value	*p* value	F value	*p* value	F value	*p* value	F value	*p* value
CO_2	14.70	<0.001	52.29	<0.001	6.16	<0.001	69.48	<0.001	476.39	<0.001	22.57	<0.001
DOC	66.60	<0.001	190.61	<0.001	31.85	<0.001	12.69	<0.001	140.41	<0.001	2.17	0.006
DON	47.97	<0.001	29.65	<0.001	7.09	<0.001	125.26	<0.001	85.58	<0.001	17.33	<0.001
AlaAP	7.12	<0.00	13.90	0.002	2.03	0.079	17.58	<0.001	29.91	<0.001	7.95	0.490
BGLU	1.95	0.079	94.68	<0.001	1.56	0.094	1.24	0.296	2.71	0.115	1.06	0.547
EST	5.57	<0.00	38.54	<0.001	1.28	0.226	27.50	<0.001	22.78	<0.001	9.03	0.530
NAGase	4.36	0.001	5.45	0.025	2.18	0.051	3.58	0.003	7.75	0.009	2.60	0.046
POX	4.16	0.001	57.24	<0.001	1.67	0.066	7.31	<0.001	12.39	0.002	1.76	0.047
NH_4^+-N	1.79	0.104	7.41	0.005	0.97	0.524	116.70	<0.001	263.04	<0.001	41.56	0.070
NO_3^--N	12.52	<0.001	98.91	<0.001	7.61	0.041	273.29	<0.001	152.28	<0.001	51.02	0.051

[a].Abreviations: CO_2: carbon dioxide emission, NAGase : N-acetyl glucosaminidase, BGlu: β-glucosidase, POX: phenoloxidase, EST: esterase, AlaAP: L-alanine amino peptidase, NH_4^+-N: ammonium, NO_3^--N: nitrate, DOC: dissolved organic carbon, DON: dissolved organic nitrogen

Table 3-3 Effects of mixture of organic substrates on microbial processes as calculated by subtracting expected value from the observed value followed by conducting a paired *t* test in materials used in oil sands reclamation at termination (60 days after incubation) of experiment

Soil process*	Glu+Acet**		Gluc+Ala		Acet+Ala		Glu+ Acet+ Ala	
	Difference	*p* value	Difference	*p* value	Difference	*p* value	Difference	*p* value
				LFH-MS				
CO_2 emision rate (μg CO_2-C g^{-1} h^{-1})	-3.7	0.261	5.1	0.042	-1.9	0.670	4.0	0.025
DOC (mg C kg^{-1})	79.0	0.035	8.0	0.392	77.0	0.124	105.0	0.028
DON (mg C kg^{-1})	-6.1	0.603	99.5	0.007	152.7	0.008	92.2	0.009
BGLU (nmol of substrate g^{-1} h^{-1})	0.7	0.335	0.5	0.224	1.0	0.380	0.3	0.805
Esterase (nmol of substrate g^{-1} h^{-1})	0.0	0.787	0.0	0.787	0.1	0.239	0.2	0.156
POX (nmol of substrate g^{-1} h^{-1})	-1.1	0.939	2.2	0.048	2.1	0.087	1.1	0.231
NAGase (nmol of substrate g^{-1} h^{-1})	542	0.090	275	0.024	249	0.033	1057	0.015
AlaAP (nmol of substrate g^{-1} h^{-1})	0.4	0.007	0.9	0.106	-0.1	0.995	3.0	0.130
NH_4^+-N (mg N kg^{-1})	1.6	0.051	2.0	0.003	1.5	0.047	2.3	0.014
NO_3^--N (mg N kg^{-1})	1.1	0.069	9.8	0.004	3.7	0.033	9.3	0.004
				PMM				
CO_2 emision rate (μg CO_2-C g^{-1} h^{-1})	29.1	0.150	152.3	0.558	156.1	0.139	78.0	0.020
DOC (mg C kg^{-1})	-3.7	0.051	5.1	0.345	-1.9	0.268	4.0	0.041
DON (mg C kg^{-1})	114.7	0.004	19.7	0.004	36.4	0.013	76.1	0.031
BGLU (nmol of substrate g^{-1} h^{-1})	-16.8	0.916	2.2	0.132	-19.3	0.782	-7.2	0.798
Esterase (nmol of substrate g^{-1} h^{-1})	-0.2	0.789	2.9	0.016	1.3	0.124	3.6	0.033
POX (nmol of substrate g^{-1} h^{-1})	2.4	0.226	1.3	0.321	-3.9	0.965	-1.8	0.958
NAGase (nmol of substrate g^{-1} h^{-1})	-48	0.598	142	0.082	318	0.040	771	0.009
AlaAP (nmol of substrate g^{-1} h^{-1})	0.6	0.100	1.0	0.047	-0.5	0.822	0.3	0.120
NH_4^+-N (mg N kg^{-1})	2.9	0.002	0.8	0.068	1.8	0.003	5.1	0.006
NO_3^--N (mg N kg^{-1})	-2.7	0.910	8.7	0.002	7.4	<0.001	9.5	<0.001

*Abbreviations: CO_2: carbon dioxide, DOC: dissolved organic carbon, DON: dissolved organic nitrogen, NH_4^+-N: ammonium, NO_3^--N: nitrate, NAGase: β-N-acetyl glucosaminidase, BGLU: β-glucosidase, POX: phenoloxidase, AlaAP: L-alanine amino peptidase

** Cont: control, Glu: glucose, Acet: acetic acid, Ala: alanine, Glu + Acet: glucose + acetic acid, Glu + Ala: glucose + alanine, Acet + Ala: acetic acid + alanine, Glu + Acet + Ala: glucose + acetic acid + alanine

Table 3-4 Pearson correlation coefficient (*r* -value) and significance[+] among soil variables in LFH and PMM organic capping materials used for oil sands reclamation (n=54).

Variable*	CLPPs	NAGase	BGLU	EST	POX	AlaAP	NH_4^+-N	NO_3^--N	CO_2	pH	DOC	DON
	LFH-MS											
NAGase	0.20											
BGLU	-0.03	-0.02										
EST	-0.06	-0.17	0.31									
POX	-0.02	0.01	-0.16	0.21								
AlaAP	0.35*	0.18	0.14	0.33	0.17							
NH_4^+-N	-0.23	0.50*	0.19	0.08	0.11	0.44*						
NO_3^--N	-0.03	0.25	-0.14	0.28	0.50*	0.44*	0.48*					
CO_2	0.08	-0.05	-0.01	-0.02	-0.08	0.32	0.14	0.44*				
pH	0.62*	0.03	0.05	-0.06	-0.50*	0.11	-0.3	-0.61	-0.14			
DOC	0.43*	0.45*	0.19	0.15	-0.41	0.42*	0.29	0.43*	0.33	0.07		
DON	0.07	0.41	-0.32	0.2	0.54*	0.01	-0.03	0.66*	-0.06	-0.50*	0.28	
DOC:DON	0.05	-0.06	0.39*	-0.13	-0.53*	-0.11	0.15	-0.41*	0.12	0.40*	-0.02	-0.73*
	PMM											
NAGase	0.58*											
BGLU	-0.13	-0.13										
EST	0.72*	0.39	-0.11									
POX	-0.12	-0.38	0.08	-0.34								
AlaAP	-0.04	-0.28	-0.11	-0.17	0.37							
NH_4^+-N	0.39*	0.06	-0.21	0.22	0.28	0.58*						
NO_3^--N	0.55*	-0.05	-0.04	0.57*	0.06	0.47*	0.65*					
CO_2	0.73*	0.83*	-0.2	0.51*	-0.24	-0.21	0.34*	0.21				
pH	-0.08	-0.32	0.16	0.22	-0.14	-0.1	-0.25	0.26	-0.14			
DOC	0.67*	0.45*	-0.35	0.64*	-0.09	0.05	0.49*	0.58*	0.65*	0.14		
DON	0.22	-0.17	-0.13	0.18	0.19	0.81*	0.63*	0.76*	-0.03	-0.03	0.12	0.3
DOC:DON	-0.29	0.09	0.09	-0.38	-0.02	-0.43*	-0.58*	-0.72*	0.04	-0.40*	-0.35*	-0.35*

[*] Abbreviations:, CLPPs: community level physiological profiles, NAGase : N-acetyl glucosaminidase, BGLU: β-glucosidase, POX: phenoloxidase, EST: esterase, AlaAP: L-alanine amino peptidase, NH_4^+-N: ammonium, NO_3^- -N: nitrate, CO_2: carbon dioxide, DOC: dissolved organic carbon, DON: dissolved organic nitrogen, DOC:DON: dissolved organic carbon to dissolved organic nitrogen ratio

[+*], $P<0.05$

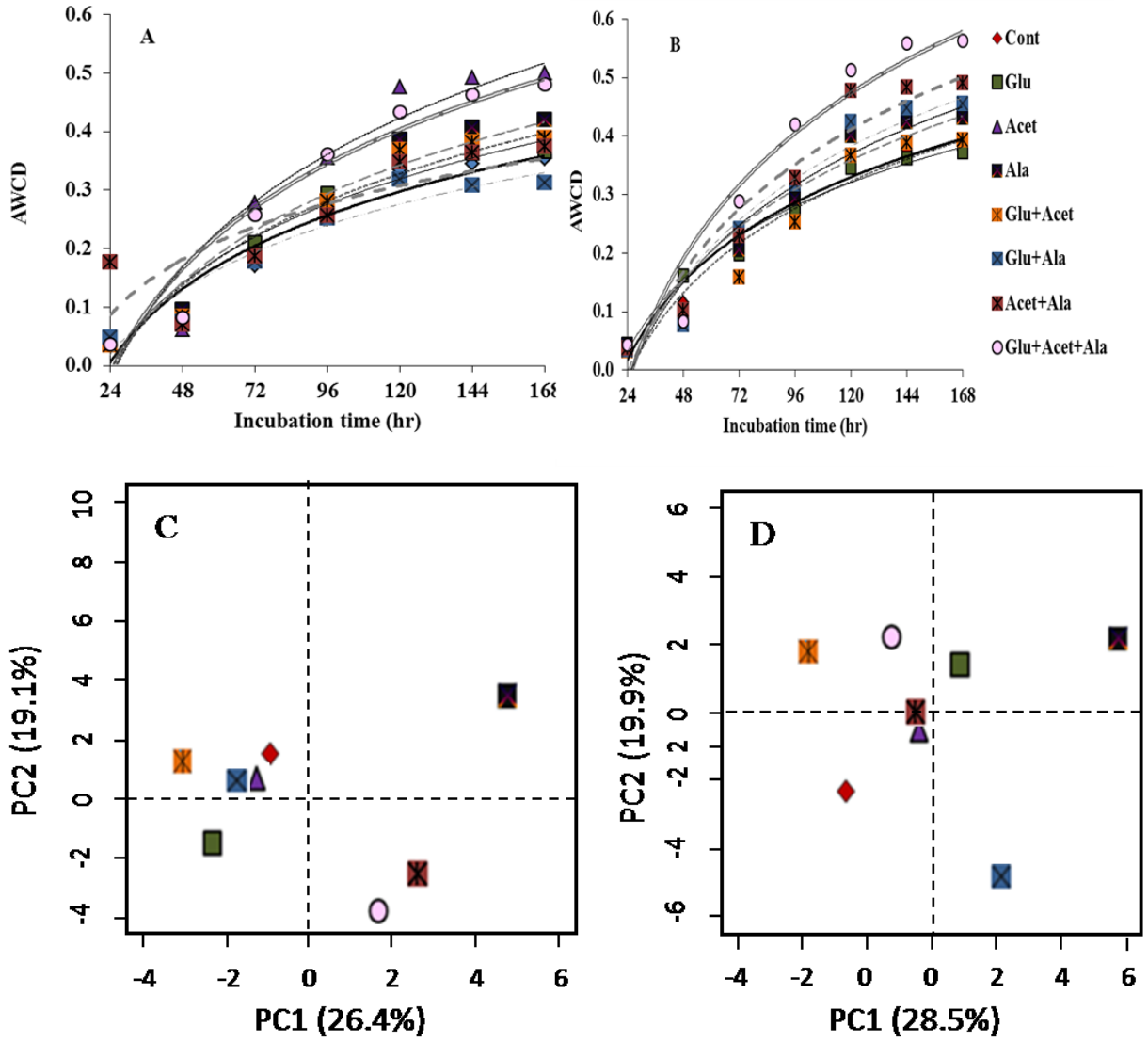

Figure 3-1 Changes in average well color development (AWCD) in (A) LFH-MS and (B) PMM and community level physiological profiles (CLPPs) in (C) LFH-MS and (D) PMM affected by organic substrate type and diversity in organic capping materials used in oil sands reclamation. Abbreviations: Cont: control, Glu: glucose, Acet: acetic acid, Ala: alanine, Glu + Acet: glucose + acetic acid, Glu + Ala: glucose + alanine, Acet + Ala: acetic acid + alanine, Glu + Acet + Ala: glucose + acetic acid + alanine

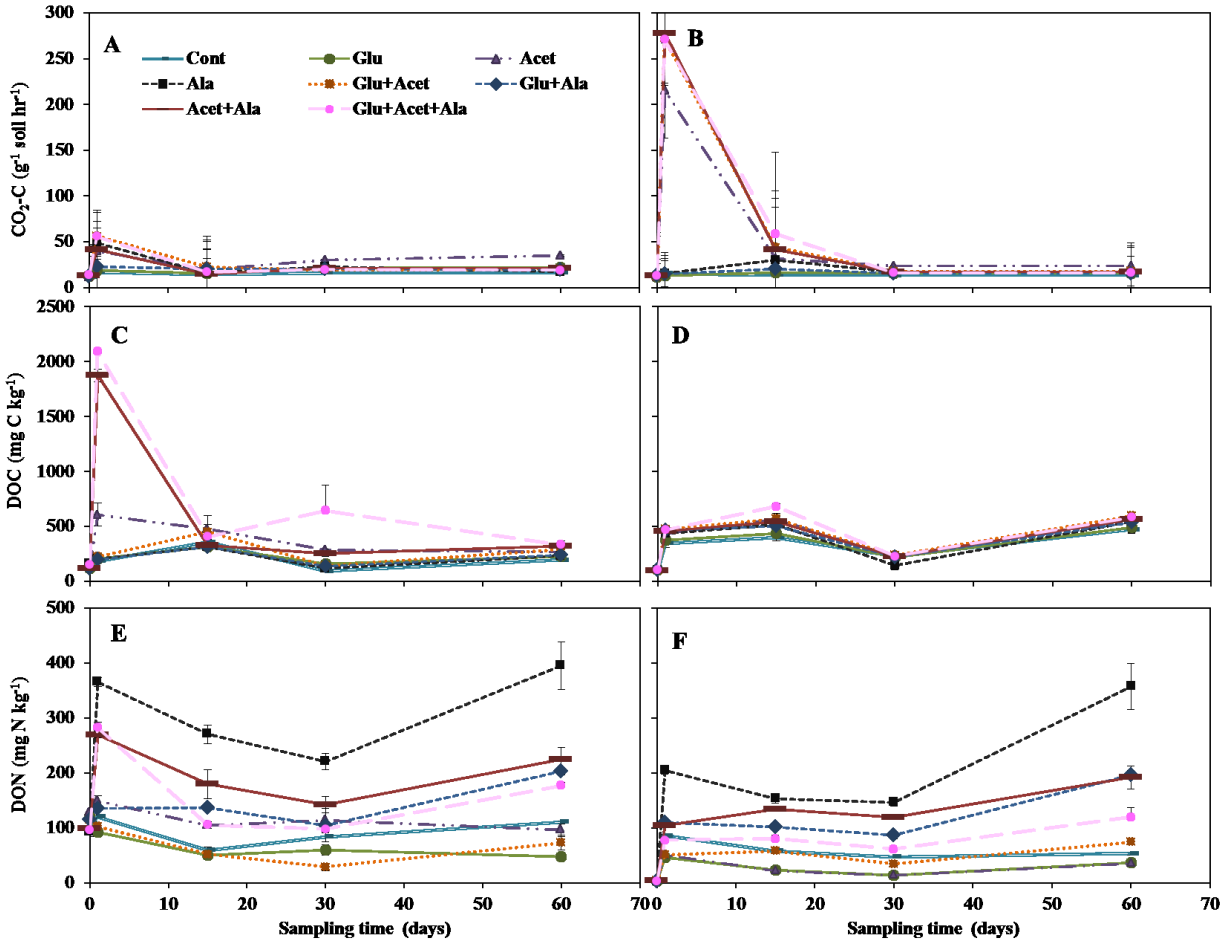

Figure 3-2 Changes in CO_2 emission rates in (A) LFH-MS and (B) PMM dissolved organic carbon (DOC) in (C) LFH-MS and (D) PMM and dissolved organic nitrogen (DON) in (E) LFH-MS and (F) PMM affected by organic substrate type and diversity in organic capping materials used in oil sands reclamation. Abbreviations: Cont: control, Glu: glucose, Acet: acetic acid, Ala: alanine, Glu + Acet: glucose + acetic acid, Glu + Ala: glucose + alanine, Acet + Ala: acetic acid + alanine, Glu + Acet + Ala: glucose + acetic acid + alanine

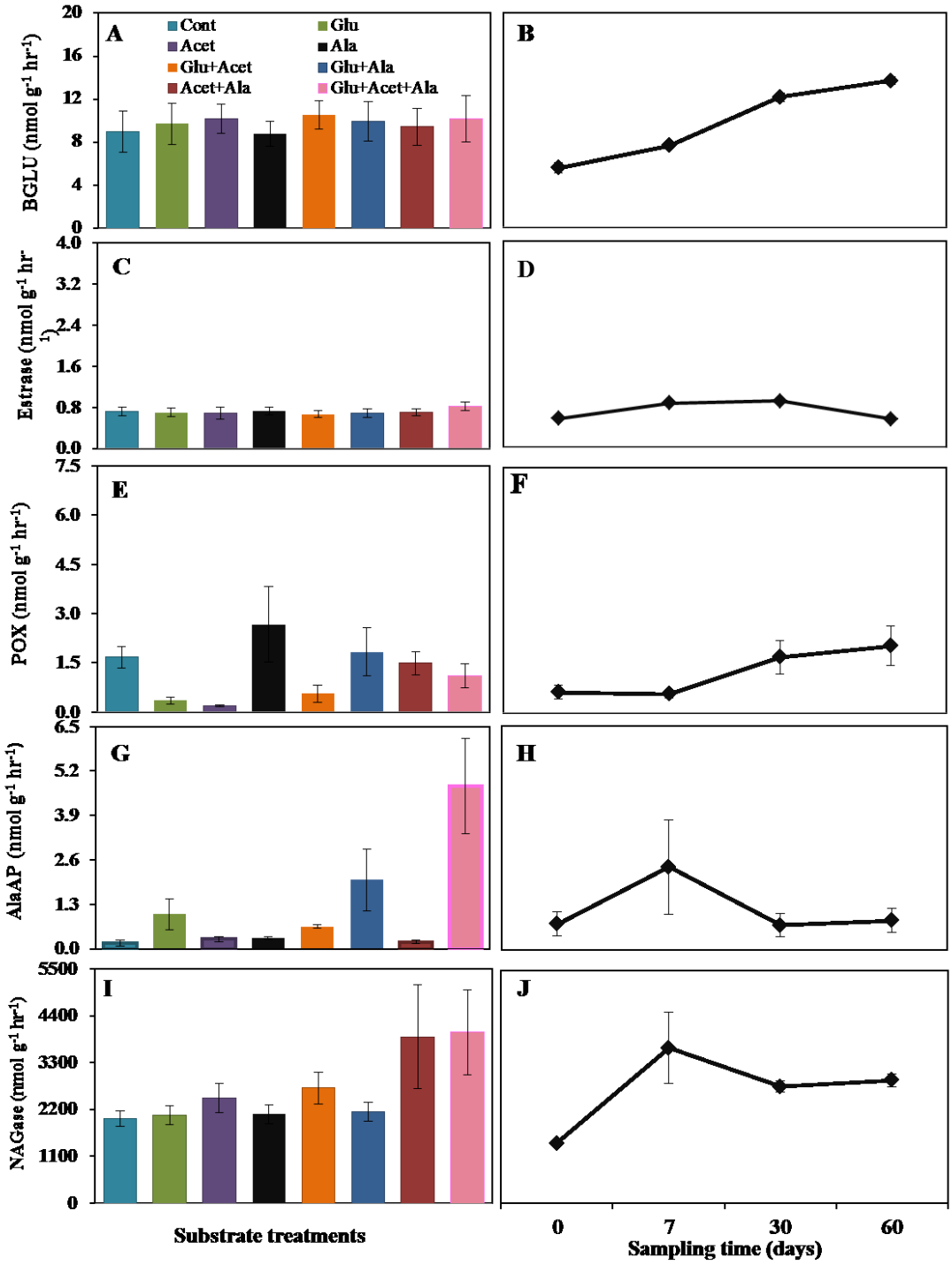

Figure 3-3 Changes in soil enzyme activities in (A, C, E, G and I) LFH-MS affected by organic substrate type (left columns) and (B,D,F, H and J) time (right columns) of measurement in organic capping materials used in oil sands reclamation. Abbreviations: Cont: control, Glu: glucose, Acet: acetic acid, Ala: alanine, Glu + Acet: glucose + acetic acid, Glu + Ala: glucose + alanine, Acet + Ala: acetic acid + alanine, Glu + Acet + Ala: glucose + acetic acid + alanine

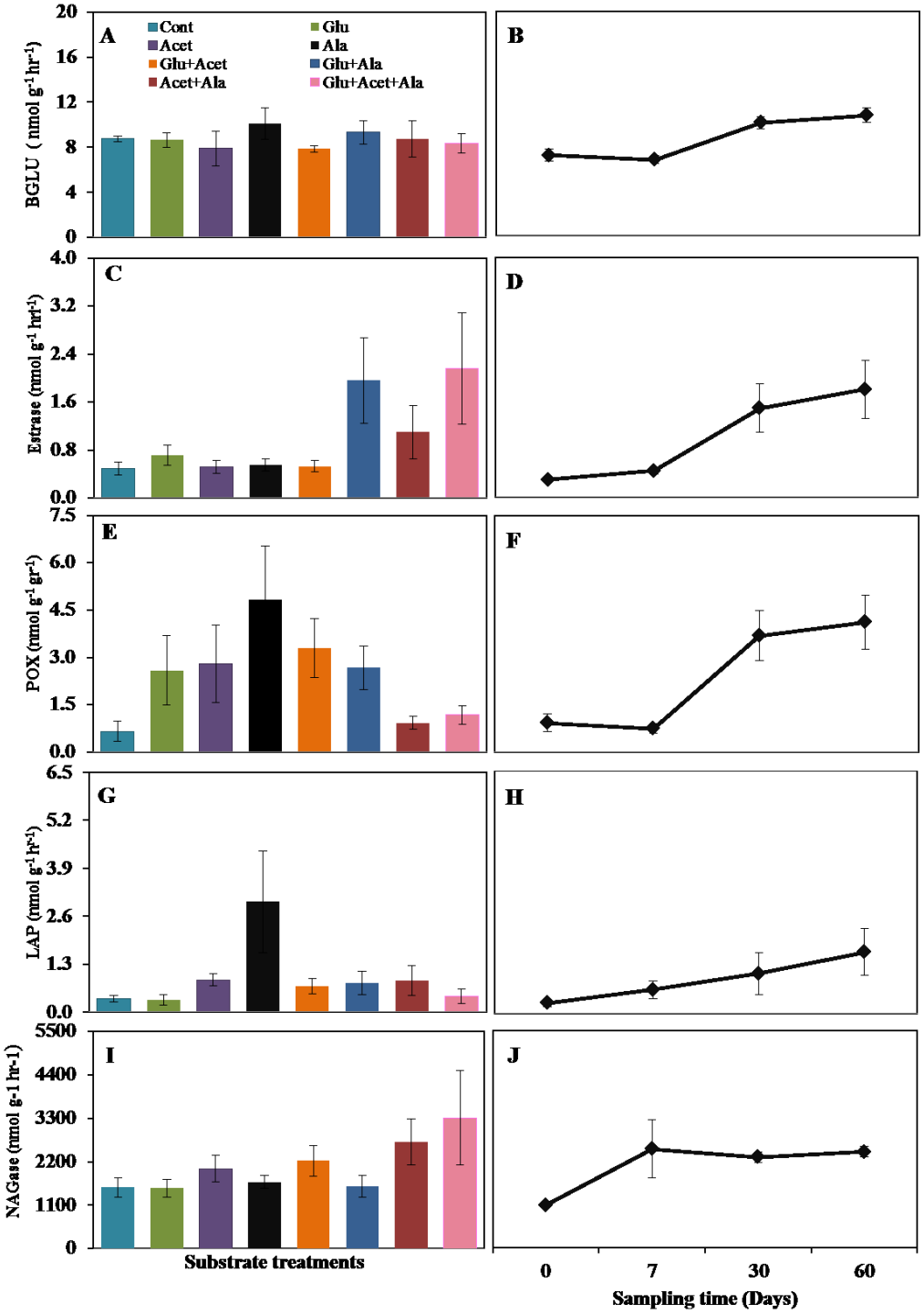

Figure 3-4 Changes in soil enzyme activities in (A, C, E, G and I) PMM affected by organic substrate type (left columns) and (B, D, F, H and J) time (right columns) of measurement in organic capping materials used in oil sands reclamation. Abbreviations: Cont: control, Glu: glucose, Acet: acetic acid, Ala: alanine, Glu + Acet: glucose + acetic acid, Glu + Ala: glucose + alanine, Acet + Ala: acetic acid + alanine, Glu + Acet + Ala: glucose + acetic acid + alanine

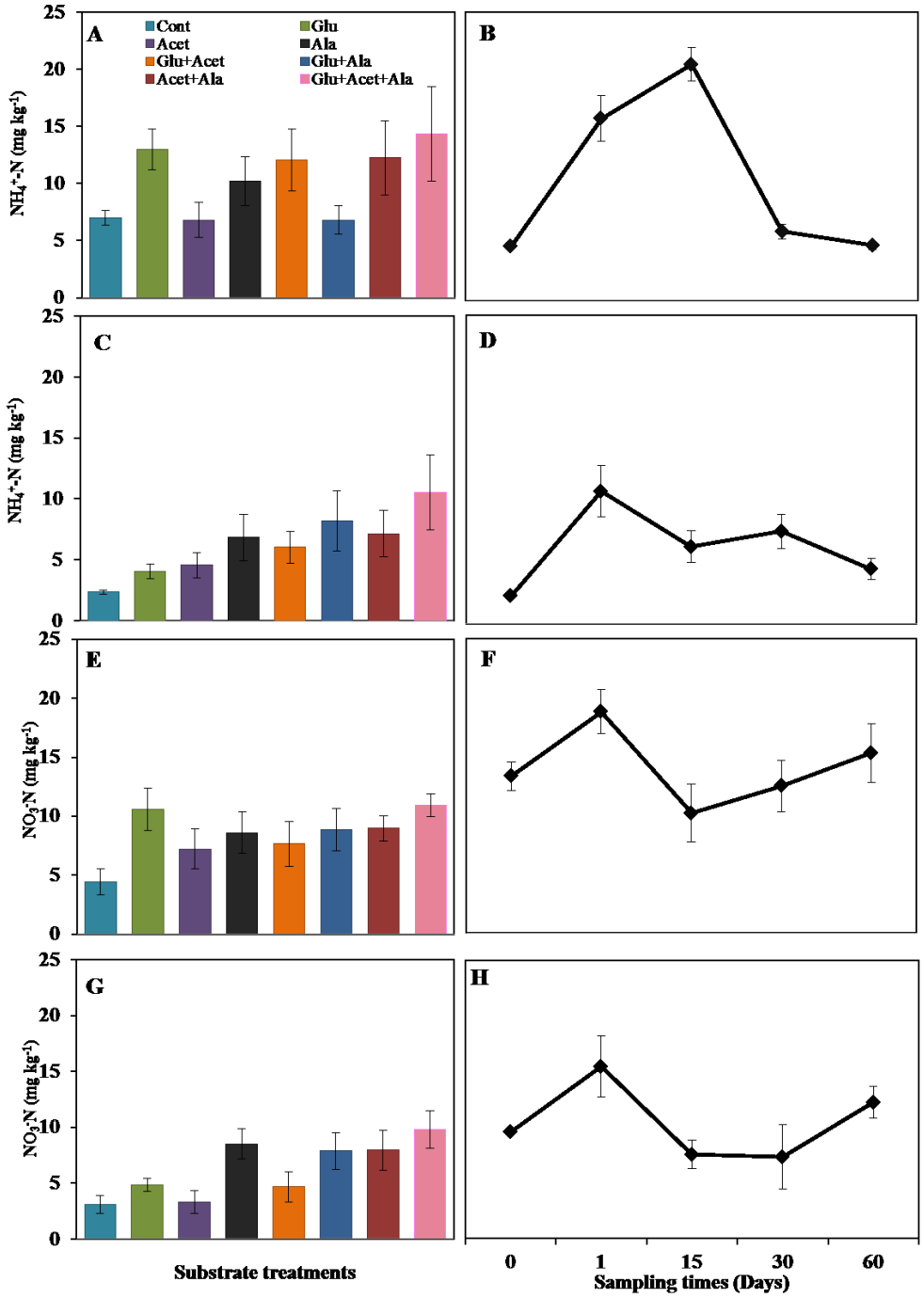

Figure 3-5 Changes in NH_4^+-N in (A) LFH-MS and (C) PMM and NO_3^--N in (E) LFH-MS and (G) PMM affected by organic substrate type (left columns) and (B, D, F and H) time (right columns) of measurement in materials used oil sands reclamation, Codes: Cont: control, Glu: glucose, Acet: acetic acid, Ala: alanine, Glu + Acet: glucose + acetic acid, Glu + Ala: glucose + alanine, Acet + Ala: acetic acid + alanine, Glu + Acet + Ala: glucose + acetic acid + alanine

CHAPTER 4 ORGANIC TO MINERAL SOIL RATIO OF ORGANIC CAPPING MATERIALS USED FOR OIL SANDS RECLAMATION ALTERED THE BIOGEOCHEMICAL PROCESSES

1. Introduction

Soil is the most complex biological system in global terrestrial ecosystems, composed of various minerals, organic components microorganisms, water and air (Coleman et al., 1998; Kögel-Knabner et al., 2008). The interactions among these components have strong influences on soil biogeochemical processes and functions such as decomposition of organic matter (OM), nutrient cycling and enzyme activities (Huang et al., 2005; Allison, 2006; Totsche et al., 2010) since these processes are altered by organo-mineral interactions (Huang et al., 2005; Kögel-Knabner et al., 2008; Pronk et al., 2012). The effect of organo-mineral interactions on soil biogeochemical processes is mainly regulated by the type (Jamro et al., 2014; Kwak et al., 2015a) composition (Turcotte et al., 2009) and quantity of OM (Schnecker et al., 2014) and minerals in the soil (Shindo and Huang, 1985; Marx et al., 2005; Kögel-Knabner et al., 2008) and other soil properties, particularly texture (Giardina et al., 2001), pH (Sinsabaugh et al., 2003) and the soil microenvironment (Scott et al., 1996).

The OM composition and quantity differences are potential to change in soil biogeochemistry (Sorenson et al., 2011; Noah et al., 2014) due to their involvement in organic substrate availability for biogeochemical processes (Schnecker et al., 2014). The carbon to nitrogen (C to N) ratio is a common indicator of the measurement of OM quality (Mohanty et al., 2013) which regulates N mineralization and organic substrate availability (Mohanty et al., 2013) and affects enzyme activities in the soil (Jamro et al., 2014; Kwak et al., 2015a). The inherent chemical recalcitrance (e.g., lignin) of OM also affects its decomposition (Smith et al., 2015), which may reduce enzyme activity (Sinsabaugh, 1994) and consequently affects N mineralization. Enzyme activities are also inhibited by the addition of humic substances due to complexity and dynamic nature of OM (Huang et al., 2005) In addition, OM quantity may also affect organic substrate availability for microbial activity which is either affected by the amount of OM applied (Li et al., 2011) and soil depth (Dimitriu et al., 2010; Schnecker et al., 2014) due to change in C to N ratio (Jamro et al., 2014) and other soil properties such as soil pH (Blume et al., 2002; Eilers et al., 2012; Jamro et al., 2014). Altering these soil properties may favor

different microorganisms having different functional properties which could alter soil enzyme activities and decomposition of OM, consequently N availability (Waldrop and Firestone, 2006; Schnecker et al., 2014). Thus, the decomposition of OM is controlled by the amount and type of OM, microorganism efficiency in utilizing C and retaining capacity of the soil (Gadd, 2007; Cotrufo et al., 2013; Smith et al., 2015).

Minerals, such as clay minerals, provide active sites for adsorption of OM and biochemical processes (Pronk et al., 2012), help stabilize OM and decrease microbial activity via physical occlusion of compounds to OM (Huang et al., 2005; Kögel-Knabner et al., 2008). Sorption of organic compounds and enzyme substrates to molecular mineral pores also limit their accessibility to microbes and enzymes (Kaiser and Guggenberger, 2003), reducing the enzyme activities (Allison, 2006), slowing OM decomposition (Watanabe et al., 2005) and decreasing nutrient availability (Silver et al., 2000). In artificial enzyme-clay- complexes studies show that the presence of clay either reduces the enzyme activities (Gianfreda et al., 1992) or enhances the enzyme activities (Bayan and Eivazi, 1999) and affects the OM decomposition (Fissore et al., 2008). In addition, iron (Fe) or aluminum (Al) oxide mineral form complexes with enzyme substrates, limit the organic substrate availability (Allison and Jastrow, 2006) and consequently reduce N availability. Thus, variation in soil minerals composition, their assemblage and their interactions with OM can cause differential biogeochemical processes (Huang et al., 2005; Gadd, 2007; Turner et al., 2014). The biogeochemical processes of disturbed and reconstructed ecosystems, such as the Athabasca oil sands region (AOSR) in northern Alberta, Canada, is completely different from that of natural ecosystems, since it is associated with exogenous organic inputs (Noah et al., 2014) and mineral soil mixed with organic capping materials (Fung and Macyk, 2000, MacKenzie and Quideau, 2012). Thus, better comprehension of mineral–organic matter biogeochemical interactions is essential to evaluate restoration practices and the sustainability of the ecosystem (Huang et al., 2005).

The AOSR is recognized as the single largest oil reserve in the world. Approximately 813 km^2 of boreal forest in the region has been disturbed by surface mining as of December 2013, and government regulations state that mining companies are bound to reclaim all disturbed areas (Alberta Government, 2015). LFH (litter (L), fragmented litter (F) and humus (H)) mineral soil mix (LFH) and peat mineral soil mix (PMM) are common sources of organic capping materials for oil sands reclamation (MacKenzie and Naeth, 2007) and are applied in different ratios of OM

(LFH or peat) to mineral soil (Naeth et al., 2013). The peat to mineral soil ratios (vol: vol) varied from 1:3 (Moskal et al., 2001) to 1:1 (Fung and Macyk, 2000; Hemstock et al., 2010) with some exceptions (Moskal et al., 2001). The LFH to mineral soil ratio (vol: vol) ranged from 1:5 to 1:1 depending on the salvaging depth and the type of mineral soil in the mixture (Alberta Environment and Water, 2012). Maintaining a consistent salvaging depth for organic capping materials is not operationally possible for reclamation (Naeth et al., 2013). However, no study has evaluated how the organic to mineral soil ratio affects the biogeochemical processes of organic capping materials used in oil sands reclamation.

The objective of this study was to evaluate the effects of the ratio of organic to mineral soil of organic capping materials used for oil sands reclamation on the biogeochemical processes. We hypothesized that changes in organic to mineral soils ratio would alter the biogeochemical processes in organic capping materials used for oil sands reclamation due to changes in OM content and organo-mineral interactions. Thus, greater enzyme activities and N availability will be expected with a greater organic to mineral soil ratio in organic capping materials regardless of organic capping material type used. The results will be helpful in understanding organo-mineral biogeochemical interactions and developing an optimal ratio of materials to design the current reclamation practices and further improvement required for long term sustainability of ecosystems in the oil sands region.

2. Materials and Methods

2.1 Collection and processing of materials

Forest floor (including L, F and H horizons), peat and mineral soil samples were collected within the AOSR, Alberta, Canada, materials that are representatives of those used for oil sands reclamation. Forest floor was collected at 0-5 cm depth from a 60-year-old trembling aspen (*Populus tremuloids* Michx.) dominated upland forest. Mineral soil was collected after removing the forest floor at 0-10 cm depth from the same area. The soil was classified as Gray Luvisol (Soil Classification Working Group, 1998) or Cryralf (Soil Survey Staff, 1998). The soil had a sandy loam texture (Jung and Chang, 2012). Peat moss (*Sphagnum spp*.) was collected from the surface (>5 cm) at a lower slope in the landscape, close to where the forest floor and mineral soil samples were collected. The samples were transported to the laboratory in cooler containing ice

packs. In the laboratory, samples were air-dried and passed through a 4-mm sieve to remove coarse fragments and debris and homogenized properly before analyses.

2.2 Experimental design and incubation experiment set up

The study involves a factorial design with two organic materials (forest floor (LFH), peat and mineral soil (MS) and their mixtures at five ratios described in Table 4-1. The treatments were replicated three times. The materials were mixed on a volumetric basis using a100 mL cylinder and final volume was kept to 100 mL in each organic to mineral soil ratio, then material was put in 250 mL flask. The weight of materials varied from 50 to 110 g in LFH to mineral soil and 20 to 110 g in peat to mineral soil ratios (Table 4-1). The ratios of organic to minerals soils were selected based on the current practice of organic capping materials used in oil sands reclamation (Fung and Macyk, 2000; Moskal et al., 2001; Hemstock et al., 2010; Alberta Environment and Water, 2012; Naeth et al., 2013). Five experimental units of each ratio treatment were set up based on the soil sampling frequency (discussed below) for measurement of soil processes and properties. The experimental design allowed us to avoid soil disturbance caused by subsampling, which would affect microbial activity and carbon dioxide (CO_2) emission rates. Four experimental units were designed for the measurement of soil enzyme activities, dissolved organic carbon (DOC) and nitrogen (DON) and CO_2 emission rate (0, 1, 7, 15, 30 days after treatment). However, the 30 days after treatment unit was reserved for measurement of CO_2 emission rate to avoid the disturbance effect. Prior to the experiment, pre-incubation was done at 25 °C for 10 days to allow the microorganisms to acclimatize to the laboratory conditions. During incubation, flasks were weighed every four days to maintain 60% water holding capacity of the mixtures; water content was adjusted with deionized water.

2.3 Organic substrate preparation and application

Organic substrate solution was prepared by using synthetic substrates (Sigma-Aldrich St. Louis MO.) for enzymes involved in carbon cycling, including β-glucosidase, cellobiohydrolase and phenol oxidase; and N cycling, including β-N-acetyl glucosaminidase (NAGase) and leucine aminopeptidase (LAP). These enzymes were selected because these are commonly found in terrestrial ecosystems (Sinsbaugh, 1994; Kandeler et al., 1999; Stursova et al., 2006). D-glucose ($C_6H_{12}O_6$) was mixed in the organic substrate solution to provide a labile organic substrate for

microorganisms for initiation of activity, since glucose is a common component in soil organic carbon pools that initiates activities of microorganism immediately (Van Hees et al., 2005; Bradford et al., 2010).

One mg of each organic substrate/compound was used to prepare an organic substrate solution with Milli-Q deionized water at a ratio of 1:1 (m/v) to avoid any inhibiting effect of any organic substrate. Two mL of organic substrate solution was applied to each treatment using a 10 mL syringe with a needle tip considering that microorganisms utilize organic substrates in excess of demand under in-situ conditions (Van Hees et al., 2005). Addition of organic substrate in excess of demand is a common approach for assessing the potential respiration and microbial responses of organic substrate utilization in soils (Bradford et al., 2010). This was also confirmed by a preliminary study for 48 hours incubation where the highest CO_2 emissions were recorded by testing basic materials used for preparation of organic to mineral soil ratios (data not shown) at two mL of organic substrate mixture. The organic substrate mixture was applied to avoid any limitation of substrate for biogeochemical reactions in artificial soil mixtures during incubation (Allison, 2006; Pronk et al., 2012) since mineral and organic soils vary in their C to N ratios (Hemstock et al., 2010; Jung and Chang, 2012) and ultimately have different decomposition potentials. The conical flasks were sealed with gas tight lids fitted with a butyl rubber septum throughout the experiment to allow the accumulation of gas in the flasks; the flasks were kept open for one and half hours after each gas sampling to ensure adequate gas exchange between atmosphere and flasks. Gas samples were collected every day for the first three days and then every four days for ensuring gas exchange between atmosphere and flasks.

2.4 Soil properties measurement

Total carbon (C) and nitrogen (N) concentrations were evaluated at day 0 and 30 of the incubation. Soil samples collected at each sampling time (0 and 30 days) were air-dried and ground with a ball mill (Mixer Mill MM 200, Thomas Scientific, Swedesboro NJ). Total C and N were analyzed by a dry combustion using an automated elemental analyzer (NA-1500 series, Carlo Erba, Milan, Italy). Soil pH was measured in a 1:2 (m:v, soil:0.01 mol L^{-1} $CaCl_2$) ratio using a digital DMP-2 mV/pH meter (Thermo Fisher Scientific Inc., Waltham, MA) (Kalra and Maynard, 1991). For ammonium (NH_4^+-N) and nitrate (NO_3^--N) analyses, soil samples were extracted using 2 mol L^{-1} KCl (Mulvaney, 1996). The extract was analyzed colorimetrically by

the indophenol blue method for NH_4^+-N (Keeney and Nelson, 1982) and by the vanadium oxidation method for NO_3^--N (Miranda et al., 2001). The NH_4^+-N, NO_3^--N and soil pH were measured at 0 and 30 days of incubation. Since NO_3^--N was detected only in a few samples the data are only presented as available N by adding NH_4^+-N and NO_3^--N. For DOC and DON concentrations, soil samples were extracted with 0.5 mol·L^{-1} K_2SO_4 solution at 1:10 (w:v) soil to solution ratio. After shaking for one hour on a reciprocating shaker (Eberbach Corp., Michigan, U.S.A), the extracts were filtered using Fisher Q2 filter papers. The C and N concentrations in the extracts were measured with a TOC-VCSN (Shimadzu, Kyoto, Japan).

2.5 Identification of minerals

The mineralogy of the soil used in this experiment was determined by X-ray diffraction (XRD) after the removal of organic matter with H_2O_2 using a Siemens D-5000 diffractometer with a Co-K radiation source. Powder samples were analysed to characterize all minerals, and oriented deposits were analysed to specifically identify clay minerals. Samples were analysed after individual treatments of K saturation, addition of ethylene glycol, heating to 550 °C or without any of the treatments listed above.

2.6 CO_2 measurement

Separate flasks were used for measuring CO_2 and other biogeochemical processes at each sampling time to avoid disturbance effects. The CO_2 gas samples were collected at day 0, 1, 3, 7, 10 and 30 of incubation. At each sampling time, gas samples were taken at time 0, 1, 12 and 24 h initially for one week and later at 24 hr at each sampling time using a 20 mL gas-tight syringe (BD Luer-Lok™ Tip) and injected into pre-evacuated exetainer vials (Isomass Exetainers) fitted with septa. The gas samples were analyzed on a gas chromatograph (Varian GC-3800). The rate of CO_2 emission (mg CO_2 h^{-1} g^{-1} soil) was calculated from concentration changes between the differences of each sampling time and pooled to determine CO_2 emission rate per day.

2.7 Soil enzymes assay

The β-glucosidase (Enzyme classification number ECN: 3.2.1.21), cellobiohydrolase (ECN: 3.2.1.91), phenol oxidase (ECN:1.10.3.2), N-acetyl-b-D- glucosaminidase (NAGase, ECN 3.2.1.14) and Leucine amino peptidase (LAP, ECN: 3.4.11.1) were evaluated after 0, 7, 15 and 30 days of incubation using microplate assays as described by Sinsabaugh et al. (2003). The

activities of β-glucosidase, cellobiohydrolase and NAGase were evaluated using the fluorescence method. Substrates of 200 mM of 4-methylumbelliferyl (MUB) for each enzyme namely *β*-D-glucosidase, *β*-D-cellobioside and N- acetyl-*β*-glucoaminide were used. One gram of fresh soil was placed in a 250 mL Nalgene bottle for preparation of soil suspensions with a 125 mL of sodium acetate buffer (50 mmol L^{-1}, pH 5). The soil suspensions were homogenized using a magnetic stirrer until suspensions were transferred into black 96 well microplates. A 200 µL soil suspension and 50 µL of 200 mmol L^{-1} substrate of each enzyme were pipetted onto each plate. Soil background wells (200 µL soil suspension + 50 µL buffer) and quench wells [(200 µL soil suspension + 50 µL 4-MUF (4-methylumbelliferone)] were then added to each plate. The plates were placed in an incubator for three hours for measurement of β-glucosidase and NAGase, and seven hours for cellobiohydrolase at 20 °C in the dark. After incubation, enzyme reaction was stopped by adding 20 µL of 0.5 mol L^{-1} NaOH to each plate. Fluorescence was measured at 360 nm excitation and 460 nm emissions using a Synergy HT multi-detection microplate reader (Bio-Tek Instruments, Winooski, VT).

The phenol oxidase and LAP activities were determined using the colorimetric method (Stursova et al., 2006). The phenol oxidase was determined in clear microplates containing 50 µL of 25 mM L-dihydroxyphenylalanine (L- DOPA) incubated for 24 hours at 20 °C. The 7-amino-4-methylcourmarin linked substrate was used to evaluate LAP activity. The incubation was done at 20 °C in the dark for 24 hours. The color development was determined at 460 nm after incubation. All measured enzyme activities (nmol of substrate g^{-1} h^{-1}) were calculated on an oven-dry (105 °C) mass basis of soil.

2.8 Statistical analyses

A repeated measures analysis of variance (ANOVA) was used to assess the effect of ratio of materials and sampling time on total C, total N, C to N ratio, enzyme activities, available N, DOC, DON concentrations and CO_2 emission rates. Tukey's honestly significant difference (HSD) test was used to determine significant effect of ratio on biogeochemical processes, time of incubation and their interactions. Pearson correlation analysis was conducted to determine the relationship between biogeochemical processes and soil properties. Assumptions of normality and homogeneity of variance were tested prior to performing ANOVA using a Shapiro-Wilk test

and Bartlett test, respectively. An α value of 0.05 was used to indicate statistical significance. All analyses were performed using a SAS 9.3 software (SAS Institute Inc., USA).

3. Results

3.1 Basic properties of soil materials

The ratio of organic to mineral soils (either LFH or peat) significantly altered the total C, total N, C to N ratio and pH (Table 4-2). With an increasing amount of LFH or peat, total C, and total N were increased ($p<0.05$) at day 0. The mean lowest total C (14.3 g kg^{-1}) was found in mineral soil only (LFH-0 and peat-0) treatments, whereas the greatest total C was found in LFH-100 (117.7 g kg^{-1}) and in peat-100 (347.7 g kg^{-1}). Organic substrate addition increased the total C concentrations in both LFH and peat ratios, except for peat-100 from days 0 to 30 of . However, no differences existed between LFH-0 or peat-0 (mineral soil) and LFH-30 and peat-30 (Table 4-2). Total N followed a similar trend as that of total C in both LFH and peat, with the initial total N concentrations ranging from 0.6 (LFH-0) to 5.8 g kg^{-1} (LFH-100) in LFH 0.6 g kg^{-1} (peat-0) 15.5 g kg^{-1} (peat-100). The total N concentrations increased approximately by 32 (LFH-70) to 67% (LFH-30) in LFH and by 64 (peat-70) to 100% (peat-50) in peat from day 0 to 30 of the incubation. On average (values not shown), the C to N ratio ranged from 19.7 (LFH-100) to 34.9 (LFH-30) in LFH and from 24.8 (peat-100) to 33.4 (peat-30) in peat, regardless of the time of measurement (Table 4-2). The C to N ratio increased in mineral soil (LFH-0 and peat-0), whereas it decreased in the rest of the treatments in both LFH and peat (Table 4-2) from day 0 to 30. The mean pH was higher in organic soils and in the mixture of mineral and organic soil treatments in both LFH and peat than in the mineral soil alone (i.e., LFH-0 or peat-0: pH=4.30). The ratio of organic to mineral soils slightly increased pH in LFH and peat from day 0 to 30 (Table 4-2). The XRD diffractograms indicated that the mineral soil predominantly contained quartz, with traces of albite and pyrite (Figure 4-1).

3.2 Dissolved organic carbon and nitrogen

The DOC concentrations were significantly affected by the organic to mineral soil ratios for LFH and peat, regardless of the time of measurement (Table 4-3). The DOC concentrations were the lowest in mineral soil (LFH-0 or peat-0) and the greatest in both organic soils (LFH-100 and peat-100); other treatments were not changed by the organic to mineral soils (Figure 4-2A

and C). The DOC concentrations were not affected by the time of measurement (Table 4-3; Figure 4-2B and D). The DON concentrations were significantly affected by the ratio of materials and time of measurement in both LFH and peat (Table 4-3). The mean DON concentrations ranged from 33 mg N kg^{-1} (LFH-0) to 72 mg N kg^{-1} (LFH-100) in LFH and 33 mg N kg^{-1} (peat-0) 78 mg N kg^{-1} (peat-100) in peat (Figure 4-2E and G). However, in both LFH and peat, the mean DON concentrations did not change from day 0 to 30 (Figure 4-2F and H).

3.3 CO_2 emission rate

The CO_2 emission rate was significantly affected by the organic to mineral soil ratio, measurement time and the interactions of ratio and time in LFH and peat soils (Table 4-3). In LFH, the highest cumulative CO_2 emission rate (data not shown) was observed in LFH-100 (125 mg CO_2-C g^{-1} hr^{-1}) followed by LFH-70 (90 mg CO_2-C g^{-1} hr^{-1}), whereas the lowest was in LFH-0 (25 mg CO_2-C g^{-1} hr^{-1}). No significant differences were observed between LFH-70 and LFH-50 and between LFH-30 and LFH-0; other treatments were significantly different from each other. The peat consistently showed a similar trend, although the emission rate was lower than that of LFH and ranged from 25 to 98 mg CO_2-C g^{-1} hr^{-1}. The CO_2 emission rate was gradually decreased along the increasing mineral soil ratios in both LFH and peat (Figure 4-3A and B). There was a greater decline of CO_2 emission rate in both organic soils (LFH-100 or peat-100) than in the mineral soil alone (LFH-0 or peat-0) between days 0 and 30. On average, the changes in CO_2 emission rate ranged from −90 (LFH-100) to −91% (LFH-70) in LFH, whereas in peat, they decreased from −70 (peat-0) to −47% (peat-100) from days 0 to 30.

3.4 Soil enzyme activities

The organic to mineral soil ratio and the time after treatment application significantly interacted in all enzyme activities in LFH and peat (Table 4-3). The β-glucosidase activity was significantly greater in the peat-100 treatment than the rest of the treatments in LFH (Figure 4-4A) and peat (Figure 4-4B). The mean values ranged from 102 nmol of substrate g^{-1} h^{-1} (mineral soil only) to 949 nmol of substrate g^{-1} h^{-1} (LFH-100) in LFH and to 1919 nmol of substrate g^{-1} h^{-1} (peat-100) in peat. The β-glucosidase activity declined in LFH (Figure 4-4A) and increased in peat (Figure 4-4B) from day 0 to 30 of measurement. The cellobiohydrolase activity followed a similar trend to that of β-glucosidase in LFH and peat (Figure 4-4C and D). The

cellobiohydrolase activity approximately doubled from day 0 to 30 in LFH and peat (Figure 4-4C and D) with a marked increase in the mineral soil only treatment (LFH-0 or peat-0). The inconsistent trends of phenol oxidase were observed in LFH and peat to mineral soils ratio along measurement times (Figure 4-4E and F). However, the mean values over time followed an order of LFH-0 < LFH-30 ≤ LFH-70 < LFH-50 < LFH-100 and peat-0 < peat-30 = peat-100 < peat-50 <peat-70. The phenol oxidase did not differ between day 0 and 30 (Figure 4-4E and F). The LAP activity consistently increased along the LFH to mineral soil ratio and over time (Figure 4-4G) with an inconsistent trend in peat to mineral soil ratio and over time (Figure 4-4H). The LAP activity ranged from 0.11 nmol of substrate g^{-1} h^{-1} (LFH-0) to 0.25 nmol of substrate g^{-1} h^{-1} (LFH-100) [1] in LFH and from 0.09 nmol of substrate g^{-1} h^{-1} (peat-70) to 0.14 nmol of substrate g^{-1} h^{-1} (peat-30) nmol of substrate g^{-1} h^{-1} in peat.

The NAGase activity was not affected by the organic to mineral soil ratios (Table 4-3). The NAGase activity had some initial increasing trend but then dramatically declined from day 0 to 30 (Figure 4-4I and J) in LFH and peat. The differences between the beginning and end of the incubation were −91 in LFH-100, −44 in peat-100, and −16 nmol g^{-1} substrate hr^{-1} in mineral soil (LFH-0 and peat-0).

3.5 Available N

The available N was significantly affected by the ratio of organic to mineral soils in LFH and peat and by the measurement time in LFH only (Table 4-3). In LFH and peat, the available N increased with the order LFH-0 or peat-0 < 30 < 50 < 70 <100 (Figure 4-5A and C). The available N significantly increased from day 0 (78.9 mg N kg^{-1}) to 30 (111.9 mg N kg^{-1}) in LFH (Figure 4-5B and D).

3.6 Relationships between soil properties and biogeochemical processes

In LFH, β-glucosidase, cellobiohydrolase, phenol oxidase and NAGase activities were positively correlated with soil pH, total C and total N and negatively correlated with the C to N ratio (Table 4-4). Several enzymes were correlated to one another; for example, β-glucosidase was positively correlated with phenol oxidase, LAP, cellobiohydrolase and NAGase activities. The CO_2 emission rate was positively correlated with β-glucosidase, phenol oxidase and

NAGase activities. The available N concentration was positively correlated with β-glucosidase, phenol oxidase and cellobiohydrolase activities and negatively correlated with the C to N ratio.

In peat, all the measured soil enzyme activities were strongly correlated with soil pH, total C and total N. The β-glucosidase was positively correlated with phenol oxidase, cellobiohydrolase and LAP activities. The CO_2 emission rate was positively correlated with phenol oxidase and NAGase activities. Available N was positively correlated with glucosidase, phenol oxidase, and cellobiohydrolase activities, whereas LAP and NAGase activities were negatively correlated with the C to N ratio (Table 4-4).

4. Discussion

The findings of this study suggest that soil biogeochemical processes are closely linked to the ratio of organic to mineral soils. The key contributing factors were changes in total C, total N, C to N ratio and pH, which caused differences in CO_2 emission rate, soil enzyme activities and N availability along the ratios of organic to mineral soils. The increasing organic to mineral soils ratio likely increased the OM content which likely increased the microbial population, resulting in a faster turnover rate of OM. That might have affected the total C and total N concentrations and consequently it changed C to N ratio due to change in microbial demand of C and N (Jonasson et al., 1996; Khan et al., 2016). The wider C to N ratio in high mineral soil ratio treatments with organic substrate addition was likely due to either (a) applied organic C substrate was not utilized by microorganisms which increased the C to N ratio or (b) applied organic C substrate was less protected by mineral soil predominantly by the quartz mineral which lacks the reactive mineral surface area (approximately 0.1 m^2/g)required for biochemical reactions (Jastrow et al., 2007; Harris, 2011, Pronk et al., 2012; Mulder et al., 2013). The presence of reactive mineral surface area typically determines the capability of protection of applied organic C to mineral (Baldock and Skjemstad, 2000). The C to N ratio increases with a decrease in the physical protection of soil particles due to presence of more intert C which is slow in decomposition (Hassink, 1992; Kaiser and Guggenberger, 2003) and increased the C to N ratio in high mineral soil ratio treatments in this study .

Earlier studies in oil sands reclamation suggest that the C to N ratio could contribute to a change in microbial processes because of a change in substrate availability in both LFH and PMM (Jamro et al., 2014; Kwak et al., 2015a). A change in organic to mineral soils ratio affected

the C to N ratio which affected the biogeochemical processes of LFH and peat. In turn, the organic to mineral soils ratio is expected to expedite the reclamation process, which needs to be tested in field condition.

The greater CO_2 emission rate, along with the increase in organic to mineral soils ratio in LFH and peat, indicated a greater amount of substrate associated with an increase in OM content (Jamro et al., 2014, Kwak et al., 2015b). This result is consistent with those of the DOC and DON concentrations, which increased with organic soils proportion in the ratios. The C to N ratio was decreased more in the presence of a high amount of organic soils in the ratio treatments, which likely enhanced decomposition of OM with increasing DOC and DON concentrations, than in the presence of high mineral soil, in which a high C to N ratio likely immobilized the DOC (Godde et al., 1996; Smith et al., 2015) and ultimately affected CO_2 emission. Alternatively, abiotic factors, such as the C to N ratio and pH changes, might shift the microbial community composition (Hogberg et al., 2007; Wan et al., 2015) along the change in the ratio of organic to minerals soils; such factors indirectly affected CO_2 emission because they strongly affect changes in microbial community composition in soils that are not inoculated with microorganisms, such as the soil in the current study (Pronk et al., 2012). In general, fungal biomass increases more with a decreasing pH and increasing C to N ratio than bacterial biomass does (Hogberg et al., 2007; Wan et al., 2015). Wei et al. (2014) showed that a shift in microbial community composition and structure increases respiration rates in artificial soils and consequently increases decomposition rate. However, concluding that changes in CO_2 emission rate along the ratio of organic to mineral soils is likely caused by a shift in microbial community composition has not been determined in this study.

Soil enzyme activities are mainly influenced by organic substrate availability and pH changes in oil sands reclamation (Jamro et al., 2014; Kwak et al., 2015a; Quideau et al., 2013). The DOC and DON provide energy to microorganisms and increase enzyme activity (Kandeler et al., 1999). Thus enhancement of the enzyme activities along the increase in ratio of organic to mineral soils was likely associated with the increase in DOC and DON concentrations and organic substrate availability (Jamro et al., 2014; Kwak et al., 2015a). However, different enzymes showed different relationships with DOC and DON, suggesting that enzymes are organic substrate specific (Burns, 1982; Allison, 2006). The amount of materials in each ratio treatment controls the distribution of organic microbial substrates (Scott et al., 1996) which may

change the structure and size of the biogeochemical interface (Quiquampoix et al., 2002), and in turn affect enzyme activities. The increase in soil enzyme activities at some time measurements in organic to mineral soils ratio (such as LFH-0, 30 and peat-0, 30) was likely associated with production of new enzymes by the microbial community because of organic substrate addition during incubation, with microorganisms being reallocated to enzyme production (Allison and Vitousek, 2005). Other ratios such as LFH-50, 70, 100 or in peat-50, 70, 100 did not stabilize/increase enzyme activity, as predicted, likely due to the increase in soil pH, as a result of the increased amount of organic soils in mixtures (Allison, 2006), because most enzyme activities function at the optimal pH (Baldrian et al., 2008).

The N availability is another important indicator of success in oil sands reclamation (Duan et al., 2015; Jamro et al., 2014; Kwak et al., 2015b; Pokharel and Chang, 2016). Our results showed that increasing the organic to mineral soils ratio increased the N availability, due to provision of more organic substrate for microbial activity which affected N mineralization and increased N availability. This is related to decrease C to N ratio along the organic to mineral soils ratio. These results are consistent with those of previous studies, in which N availability in oil sands reclamation was closely linked to the C to N ratio of materials (Jamro et al., 2014; Kwak et al., 2016). The greater N availability was also linked to greater enzyme activity, because of the greater mineralization rate (Jamro et al., 2014; Kwak et al., 2015a), as evident in the relationship results in this study.

Although this research was not focused on the effect of time on biogeochemical processes, two common responses of biogeochemical processes were observed: a priming effect and an additive effect. Priming effects are strong short-term changes in the turnover of soil OM (Kuzyakov et al., 2000). The addition of OM to the soil might cause an acceleration of mineralization (a positive priming effect) and a reduction or immobilization of the added C (negative priming effect; Kuzyakov et al., 2000) because of microbial community shift (De Nobili et al., 2001) and change in C to N ratio (Kwak et al., 2016). Our results indicated a preferential mineralization of applied C relative to the indigenous OM of the mineral soil; the mineralization resulted in an increase in CO_2 emission from day 0 to 1 in all organic to mineral soil ratios. Consequently, it might increase most extracellular enzymes and degrade inhibited organic compounds (Schimel and Schaeffer, 2012). The increase in activities of enzymes such as cellobiohydrolase, phenol oxidase and LAP, either continuously or in late stages, could be

associated with added substrates; these likely worked as a precursor of some enzymes because degradation of organic compounds requires production of extracellular enzymes (German et al., 2011). The reduction of NAGase from 0 to 30 days after the organic substrate addition is likely associated with increase in soil pH which might have decreased the fungal population and consequently decreased NAGase activity, since NAGase was related with the fungal population (Andersson et al., 2004). Alternatively, applied substrate was inadequate for a microbial community in the soil (Allison, 2006; Wei et al., 2014). A change in enzyme activities during incubation was related to soil pH. The increase in pH from day 0 to 30 would have released a weakly bound organic substrate (Mikutta et al., 2007) and consequently increased enzyme activities and N availability. This result is also clear from the observed strong relationships of soil enzymes with pH (Table 4-4) and related to the enzyme activities functioing at optimal pH (Baldrian et al., 2008). Thus, pH has a critical role in organic–mineral biogeochemical interactions in the studied soil. However, the minimal interactions between ratio and time, regardless of the type of organic soil suggest that either a one-month experiment was not long enough to establish the organo-mineral-biogeochemical association (Allison, 2006) or mineral soil mixed with organic soils had quartz mineral and mixture of organic and mineral soils lacked suitable minerals, such as clay, which could help in aggregation (Huang et al., 2005). Hence, it is needed to manipulate materials like clayey soil and good quality overburden material enhancing the aggregation of soil particles and stability of soil processes.

The differences in LFH and peat in measured biogeochemical processes were likely due to inherited characteristic differences in LFH and PMM (Jamro et al., 2014; MacKenzie and Naeth, 2010; MacKenzie and Quideau, 2012). In previous studies, LFH-MS had greater microbial biomass carbon (Kwak et al., 2015a), soil enzyme activities (Jamro et al., 2014), changed microbial community (Kwak et al., 2015a) and OM chemistry (Turcotte et al., 2009). Greater cellobiohydrolase and phenol oxidase activities in peat indicate slower decomposition of organic compounds, such as cellulose and phenolic compounds, than in LFH. Greater LAP activity in LFH than in peat suggests greater N mineralization in LFH, likely associated with a lower C to N ratio in LFH than in PMM. Thus, caution is required in considering the effects of organic to mineral soil ratio on functionality of reclaimed ecosystem; differences of inherited properties of LFH and peat need to be considered. Future expansion of mining will require more LFH for land reclamation, although LFH is limited (Naeth et al., 2013). Under these circumstances, reducing

the application ratio of LFH to mineral soils could help to overcome this issue. This study supports the claim that a 50:50 ratio of organic to mineral soils (LFH-50 or peat-50 i.e LFH: mineral soil 50:50 or peat: mineral soil 50:50) would be a better strategy in oil sands reclamation as the biogeochemistry of organic soils will not be changed.

5. Conclusions

The study demonstrated that the increasing ratio of organic to mineral soils improved soil functions and processes of organic capping materials used in oil sands reclamation. Changes in soil biogeochemical processes were closely associated with changes in C to N ratio and pH along with organic to mineral soils ratio. LFH-50 and peat-50 ratios were optimum and could increase if organic material was available at the reclamation site. Field evaluation for these kinds of effects will need to be conducted for improvement of current reclamation practices.

Table 4-1 Description of treatments used in this experiment

Treatment code	LFH to mineral soil ratio (Vol:Vol)	Weight of material (g)	LFH: MS (g)	Treatment code	Peat to mineral soil ratio (Vol:Vol)	Weight of material (g)	Peat: MS (g)
LFH-0	0 : 100	110	110:0	Peat-0	0 : 100	110	100
LFH-30	30 : 70	87	17:70	Peat-30	30 : 70	90	8:82
LFH-50	50 : 50	80	25:55	Peat-50	50 : 50	72	10:62
LFH-70	70 : 30	68	33:45	Peat-70	70 : 30	45	13:32
LFH-100	100 : 0	50	50:0	Peat-100	100 : 0	20	20

Table 4-2 Basic properties of materials used in this experiment

Treatments*	Total organic carbon (g kg^{-1})		Total organic nitrogen (g kg^{-1})		Carbon to nitrogen ratio		pH	
	0 DAT	30 DAT	0 DAT	30 DAT	0 DAT	30 DAT	0 DAT	30 DAT
LFH								
LFH-0	1.43 (0.02)d	2.92 (0.06)d	0.06 (<0.01)c	0.08 (<0.01)d	25.3 (0.7)bc	36.8 (1.2)a	4.10 (<0.01)b	4.50 (0.11)b
LFH-30	2.36 (0.02)d	3.05 (0.03)d	0.06 (<0.01)c	0.10 (<0.01)d	39.4 (0.3)a	30.5 (0.3)b	4.74 (0.01)ab	5.09 (0.17)ab
LFH-50	4.39 (0.01)c	4.64 (0.01)c	0.15 (0.01)bc	0.22 (<0.01)c	29.4 (0.7)b	21.1 (<0.01)c	4.78 (0.15)a	5.17 (0.10)ab
LFH-70	6.52 (0.16)b	6.55 (<0.01)b	0.25 (0.01)b	0.33 (<0.01)b	26.1 (0.2)bc	20.1 (0.1)c	4.90 (0.12)a	5.31 (0.03)ab
LFH-100	11.72 (0.29)a	15.97 (<0.01)a	0.58 (0.02)a	0.87 (0.02)a	20.6 (0.9)c	18.7 (0.2)c	5.01 (0.02)a	5.51 (0.14)a
Peat								
Peat-0	1.43 (0.02)d	2.92 (0.05)d	0.06 (<0.01)d	0.08 (0.03)c	25.3 (0.7)b	36.8 (1.3)a	4.10 (<0.01)b	4.50 (0.11)b
Peat-30	3.33 (0.12)c	4.06 (0.02)cd	0.09 (0.02)cd	0.14 (0.02)c	37.1 (0.6)a	29.7 (0.5)b	4.56 (0.02)a	4.59 (0.04)b
Peat-50	4.37 (<0.01)c	6.94 (0.47)bc	0.14 (<0.01)c	0.28 (0.01)b	31.2 (0.1)ab	24.7 (0.4)bc	4.56 (0.01)a	4.70 (0.04)b
Peat-70	23.61 (0.31)b	8.97 (0.48)b	0.87 (0.06)b	0.31 (0.01)b	26.2 (1.0)ab	28.5 (0.2)bc	4.68 (0.06)a	5.16 (0.09)ab
Peat-100	34.75 (0.01)a	27.99 (0.31)a	1.55 (0.02)a	1.23 (0.02)a	26.8 (2.1)ab	22.7 (0.1)c	4.78 (0.07)a	5.68 (0.08)a
Repeated Measures ANOVA								
LFH	F value	*P* value	F value	*P* value	F value	*P* value	F value	*P* value
Ratio	1208.37	<0.001	442.21	<0.001	56.56	<0.001	11.26	<0.001
Time	121.58	<0.001	78.94	<0.001	14.62	0.002	17.55	0.002
Ratio*time	39.99	<0.001	18.75	<0.001	25.85	<0.001	0.06	0.99
Peat								
Ratio	1267.83	<0.001	1393.3	<0.001	7.27	0.001	22.85	<0.001
Time	116.69	<0.001	101.24	<0.001	0.52	0.481	34.94	<0.001
Ratio*time	113.37	<0.001	98.43	<0.001	9.94	0.002	5.2	0.060

*LFH-0= LFH:MS 0:100, LFH-30= LFH:MS 30:70, LFH-50= LFH:MS 50:50, LFH-70= LFH:MS 70:30, LFH-100= LFH:MS 100:0

Peat-0= Peat:MS 0:100, Peat-30= Peat:MS 30:70, Peat-50= Peat:MS 50:50, Peat-70= Peat:MS 70:30, Peat-100= Peat:MS 100:0,

DAT: days after treatment

Table 4-3 Analysis of variance (*F* and *P* values) of the effects of organic to mineral soils ratio on selected soil processes for oil sands reclamation and their interaction with sampling time

Soil process[a]	LFH						Peat					
	Ratio of materials		Sampling time		Ratio*sampling time		Ratio of materials		Sampling time		Ratio*sampling time	
	F value	*p* value	F value	*p* value	F value	*p* value	F value	*p* value	F value	*p* value	F value	*p* value
CO_2	46.47	<0.001	44.59	<0.001	5.55	<0.001	57.3	<0.001	18.95	<0.001	2.4	0.002
DOC	2.72	0.042	3.02	0.055	1.84	0.087	7.06	<0.001	1.04	0.083	2.03	0.506
DON	6.71	0.003	5.70	0.004	2.06	0.054	8.54	<0.001	8.92	<0.001	7.19	0.051
BGLU	217.72	<0.001	13.30	<0.001	12.37	<0.001	21.23	<0.001	20.96	<0.001	1.87	0.040
CBH	8.35	0.001	55.15	<0.001	12.18	<0.001	7.49	<0.001	27.41	<0.001	1.45	0.087
LAP	27.73	<0.001	79.58	<0.001	4.67	<0.001	9.51	<0.001	35.84	<0.001	14.25	<0.001
NAGase	1.12	0.062	141.85	<0.001	16.73	<0.001	5.05	0.041	107.11	<0.001	5.67	<0.001
POX	20.03	<0.001	3.59	0.022	1.04	0.047	81.04	<0.001	38.59	<0.001	8.42	<0.001
Avail. N	28.24	<0.001	16.70	<0.001	0.85	0.511	10.36	<0.001	2.95	0.103	0.37	0.835

[a].Abreviations: LFH: litter humifed and fermented material, CO_2: carbon dioxide, NAGase : N-acetyl glucosaminidase, BGLU: β-glucosidase, CBH: cellobiohydrolase, POX: phenoloxidase, LAP: L-leucine amino peptidase, avail. N: available nitrogen, DOC: dissolved organic carbon, DON: dissolved organic nitrogen

Table 4-4 Pearson correlation coefficient (*r* -value) and significance[+] among soil variables in LFH and peat materials used for oil sands reclamation (n=30).

Soil variable*	CO_2	DOC	DON	BGLU	CBH	POX	LAP	NAGase	TC	TN	C to N ratio	Avail. N
LFH												
DOC	0.11											
DON	0.22	0.65**										
BGLU	0.64**	0.11	0.39*									
CBH	-0.24	0.13	0.28	0.40*								
POX	0.40*	0.06	0.35	0.76**	0.39*							
LAP	-0.05	0.46*	0.43*	0.10	0.31	0.34*						
NAGase	0.94**	0.01	0.03	0.44*	-0.45*	0.27	-0.21					
TC	0.31	-0.04	0.21	0.80**	0.67**	0.74**	0.09	0.13				
TN	0.21	-0.03	0.23	0.75**	0.69**	0.72**	0.05	0.04	0.98**			
C/N ratio	-0.13	-0.01	-0.27	-0.62*	-0.42*	-0.55*	0.09	0.06	-0.65	-0.7		
Avail.N	0.08	-0.08	0.25	0.64**	0.47**	0.55**	0.55*	-0.07	0.67*	0.65*	-0.57*	
pH	-0.03	0.08	0.30	0.51**	0.49**	0.46**	0.26	0.18*	0.55**	0.57**	-0.44*	0.73**
Peat												
DOC	0.14											
DON	0.16	-0.02										
BGLU	0.11	0.29										
CBH	0.21	0.44*	-0.02	0.62**								
POX	0.66**	0.13	0.37*	0.39*	0.37*							
LAP	0.07	0.32	-0.04	0.75**	0.77**	0.35*						
NAGase	0.40*	-0.11	0.29	-0.55	-0.62**	0.13	-0.67**					
TC	0.69**	0.50**	0.16	0.31*	0.29	0.63**	0.28	0.32				
TN	0.71**	0.51**	0.14	0.32*	0.30	0.60**	0.29	0.31	0.99**			
CN ratio	-0.25	-0.21	0.06	-0.47	-0.17	-0.42*	-0.30	-0.08	-0.46	-0.47		
Avail. N	0.34*	-0.02	-0.02	0.58*	0.50**	0.51**	0.09*	-0.07	0.67**	0.68**	-0.37*	
pH	0.47*	-0.11	0.73**	0.73**	0.67**	0.37*	0.71**	-0.33	0.54*	0.54*	-0.37	0.66**

[a].Abreviations: CO_2: carbon dioxide, NAGase : N-acetyl glucosaminidase, BGlu: β-glucosidase, CBH: cellobiohydrolase, POX: phenoloxidase, LAP: L-leucine amino peptidase, avail. N: available nitrogen, DOC: dissolved organic carbon, DON: dissolved organic nitrogen, TC: total carbon, TN: total nitrogen, C to N ratio: carbon to nitrogen ratio

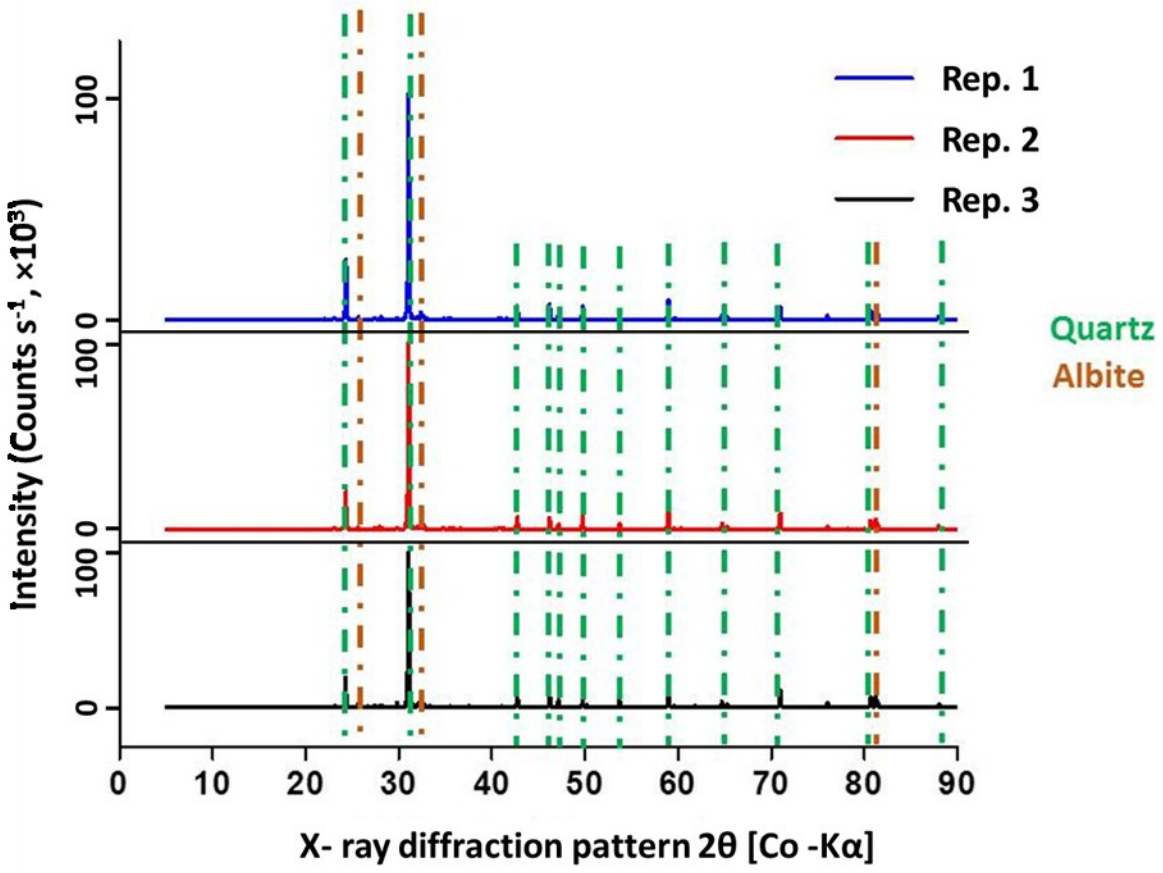

Figure 4-1 X- ray diffraction spectrum showing the minerology of the mineral soil used in the experiment

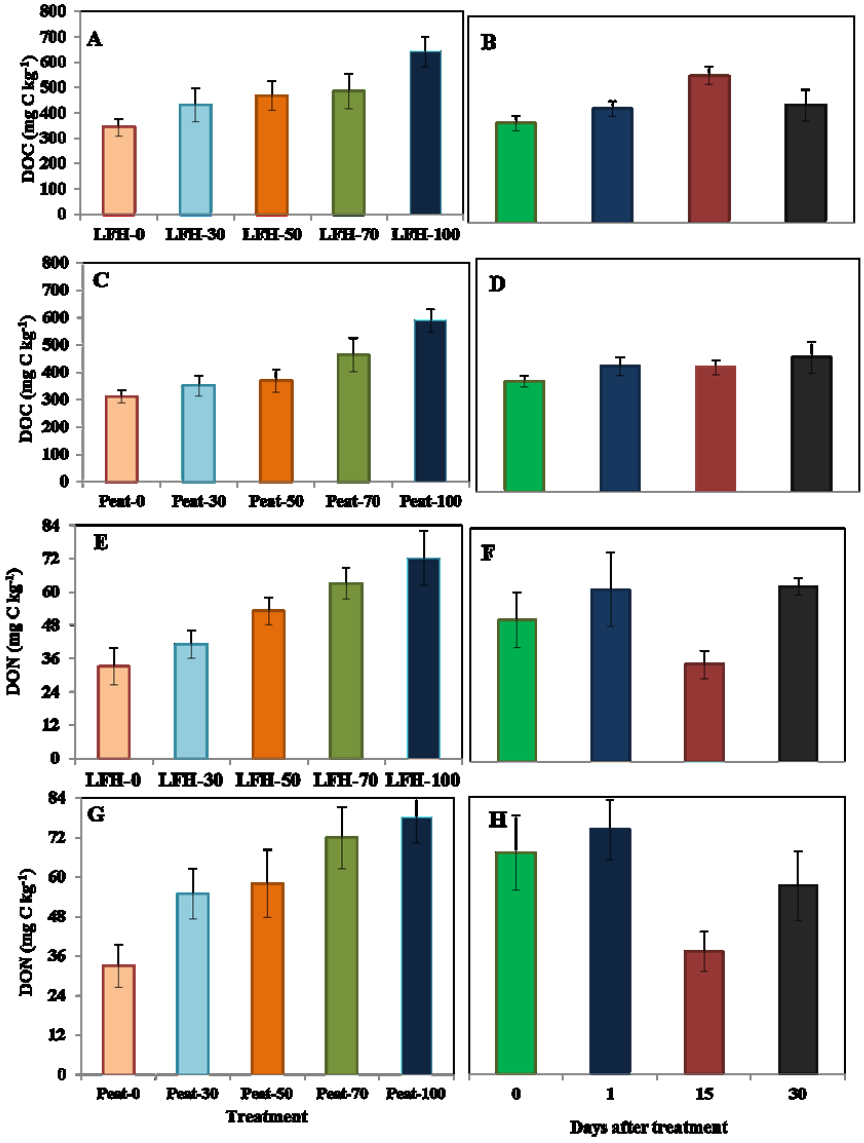

Figure 4-2 Changes in dissolved organic carbon (DOC) and nitrogen (DON) concentrations as affected by the ratios of materials and days after treatment in LFH (A, B, E, F) and in peat (C, D, G, H) materials used in the experiment

Treatments: LFH-0= LFH:MS 0:100, LFH-30= LFH:MS 30:70, LFH-50= LFH:MS 50:50, LFH-70= LFH:MS 70:30, LFH-100= LFH:MS 100:0

Peat-0= Peat:MS 0:100, Peat-30= Peat:MS 30:70, Peat-50= Peat:MS 50:50, Peat-70= Peat:MS 70:30, Peat-100= Peat:MS 100:0

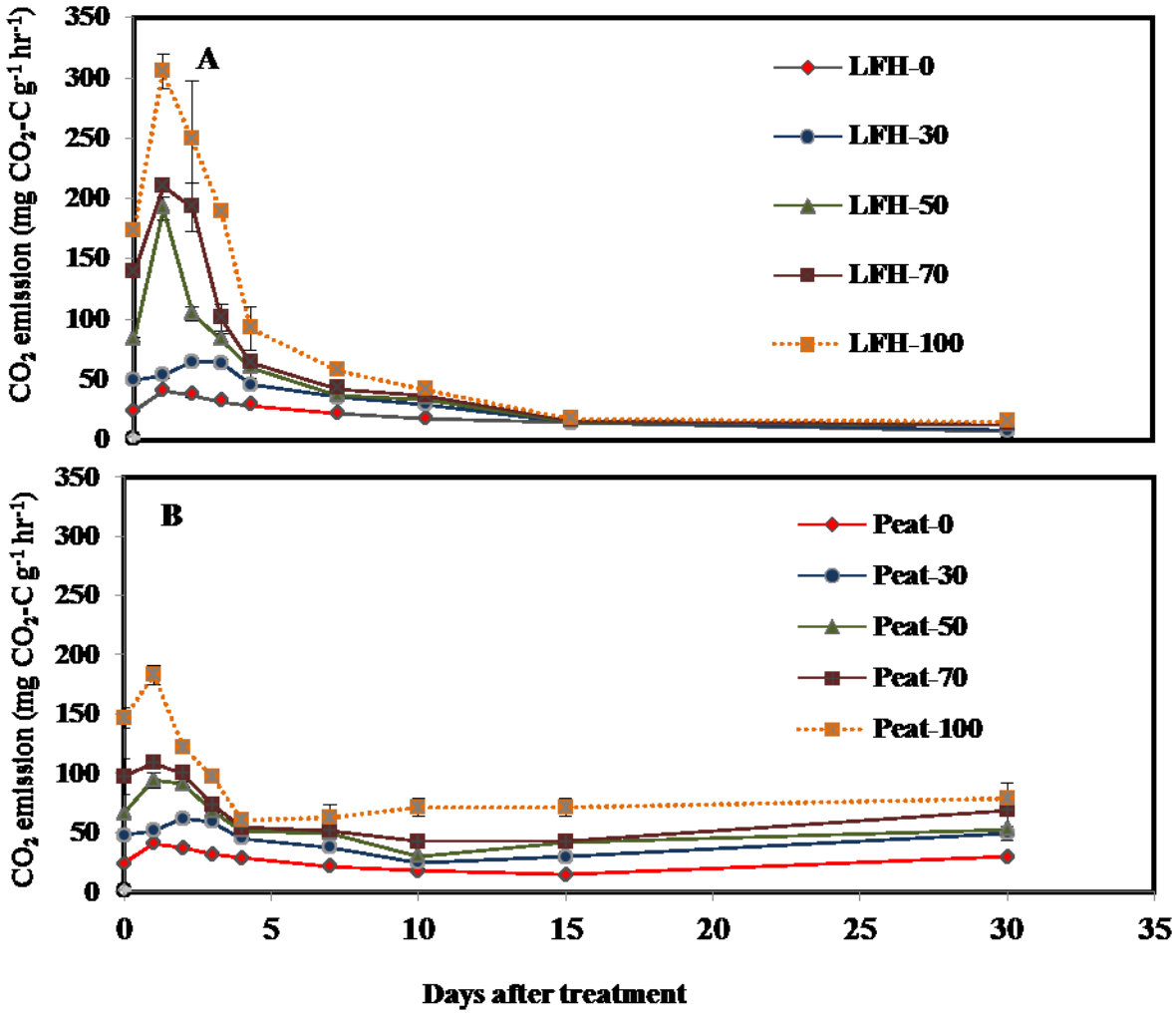

Figure 4-3 The CO_2 emission rate in LFH (A) and peat (B) affected by ratios of materials used in the experiment

Treatments: LFH-0= LFH:MS 0:100, LFH-30= LFH:MS 30:70, LFH-50= LFH:MS 50:50, LFH-70= LFH:MS 70:30, LFH-100= LFH:MS 100:0

Peat-0= Peat:MS 0:100, Peat-30= Peat:MS 30:70, Peat-50= Peat:MS 50:50, Peat-70= Peat:MS 70:30, Peat-100= Peat:MS 100:0

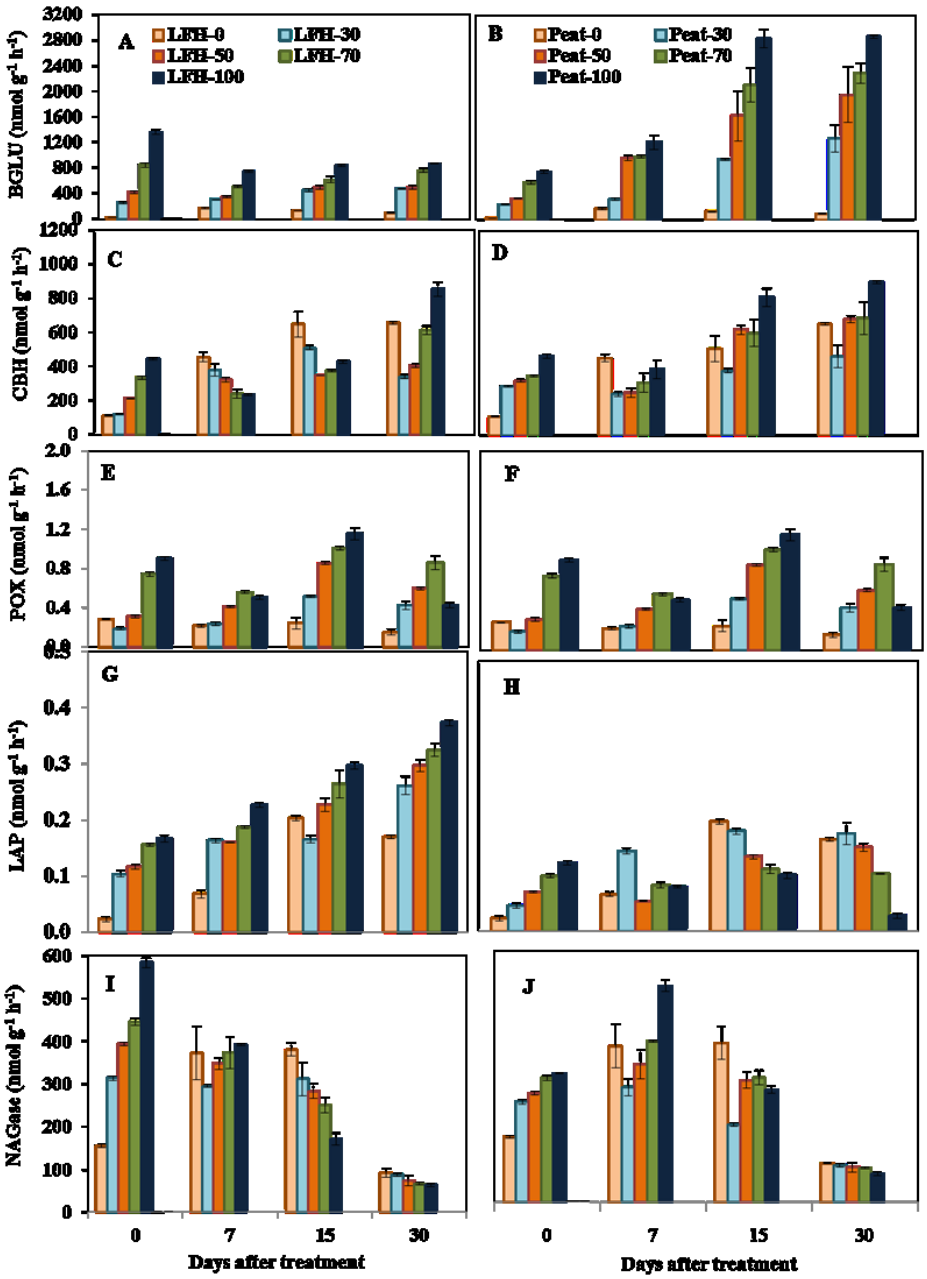

Figure 4-4 Changes in soil enzymes activities in LFH (A, C, E, G, I) and peat (B, D, F, H, J) as affected by organic to mineral soils ratio used in the experiment

Abbreviations: BGLU: β-glucosidase, CBH: cellobiohydrolase, POX: phenol oxidase, LAP: leucine aminopeptidase, NAGase: N- Acetyl glucoaminidase

Treatments: LFH-0= LFH:MS 0:100, LFH-30= LFH:MS 30:70, LFH-50= LFH:MS 50:50, LFH-70= LFH:MS 70:30, LFH-100= LFH:MS 100:0

Peat-0= Peat:MS 0:100, Peat-30= Peat:MS 30:70, Peat-50= Peat:MS 50:50, Peat-70= Peat:MS 70:30, Peat-100= Peat:MS 100:0

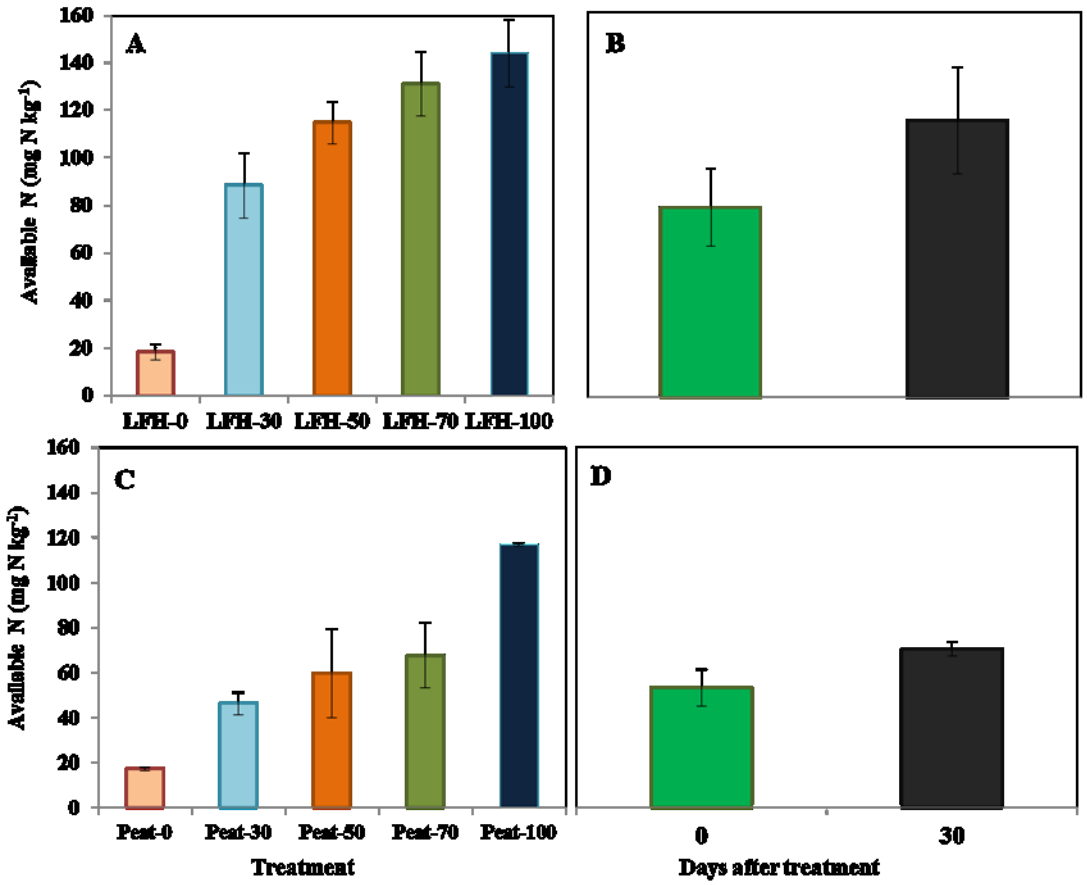

Figure 4-5 Changes in available N concentration as affected by the ratios of materials and days after treatment in LFH (A and B) and peat (C and D) materials used in the experiment

Treatments: LFH-0= LFH:MS 0:100, LFH-30= LFH:MS 30:70, LFH-50= LFH:MS 50:50, LFH-70= LFH:MS 70:30, LFH-100= LFH:MS 100:0

Peat-0= Peat:MS 0:100, Peat-30= Peat:MS 30:70, Peat-50= Peat:MS 50:50, Peat-70= Peat:MS 70:30, Peat-100= Peat:MS 100:0

CHAPTER 5 FINE ROOT DYNAMICS IN LODGEPOLE PINE AND WHITE SPRUCE STANDS ALONG PRODUCTIVITY GRADIENTS IN RECLAIMED OIL SANDS SITES

1. Introduction

Fine roots as part of the tree root system play an important role in resource (e.g., water and nutrients) capture (West et al., 2004). It is widely accepted that fine root production, turnover, and decomposition make a greater contribution to available soil nutrient pools than inputs from aboveground litter to the soil (Aerts et al., 1992). Fine root length density and root surface area are key root morphological features and have an important role to play in soil resource exploitation (Gilroy and Jones, 2000; Metcalfe et al., 2008). Fine root length density can be used to estimate the ability of roots to proliferate and to sequester nutrients, whereas root surface area can be used to estimate the stand absorptive potential for resources (Eissenstat et al., 2000). Thus, enhanced fine root growth can assist in nutrient retention and scavenging and mining resource for acquisition from the soil (Hinsinger et al., 2005; Lambers et al., 2008), and it would be particularly important in reclaimed ecosystems where availabilities of resources such as water and nutrients are often limiting (Jung et al., 2014; Boldt-Burisch et al., 2015; Duan et al., 2015). Alterations in fine root growth and architectural traits may reflect the availability of soil resources (Rosenvald et al., 2011) and stand characteristics (Jung and Chang, 2013). Thus, the proliferation of fine roots within a stand may serve as a useful indicator for assessing stand productivity in reclaimed ecosystems (Gilroy and Jones, 2000).

Surface-mining activities in the Athabasca oil sands region (AOSR) have disturbed about 750 km^2 of land, which accounts for about 0.2% of the mixed wood boreal forest ecosystem (Government of Alberta, 2014). Oil sands companies are legally bound to reclaim the disturbed land according to the Alberta Environmental Protection and Enhancement Act (Powter et al., 2012). Current oil sands reclamation practices predominantly involve the use of peat mineral soil mix (PMM) as an organic capping material over tailings sand or overburden substrates (Rowland et al. 2009). Inherent properties of these materials, such as the slow decomposition rate of PMM, means that it releases nutrients (e.g., nitrogen) slowly due to a wide carbon to nitrogen ratio (Jamro et al., 2014; Kwak et al., 2015b). The substrates below the capping materials may have high soluble salt concentrations, poor drainage, and heavy compaction, which could limit the growth of trees (Jung et al., 2014; Duan et al., 2015) and fine roots (Strand et al., 2008).

Processes associated with fine root dynamics such as fine root production and turnover are thought to be some of the main drivers of biogeochemical nutrient cycling and overall stand productivity in terrestrial ecosystems (Yuan and Chen, 2013; Gundale et al., 2014; Tripathi et al., 2014). These processes are responsive to soil environmental changes (Jagodzinski and Kałucka, 2010; Yuan and Chen, 2013) and reclamation practices (Lazorko and Van Rees, 2012; Jung et al., 2014). However, how reclamation practices influence fine root dynamics and their relationship with stand productivity is poorly understood in oil sands reclamation. In a recent study, Jung et al. (2014) reported that tailings sand and overburden substrates differ in pore size distribution, with tailings sand having more macropores with low water- and nutrient holding capacity and the overburden material having more micropores. These differences in pore structure in the soil may influence the distribution of resources through enhanced leaching of nutrients in tailings sand and decreased drainage in overburden material that may induce an anaerobic environment and limit nutrient transformation rates and their availability (Brady and Weil, 2008), affecting fine root growth. The overburden material can be severely compacted, which could lead to increased bulk density and decreased root growth (Jung et al., 2014). Overburden material can be saline sodic, containing sodium, sulfate, and chloride ions (Lazorko and Van Rees, 2012), which can cause imbalances in water and nutrient availabilities (Munns and Tester, 2008). Soil salinity could alter the morphology of fine roots (Lazorko and Van Rees, 2012) and reduce fine root biomass (Jung et al., 2014). Therefore, the life span (Self et al., 1995), turnover rate and decomposition of fine roots may be affected (Zhang et al., 2009).

The inherent soil properties of these substrates such as high salinity, compaction, nutrient, and water limitations are of concern regarding the sustainability of current oil sands reclamation practices because these soil factors affect the productivity of trees grown on reclaimed soils (Lilles et al., 2012; Duan et al., 2015; Pinno and Hawkes, 2015). In a recent study, Duan et al. (2015) found that the differences in stand productivity in oil sands reclamation were associated with differences in soil electrical conductivity (EC) and bulk density in overburden sites and volumetric water content in tailings sand sites. Electrical conductivity and bulk density were greater in low than in medium and high productivity sites planted to white spruce (*Picea glauca* (Moench.) Voss) on an overburden substrate, while volumetric water content was greater in high and medium than in low productivity sites planted to lodgepole pine (*Pinus contorta* Dougl) on a tailings sand substrate. Thus, fine root growth and their dynamics would likely be greater in high

than in medium and low productivity sites. Although a few studies on fine root distribution of boreal forest species in oil sands reclamation have been conducted (Lazorko and Van Rees, 2012; Jung et al., 2014), no one has assessed whether fine root dynamics might be related to stand productivity in oil sands reclamation.

Understanding fine root dynamics is important for improving current reclamation practices for establishing functional forest ecosystems in the oil sands (Yan et al., 2012; Jung and Chang, 2013; Jung et al., 2014). The objective of this study was to evaluate the relationship between fine root dynamics, including processes such as biomass production, turnover, decomposition, and morphological characteristics such as fine root surface area, root length density, and stand productivity in reclaimed oil sands soils. We hypothesized that fine root growth would have a positive relationship with aboveground tree growth along productivity gradients of different tree species planted in reclaimed oil sands sites. It was assumed that the differences in fine root dynamics would reflect the differences in inherited characteristics of tailings sand and overburden materials used for oil sands reclamation.

2. Materials and Methods

2.1 Site description and research plots

This study was conducted on an oil sands lease area reclaimed after open-pit mining, located approximately 24 km north of Fort McMurray in the AOSR. The area has a continental boreal climate where winters are long and cold and summers are short and warm. The long term mean annual temperature was 1.0 °C from 1981 to 2010 with a daily average temperature from 17.4 °C in January to 17.1 °C in July. Mean annual precipitation was 418.6 mm, which mostly falls as rain (316.3 mm) during summer (Environment Canada, 2015). The mean temperature was 16.7 °C and 17.3 °C in 2011 and 2012 during the study. The total precipitation was 87 mm in 2011 and 280 mm in 2012 (data not shown), indicating a dry year in 2011. The study sites were reclaimed with PMM as the organic capping material over the tailings sand or the overburden substrates. These sites were reclaimed at different times between 1984 and 1996. The selected sites had PMM depth ranging from 11 to 48 cm (Table 5-1). Lodgepole pine was planted on PMM over a tailings sand substrate and white spruce was planted on PMM over overburden substrate.

The main understory plant species on lodgepole pine sites were prickly rose (*Rosa acicularis* Lindl), raspberry (*Rubus idaeus* L.), sweet clover (*Melilotus* spp.), dandelion (*Taraxacum officinale* L.), and slender wheat grass (*Agropyron trachycaulum* Link Malte). The understory vegetation in white spruce stands was dominated by willow (*Salix* spp.), green alder (*Alnus crispa* (Ait.) Pursh), sweet clover, dandelion, and bluejoint grass (*Calamagrostis canadensis* (Michx) Beau.) (Jung et al., 2014). Some key site characteristics are summarized in Table 5-1. A total of 12 sites were set up with six of each species (lodgepole pine and white spruce) encompassing three productivity levels (low, medium, and high). Initially, stands representing different productivity levels were identified based on visual inspection of tree performance and productivity of each stand was confirmed by tree height (height pole) and diameter at breast height (diameter tape) measurements that were used to calculate mean annual growth over time (Duan et al., 2015). Each productivity level was replicated twice for each species (Table 5-1) due to less availability of sites. For this study, 10 × 10 m plots were set up randomly at each site in June 2011. Tree age, stand density, and the degree of soil penetration were different at each site (Table 5-1). Tree age was 15-20 years on lodgepole pine sites and 15-29 years on white spruce sites. The stand density was 1500-2700 and 1900-3100 stems per hectare in pine and spruce sites, respectively. Soil compaction p as measured by penetration resistance was 255-620 kpa in pine sites and 1517-2137 kpa in spruce sites (Table 5-1).

2.2 Field and laboratory methods

Soil penetration resistance was evaluated to a depth of 45 cm at three intervals (0-15, 15-30, and 30-45 cm) at five randomly selected locations in each plot using a soil penetrometer (Spectrum Technologies Inc., Aurora, IL) with a base tip of 1.27 cm diameter. Thermocouple temperature probes and CS616 time domain reflectometry (TDR) probes were installed at the depth of 10 cm below the soil surface in PMM and 10 cm below the PMM substrate interface, respectively, in each plot for the measurements of soil temperature and water content. These measurements were made hourly and datalogged. Soil was sampled at 0-20 cm using a soil auger in five randomly selected locations in each plot and mixed to form a composite sample. The composited samples were stored in plastic bags and transported to the laboratory for analyses. Soil samples were sieved (2 mm), then homogenized manually after discarding the coarse fragments and roots, and used for analyses. Soil gravimetric water content was measured after

oven drying a subsample at 105 °C for 24 h. Soil pH and EC were evaluated using a pH meter and an EC meter with a 1:2 (m:v) soil to water ratio (Kalra and Maynard, 1991). Ammonium and nitrate concentrations were analyzed using the indophenol blue method (Keeney and Nelson, 1982) and the vanadium oxidation method (Miranda et al., 2001), respectively, after extracting a subsample with 2 mol L^{-1} KCl solution (Mulvaney, 1996). Total carbon and total nitrogen concentrations were analyzed by dry combustion with a Carlo Erba NA 1500 automated elemental analyzer (Carlo Erba Instruments, Millan, Italy).

2.3 Fine root sampling and measurements

2.3.1 Fine root sampling

Both sequential and ingrowth core methods were used for root sampling (Vogt and Persson, 1991). For the sequential soil core method, five soil samples were collected from randomly selected locations in each plot each month from June to September in 2011 and 2012. The sampling period was selected to measure the differences of fine root parameters during the growing season. The soil cores were collected at 0–30 cm in each plot using a steel corer (6.6 cm inner diameter). Samples were placed in plastic bags and transported to the laboratory in cooler containing ice packs. For the ingrowth method, root ingrowth cores (30 cm long, 6.6 cm inner diameter) were constructed from plastic mesh (Quick Count plastic canvas, Uniek, Inc., Waunakee, WI) with an opening size of 1.5 × 1.5 mm. As most fine roots are located in the upper 30 cm (Yuan and Chen, 2010), our root measurement was conducted for that depth. Twelve ingrowth cores were randomly installed to the 30 cm depth in each plot in July 2011. Before installing the cores, soil cores to 30 cm were taken using a steel corer. After removing roots from soils manually by sieving, the root-free soils were placed back in the hole after an ingrowth core was inserted. Four ingrowth cores in each plot were retrieved in October 2011 and May and July 2012. Roots that penetrated through the ingrowth core and exposed were trimmed. The ingrowth core samples were placed in plastic bags and transported to the laboratory in a cooler containing ice packs.

2.3.2 Washing, sorting, and characterization of fine roots

Roots in sequential soil cores were separated from soils by washing them with tap water. Samples were soaked overnight, poured into trays, and rubbed gently. Roots floating on top the

water were collected by pouring water into a sieve (0.5 mm mesh) (Yuan and Chen, 2013). The procedure was repeated until only rocks and organic debris (which floated more readily than root fragments) were left in soils. Roots in the sieve were poured in a plastic container and dispersed in water for further manual separation of roots from the organic material and washed again. After scanning, three root samples from sequential cores and two from ingrowth cores were oven-dried at 70°C to constant weight and weighed. The sum of all root cores collected by sequential coring at each root sampling time in 2011 and 2012 was used for the measurement of total fine root biomass. Fine root biomass (kg ha^{-1}) was calculated according to McClaugherty et al. (1982) as dry mass of living roots (gram) × 10^{-3} ×10^{8} /area of the core (area of the core: $\pi d^2/4$, d = 6.6 cm)

2.3.3 Fine root production

Fine root production was calculated for sequential and ingrowth core samples. Fine root production in ingrowth cores was estimated using fine root mass (sum of live and dead roots, as some roots died in the ingrowth cores in the incubation period and it was necessarily to combine them for true representation of fine root production) divided by the period of growth on a yearly basis (Vogt et al., 1998). In sequential cores, it was calculated by summing the root increments in sampling intervals in 2011 and 2012 (Fairley and Alexander, 1985).

2.3.4 Fine root decomposition and turnover rates

Fine root decomposition rate was determined by the mesh bag technique (Mcclaugherty et al., 1984). A mesh bag, 10 × 20 cm in size, was made of fiber glass mesh with mesh size of 0.3 × 0.3 mm. Root samples collected from the top 30 cm of surface soil from the established plots of each site in June 2011 were used after being washed, dried at 65°C for 48 h, and cut into 2-5 cm lengths. Twelve mesh bags with 0.5 g of root materials were placed with ingrowth cores, at a depth of 20 cm with a slit in the soil cut to 45° to ensure good contact of the litter bag with the soil. Four bags from each plot were retrieved in October 2011 and May and July 2012. Residual root materials were carefully removed from the bags, washed in a sieve by pouring water slowly to remove adhered soil particles after brushing the mesh bag outside (if there were soil particles adhering to the litter bag), dried, and weighed. The weight loss upon drying was measured in each sample. Data of sampled mesh bags at each sampling time were pooled to represent a plot average. Decomposition rate constant (k) for each sample at each sampling time was calculated

using the following exponential model based on the relationship between root mass remaining and incubation time of the mesh bag (Wieder and Lang, 1982): M_t/M_0 ¼ e^{-kt} where M_0 is the initial dry mass, M_t is the dry mass remaining at time t, and k is the decay constant and is expressed in years. Mean residence time was calculated using the equation (Giardina and Ryan, 2000): MRT= ¼ 1/k. Turnover rates of each sample were calculated using the formula (Yang et al., 2004): Turnover rate ($year^{-1}$) = annual fine root production (kg ha^{-1} $year^{-1}$) / mean fine root biomass (kg ha^{-1}).

2.4 Statistical analyses

Repeated measures analysis of variance (ANOVA) was performed to determine whether time of sampling and stand productivity affected total fine root length density, mean root surface area, total fine root biomass, and root decomposition rate in each tree species using the PROC MIXED model. Sampling time was used as a repeated measures variable. One-way ANOVA was performed to evaluate the effect of stand productivity level on fine root production and turnover rate separately for each method of root sampling. Only two replications of each productivity level were used due to limitations of site availability for each productivity gradient. Means were separated in both repeated measures and one-way ANOVAs using Tukey's honestly significant difference test. Pearson correlation analysis was performed to examine the relationship between measured root parameters and soil properties (soil compaction, pH, EC, available N, volumetric water content, and soil temperature) for each species separately. Linear regression was conducted to determine the relationship between fine root dynamics (total fine root productivity, total turnover rate, and total fine root length density) and tree size (height and DBH) for each species. An α value of 0.05 was used to indicate significant differences in all analyses. Assumptions of normality and homogeneity of variance were tested with a Shapiro–Wilk test and Bartlett test when performing the ANOVA. All data were normally distributed except fine root production and turnover rate measured by the ingrowth core method, and data were log-transformed to meet the assumption of normality and homogeneity. All statistical analyses were performed using version 9.2 of SAS (SAS Institute Inc., Cary, NC).

3. Results

3.1 Mean root surface area, total fine root length density, and total fine root biomass

Mean monthly root surface area (m^2 m^{-2}) in 2011 ranged from 0.53 (June) to 2.63 (August) and in 2012 ranged from 0.68 (June) to 3.04 (July), respectively, in lodgepole pine stands. Further, mean monthly root surface area across white spruce productivity gradient ranged from 0.52 (September) and 2.02 (June) in 2011 and from 0.65 (September) to 2.34 (July) in 2012, respectively, in white spruce stands, across the productivity gradient (Table 5-2). It was not affected by productivity level in pine stands in 2011 ($p = 0.27$) and 2012 ($p = 0.29$) although, in spruce stands, it was significantly influenced by productivity level and time of sampling (Table 5-2, Appendix A). Mean surface area was consistently greater in medium and high than in low productivity sites in both 2011 and 2012. Mean surface area decreased from June to September in both medium (70%) and high (33%) productivity levels but not in the low productivity level in both 2011 and 2012 based on data in Table 5-2.

Fine root length density was significantly influenced by productivity level and sampling time) in pine stands (Table 5-2, Appendix A). It was higher in the high than in the low productivity sites in the first sampling in both 2011 and 2012. Fine root length density averaged over the 2 years varied by 2, 53, and 67% from low to high productivity level. It was not affected by productivity level in both 2011 ($p = 0.60$) and 2012 ($p = 0.78$) but was affected by time of sampling ($p < 0.05$) in spruce stands in both 2011 and 2012. In both pine and spruce stands, mean fine root length density decreased by approximately 50% from June to September regardless of the productivity level except for the low productivity pine stand (Table 5-2).

The mean fine root biomass was not affected by productivity level for both species (Table 5-2, Appendix A) in both 2011 and 2012, except in the July 2011 sampling in the white spruce stands. In general, in 2011, the lowest seasonal mean value (160 kg ha^{-1}) was found in the medium productivity level and the highest seasonal value (253 kg ha^{-1}) was found in the high productivity level in pine stands. In spruce stands, the lowest seasonal mean fine root biomass (125 kg ha^{-1}) was found in the low productivity level and the highest seasonal value (144 kg ha^{-1}) was found in the high productivity level. Mean fine root biomass was greater in 2012 than in 2011 in all three productivity levels of both species, except in the high productivity pine sites. From 2011 to 2012, mean fine root biomass varied by 6, 15, and 1.3% in low, medium and high productivity levels, respectively, in pine stands, and 14, 29, and 19%, respectively, in spruce stands. Mean fine root biomass in pine trees was significantly different among sampling months (Table 5-2, Appendix A). The highest mean value was found in June (289 kg ha^{-1}), and the

lowest mean value was found in September (109 kg ha^{-1}) across the productivity gradient and years in lodgepole pine stands. There were no significant differences among sampling times along the productivity gradient in spruce.

3.2 Fine root decomposition

The fine root decomposition rate, quantified as the percent mass remaining, was not affected by productivity level but was affected by incubation time for both pine and spruce stands (Table 5-3). The average fine root mass remaining ranged from 36% to 87% in pine stands and from 41 to 86% in spruce stands across the incubation periods (data not shown). Percent total fine root mass loss was not altered by the productivity level in both pine ($p = 0.33$) and spruce ($p = 0.30$) stands. The k values were not different between species along the productivity gradient. Mean k values were in the order (as a pattern, $p > 0.05$) of low < medium < high in pine stands and low > medium > high in spruce stands, with the mean residence time followed an opposite trend to that of k values for both tree species (Table 5-4)

3.3 Total fine root production and turnover rates

Total fine root production measured using the sequential core method was 1004 to 2704 and 225 to 2676 kg ha^{-1} $year^{-1}$ in pine and spruce stands, respectively, along the productivity gradients (Figure 5-1). It increased in pine stands with stand productivity ($p < 0.05$) when measured by the sequential coring method, while was not affected by stand productivity in spruce stands (Figure 5-1). Total fine root production was not different among the productivity levels in both pine ($p = 0.45$) and spruce stands ($p = 0.37$) when measured by the ingrowth core method. Turnover rate measured with the sequential core method increased ($p < 0.05$) with increasing stand productivity in pine stands (Figure 5-1), but not in spruce stands ($p = 0.33$). Turnover rate calculated using ingrowth cores did not change with stand productivity. Estimates of turnover rates in pine and spruce stands ranged from 0.04 to 0.32 and 0.02 to 0.16 $year^{-1}$, respectively, across the measurement methods and productivity levels (Figure 5-1). In general, the values were greater with the sequential coring than with the ingrowth core method.

3.4 Soil, fine root, and tree performance parameters

For pine stands, significant relationships were observed between available nitrogen and fine root decomposition rate, but not between available nitrogen and other fine root properties

such as fine root biomass, fine root surface area, fine root production, or turnover rates (Table 5-5). Fine root production and turnover rates were correlated with each other (Table 5-5). In spruce stands, most variables of fine root properties were negatively correlated with soil penetration resistance. Nitrogen availability was also negatively correlated with soil penetration resistance. Total fine root biomass was positively correlated with soil pH (Table -5)

3.5 Fine root dynamics and their relationship with tree performance

Linear regression analysis indicated that total fine root length density, fine root production, and turnover rates linearly increased with tree height and DBH in pine stands. In spruce stands, there was no significant relationship between total fine root length density, fine root production, and turnover rates and any of the tree performance parameters (Table 5-6).

4. Discussion

4.1 Fine root production and turnover rates

Increased fine root production and turnover rate with increased stand productivity in the pine stands (Figure 5-1) is consistent with Yuan and Chen (2013) and may be explained by changes in resource availability along the productivity gradient. According to the optimality theory (Espeleta and Donovan, 2002), trees growing in a nutrient and water-limited environment would be expected to maximize their nutrient uptake by increasing their fine root productivity and turnover rate. This has been demonstrated in previous studies that focused on water and nutrient limitations in reclaimed sites with tailings sand as a substrate (Naeth et al., 2011; Jung et al., 2014; Luna Wolter and Naeth, 2014). Fine root growth is typically influenced by available nutrients in forest ecosystems (Ingestad and Agren, 1991) and given the significant response for fine root biomass and turnover rate across the productivity gradient that is likely the case in our study. However, fine root growth in pine stands in our study was not significantly correlated with nitrogen availability (Table 5-5), suggesting there could be other factors such as water availability or salinity that affect fine root growth in PMM capping material over tailings sand (Duan et al., 2015). Duan et al. (2015) showed that water availability increased along the productivity gradient and subsequently improved tree performance. However, it is difficult to conclude the increase in fine root production and turnover rates along the productivity gradient in pine sites was associated with water availability as such relationships were not determined.

The negative relationship ($p < 0.05$) between fine root productivity and turnover rate and EC in pine sites (Table 5-5) indicates that higher EC reduced fine root productivity and turnover rate in some of the pine sites. Fine root production in spruce sites might also be affected by the EC of overburden material as indicated in a previous study on the same study site showing EC was also a problem for the growth of spruce trees planted on an overburden substrate (Duan et al., 2015). However, in this study, fine root productivity in spruce sites was not related to EC (Table 5-5). In general, the increase in EC may impose two stresses on plant growth (Fageria et al., 2010): (1) the increased EC may increase the osmotic potential in the rhizosphere, which may cause water stress and subsequently affect plant growth (Munns and Tester, 2008); and (2) toxic effects of high concentration of ions associated with high salinity may cause nutrient imbalance and affect plant growth (Grattan and Grieve, 1992), including root growth. Lilles et al. (2012) suggested that reduction in root growth on sites with high salinity at depth may affect long-term productivity of established forests. Khasa et al. (2002) found that lodgepole pine seedlings had lower survival at high salt concentrations. High EC levels can also reduce microbial activity and nitrogen mineralization (Pathak and Rao, 1998), which can lead to reduced turnover rate.

The lack of relationship between fine root productivity and turnover rate with available nitrogen may be linked to soil compaction in overburden substrate into which spruce was planted. Soil compaction can reduce microbial activity and nitrogen mineralization (Tan et al., 2005), which in turn can restrict fine root growth and overall tree performance (Kozlowski, 1999). Fine root production and turnover rates in this study are on the lower end of the reported range (Yuan and Chen, 2013). The sequential core method may underestimate fine root production rates relative to the ingrowth core method (Makkonen and Helmisaari, 1999; Yuan and Chen, 2013) and are affected by length of sampling interval and loss of roots between two sampling periods (Finer and Laine, 1998). The ingrowth core method takes a minimum of 12 months for roots to recolonize cores in temperate forests (Hendricks et al., 2006). It may take longer to recolonize cores in colder and more resource limited environments. More advanced methods such as the minirhizotron should be considered for root measurements in the future.

4.2 Fine root decomposition

Our species-specific differences in fine root decomposition could be due to differences in the degree of mycorrhizal colonization (Koide et al., 2011) as both pine and spruce are

mycorrhizal species (Comeau and Kimmins, 1989; Ostonen et al., 2011), chemical composition of roots (Chen et al., 2002) and differences in substrates used in oil sands reclamation. Pine is an early successional coniferous species and has a faster rate of colonization by mycorrhizae (Shishido et al., 1996). Mycorrhizal colonization can result in increased rate of root decomposition, as colonization of roots by mycorrhizae can stimulate nitrogen content of root tissues which may lead to frequent grazing by soil herbivores and result in faster fine root decomposition (Koide et al., 2011). Spruce is a late successional species and mycorrhizae have high persistence in its root system in oil sands reclamation (Onwuchekwa et al., 2014), which can lower fine root decomposition rates in spruce. The longer mean residence time in fine roots of spruce trees may be due to increased recalcitrance of carbon compounds (Lin et al., 2011), such as lignin, in root tissues. Spruce fine roots have lower water soluble extractives (3% on a dry weight basis), greater water-soluble phenols (1%), and lignin (0.3%) concentrations than pine fine roots (Chen et al., 2002) which might also lower the decomposition rate of spruce fine roots. Slower decomposition of spruce fine roots may be due to stabilization of soil aggregates by overburden compaction (Hertel et al., 2009).

Highly compacted overburden substrates lack aeration, results in reduced microbial activity and lower nutrient availability (Lazorko and Van Rees, 2012) and can therefore affect fine root decomposition. These results corroborate with the negative relationship between soil compaction and decomposition rate of fine roots of spruce (Table 5-5). High temperatures in the tailings sand substrate could increase fine root decomposition rate in pine stands (Naeth et al., 2011) as indicated by the positive relationships between soil temperature and fine root mass loss in this study (Table 5-5). The change in soil temperature may affect microbial activity and the decomposition of fine roots (Priha et al., 2001). Soil textural differences may also alter soil thermal conductivity and heat capacity (Hillel, 2005). Sandy materials have higher thermal conductivity and lower heat capacity than clayey materials (Hillel, 2005). In general, soils with low heat capacity (tailings sand) could have a higher temperature relative to soils with a high heat capacity (overburden). These differences in temperature could affect fine root decomposition in tailings sand. In a concurrent study, House (2015) showed that tailings sand had higher soil temperature than overburden during 2011 and 2012. Therefore, the greater decomposition rate of pine than spruce roots was most likely related to the higher temperature in tailings sand. In this study, we used the mesh bag technique which may underestimate the *k* value

(Chen et al., 2002) and might have not differed with productivity levels within pine and spruce stands. Large soil organisms such as soil vertebrates and invertebrates cannot enter the mesh bag (Mcclaugherty et al., 1984) and the disturbance of rhizosphere microorganisms such as bacteria, fungi, and their associations such as mycorrhizal colonization during root sample preparation while putting them in the mesh bag may also delay fine root decomposition as they need time to re-establish in the soil (Bradbury et al., 1998)

4.3 Total fine root length density and mean root surface area

The different patterns of total fine root length density and mean root surface area for the two tree species indicated varied resource exploitation abilities over the productivity levels (Table 5-2). The greater increase in total fine root length density among pine than spruce stands at the same productivity level suggests that pine roots may proliferate more quickly in reclaimed soils than spruce roots, although results are likely associated with the physical environment of substrates (water limitation) used in oil sands reclamation (Jung et al., 2014). Tailings sand is typically characterized by a lack of micropores that prevent capillary rise of water to the PMM capping layer (Li et al., 2013). The tailings sand typically has a low waterholding capacity (Khasa et al., 2005) and may cause reduction of fine root growth below the interface layer. The characteristically greater total fine root length density in pine stands may therefore confirm that lodgepole pine trees could grow in a water-stressed environment, in a reclaimed ecosystem (Jagodzinski and Kałucka, 2010) such as in a tailings sand substrate. This could be associated with the mycorrhizal colonization capacity of pine trees (Kranabetter et al., 2006), which is known as an efficient way to exploit the nutrient and water resources (Jagodzinski and Kałucka, 2010). Mycorrhizal colonization alters the root length and root architecture (Eissenstat et al., 200) and increases belowground absorptive surface area for coping with water limitations (Metcalfe et al., 2008). These changes in root architecture and production would have ecological implications for plant establishment, survival and productivity in water-limiting conditions (Auge, 2001). The differences in mean root surface area between species are likely associated with differences in soil compaction and salinity of the overburden material as shown with the negative relationships of root surface area and soil compaction in spruce. Highly compacted overburden materials do not facilitate root penetration and reduce the plant's capability to extract the large soil volume for resources such as was characteristic of the spruce stands below the

capping material (Jung et al., 2014). Under these conditions, resource limitations may be compensated by increasing root surface area (Rewald et al., 2011). However, such a compensating mechanism would be an inefficient way to increase resource availability in the zone where resources are depleted (Jagodzinski and Kałucka, 2010).

4.4 Total fine root biomass

Different patterns in total fine root biomass further show clear species-specific differences in fine root dynamics between pine and spruce in each productivity level, except the medium productivity pine sites. These results are associated with the fine root biomass being negatively correlated with the fertility of soils in boreal forests (Helmisaari et al., 2002; Yuan and Chen, 2010). Seasonal and interannual variations in fine root biomass in pine were likely associated with changes in soil water and temperature, consistent with the findings of Comeau and Kimmins (1989). In general, fine root biomass peaked in mid- to late summer and was lowest in the fall. This coincides with the reduced demand for resources in the late summer and the fall when leaf senescence will begin (Brassard et al., 2009). The greater fine root biomass in June than in the later months of the year suggests that there would be growth of fine roots in earlier months such as May, when the soil temperature starts to increase. In general, fine root growth of the woody species exponentially increases with the increasing soil temperature (Pregitzer et al., 2000; Steinaker et al., 2010).

The positive relationship between temperature and fine root biomass (Table 5-5) in this study suggests that most of the root growth occurred in the growing season (June-September) in the oil sands, as the soil temperature would be very low outside of that period to allow substantial fine root production. The increase in soil pH appeared to be strongly related to the increase of fine root biomass in both of the tree species due to their positive relationship (Table 5-5). Yuan and Chen (2010) found fine root biomass increased with increased soil pH but that it decreased with soil nutrient availability. This might be due to the inhibited microbial activity under low pH, while at higher pH, the microorganisms can compete with the fine roots for nitrogen (Brunner et al., 2002). Thus, the fine roots may proliferate with increasing nitrogen availability and subsequently the fine root biomass increased with pH. Hahn and Marschner (1998) found increased root growth with lime application was associated with improved nutrient supply and biological activity.

4.5 Contrasting relationships between lodgepole pine and white spruce

The stronger relationships of fine root properties with stand productivity in pine than in spruce are likely associated with interspecies differences in rooting habits, growth potentials, tolerance to environmental stresses, and site-specific differences in substrate materials (Jung et al., 2014; Duan et al., 2015). Lodgepole pine has a potential to thrive in extreme environmental conditions (Stuart et al., 1989) and site types (Pinno et al., 2012). Pine species are less sensitive to compaction (Bulmer and Simpson, 2005) and more tolerant to nutrient deficiencies (Bothwell et al., 2001; Jung et al., 2014), salinity (Khasa et al., 2005), and water limitation. This is likely associated with the pine having a taproot system with vertical sinkers on well-drained sites. Its roots can go deeper in the soil for extracting resources (Kranabetter et al., 2006) increasing root growth and tree productivity (Ostonen et al., 2011). Pine has a greater nutrient retranslocation efficiency and maintains adequate nitrogen status in resource-limited soils during maturity stages (Miller, 1995) than spruce; those might have contributed to increased tree growth in reclaimed oil sands soils and maintaining the relationship between aboveground and belowground pine tree performance in this study.

The lack of relationships between fine root properties and stand productivity in spruce is likely associated with its slow rate of growth (Khasa et al., 2002), shallow rooting habit (Burns and Honkala, 1990), sensitivity to salinity (Renault et al., 1998), compaction (Bothwell et al., 2001), and nutrient deficiency (Duan et al., 2015). Staples and Van Rees (2001) indicated that spruce growth could be affected at EC levels > 0.5 dS m^{-1}. The upward movement of salts through diffusion from capping materials such as a sodic overburden material, if the capping layer is thin (Kessler et al., 2010), would increase the EC level and affect root growth (Duan et al., 2015). This is consistent with previous findings that the reduction of height in spruce was linked to reduced spruce root growth in saline soils (Lilles et al., 2012). The morphology and structure of spruce roots varies with nitrogen availability (Krasowski and Owens, 1999) and soil chemical properties (Bredemeir et al., 1995). Site difference is also important in affecting tree growth and relationships between fine root properties and tree growth. The reader is cautioned that the differences between pine and spruce sites were confounded between species and site differences. Therefore, when interpreting differences between the stand types, both species and site differences need to be considered.

5. Conclusions

The contrasting relationships of fine root properties with stand productivity in pine and spruce stands demonstrate the need for species- and site-specific management in oil sands reclamation. The selection of tree species and substrate material could affect the success of oil sands reclamation. This study showed that most fine root parameters systematically changed along the productivity gradient in pine stands but not in spruce stands. The species-specific differences in fine root dynamics were likely due to differences in the properties of tailings sand and overburden substrates. Fine root dynamics were strongly linked to EC in lodgepole pine stands, but were more affected by soil compaction in white spruce stands. The negative relationships of EC and soil compaction with nitrogen availability indirectly influenced fine root dynamics of both stand types. Thus, the effects of EC and compaction on reduction in resource acquisition and their relationships to fine root dynamics in reclaimed oil sands sites should be evaluated in more sites in the future. Fine root dynamics of both species should be measured under similar conditions with either PMM over tailings sand or PMM over overburden, for selection of suitable substrate and tree species for sustainable ecosystem development after reclamation in the oil sands region.

Table 5-1 Characteristics of studied reclaimed oil sands sites as determined in 2011 in the Athabasca oil sands region, Alberta, Canada

Site no.	Location		Year[a]	Substrate[b]	Tree species	Productivity gradient	Amend-ment Depth (cm)	Total C (g kg^{-1})	Soil penetration resistance (kpa)[c]	EC (dSm^{-1}) [c]	Stand density (stem ha^{-1})
	Latitude	Longitude									
1	N 56°59'02"	W 111°27'04"	1996	Tailings sand	Lodgepole pine	Low	17	67	448	3.75	1500
2	N 56°58'38"	W 111°27"39"	1991	Tailings sand	Lodgepole pine	Low	14	65	620	0.90	2300
3	N 56°59'30"	W 111°27'15"	1996	Tailings sand	Lodgepole pine	Medium	14	50	517	0.60	1700
4	N 56°58'55"	W 111°29'58"	1992	Tailings sand	Lodgepole pine	Medium	30	79	483	0.63	2700
5	N 56°58'42"	W 111°27'51"	1991	Tailings sand	Lodgepole pine	High	18	62	276	0.39	2300
6	N 56°59'47"	W 111°28'14"	1991	Tailings sand	Lodgepole pine	High	24	160	255	0.54	2100
7	N 56°58'54"	W 111°31'04"	1992	Overburden	White spruce	Low	12	15	2137	4.71	2000
8	N 56°58'45"	W 111°27'24"	1991	Overburden	White spruce	Low	22	85	2137	5.04	2300
9	N 56°59'25"	W 111°27'04"	1996	Overburden	White spruce	Medium	30	51	1724	2.71	3100
10	N 56°59'09"	W 111°32'08"	1982	Overburden	White spruce	Medium	20	49	1792	1.01	2800
11	N 56°59'24"	W 111°32'09"	1991	Overburden	White spruce	High	11	45	1655	0.76	1900
12	N 56°59'51"	W 111°32'40"	1991	Overburden	White spruce	High	27	48	1517	0.80	2600

[a] Year indicates the year the trees were planted after soil reconstruction

[b] Substrate below the organic capping material

[c] Average of three soil depths (0-15, 15-30 and 30-45 cm)

Table 5-2 Fine root paramters of lodgepole pine and white spruce stands along stand productivity gradients in oil sands reclamation at different time of samplings

Root parameter	Stand productivity	Lodgepole pine				White spruce			
		Jun.	Jul.	Aug.	Sept.	Jun.	Jul.	Aug.	Sept.
Root surface area (m^2 m^{-3})	Low	0.002A	0.003 A	0.007A	0.006A	0.003C	0.004C	0.003A	0.004A
	Medium	0.005A	0.009A	0.006A	0.005A	0.006A	0.007A	0.003 A	0.002BC
	High	0.007A	0.007A	0.005A	0.004A	0.005AB	0.006AB	0.002AB	0.003AB
Fine root length density (m m^{-3})	Low	180B	224B	207B	184A	318A	362A	185B	172B
	Medium	403A	476AB	322AB	188A	397A	436A	189B	138B
	High	571A	620A	273AB	180A	360A	413A	165B	161B
Fine root biomass (Mg ha^{-1})	Low	94A	74A	47A	40A	26A	50A	40A	21A
	Medium	65A	62A	23A	16A	45A	53A	39A	24A
	High	78A	83A	68A	30A	80A	36A	33A	16A

*Means with different capital letters indicate significant differences between stand productivity levels for each root parameter in each column

Table 5-3 Percent mass of fine roots of lodgepole pine and white spruce remaining after each incubation period along stand productivity gradients in oil sands reclamation

	Lodgepole pine			White spruce		
Stand productivity level	Oct. 2011	May. 2012	Jul. 2012	Oct. 2011	May. 2012	Jul. 2012
Low	95 A*	76 A	46 A	86 A	67 A	39 A
Medium	80 A	42 A	32 A	94 A	76 A	51 A
High	87 A	46 A	32 A	79 A	79 A	32 A
Repeated measures ANOVA						
	F value	*p* value		F value	*p* value	
Stand productivity	5.32	0.103		0.43	0.686	
Time of incubation	222.7	<0.001		71.46	<0.001	
Stand productivity * time of incubation	5.04	0.060		0.41	0.799	

*Means with same capital letters indicate non- significant differences between stand productivity in each column

Table 5-4 Percent total fine root mass loss, decomposition rate (*k*-value in years) and mean residence time in years in of lodgepole pine and white spruce stands along a stand productivity gradient in oil sands reclamation

Stand productivity	Lodgepole pine			White spruce		
	Total fine root mass loss	k-value	Mean residence time	Total fine root mass loss	k-value	Mean residence time
Low	54 A	0.190 A	5.52 A	61 A	0.241 A	4.68 A
Medium	68 A	0.262 A	3.84 A	49 A	0.175 A	6.12 A
High	68 A	0.265 A	3.84 A	68 A	0.165 A	6.11 A
One-Way ANOVA						
F value	1.63	1.73	1.63	2.29	1.64	2.25
p value	0.331	0.320	0.331	0.304	0.379	0.308

*Means with same capital letters indicate non- significant differences between stand productivity in each column

Table 5-5 Pearson correlation coefficient (*r* -value) and significance[+] among soil variables in lodgepole pine and white spruce stands in oil sands reclamation (n=24)

Variable [a]	Avail.N	Compaction	EC	pH	FRLD	FRP	RSA	FRB	TOR
Lodgepole pine									
Available N									
Soil penetration resistance	0.50*								
EC	-0.27	0.72*							
pH	-0.16	0.07	-0.45*						
FRLD	0.26	-0.50*	0.43*	0.27					
FRP	0.07	0.11	-0.52*	0.33	-0.10				
RSA	0.08	-0.18	-0.22	0.46*	0.66**	-0.16			
FRB	0.34	0.19	-0.37	0.50*	-0.1	0.85*	-0.01		
TOR	-0.23	-0.32	-0.52*	-0.24	-0.07	0.51*	-0.30	-0.02	
FRdecomp.	-0.03	0.20	0.28	-0.02	0.19	-0.19	-0.10	-0.04	-0.30
White spruce									
Available N									
Soil penetration resistance	-0.66*								
EC	0.75*	-0.56*							
pH	-0.77*	0.36	-0.83*						
FRLD	-0.08	-0.09	0.07	-0.05					
FRP	0.24	-0.05*	0.33	0.09	-0.07				
RSA	0.31	-0.45*	0.22	-0.22	0.73*	0.05			
FRB	0.04	-0.27	-0.15	0.52*	-0.17	0.82*	-0.02		
TOR	-0.37*	-0.54*	0.49*	-0.13	-0.02	0.97*	0.10	0.66*	
FR decomp.	-0.01	-0.14	-0.28	0.20	0.57*	-0.09	-0.41*	0.12	-0.14

[+*]Significant at the $p < 0.05$ level

[a] Abbreviations: Available N: available nitrogen, EC: electrical conductivity, FRLD: fine root length density, FRP: fine root production, RSA: root surface area, FRB: fine root biomass, TOR: Turnover rates, FR decomp: fine root decomposition

Table 5-6 Regression equations for the relationships between fine root dynamics (y) and tree performance (x) in oil sands reclamation

Fine root dynamics processes[a]	Lodgepole pine		White spruce	
	Equation	R^2	Equation	R^2
Fine root length density and diameter at breast height	y = 121.7x –467.7	0.88*	y = 9.0x + 297.3	0.12
Fine root length density and height	y = 231.4x - 637.7	0.97*	y = 11.2x + 298.2	0.13
Fine root production and height	y = 231.4x – 637.7	0.94*	y = 11.2x + 298.2	0.13
Fine root production and diameter at breast height	y = 121.7x – 467.7	0.76*	y = 0.11x + 0.50	0.04
Turnover rate and height	y = 231.4x - 637.7	0.98*	y = 0.01x + 0.06	0.04
Turnover rate and diameter at breast height	y = 121.7x - 467.7	0.89*	y = 0.01x + 0.01	0.15

[+*]Significant at the $p < 0.05$ level

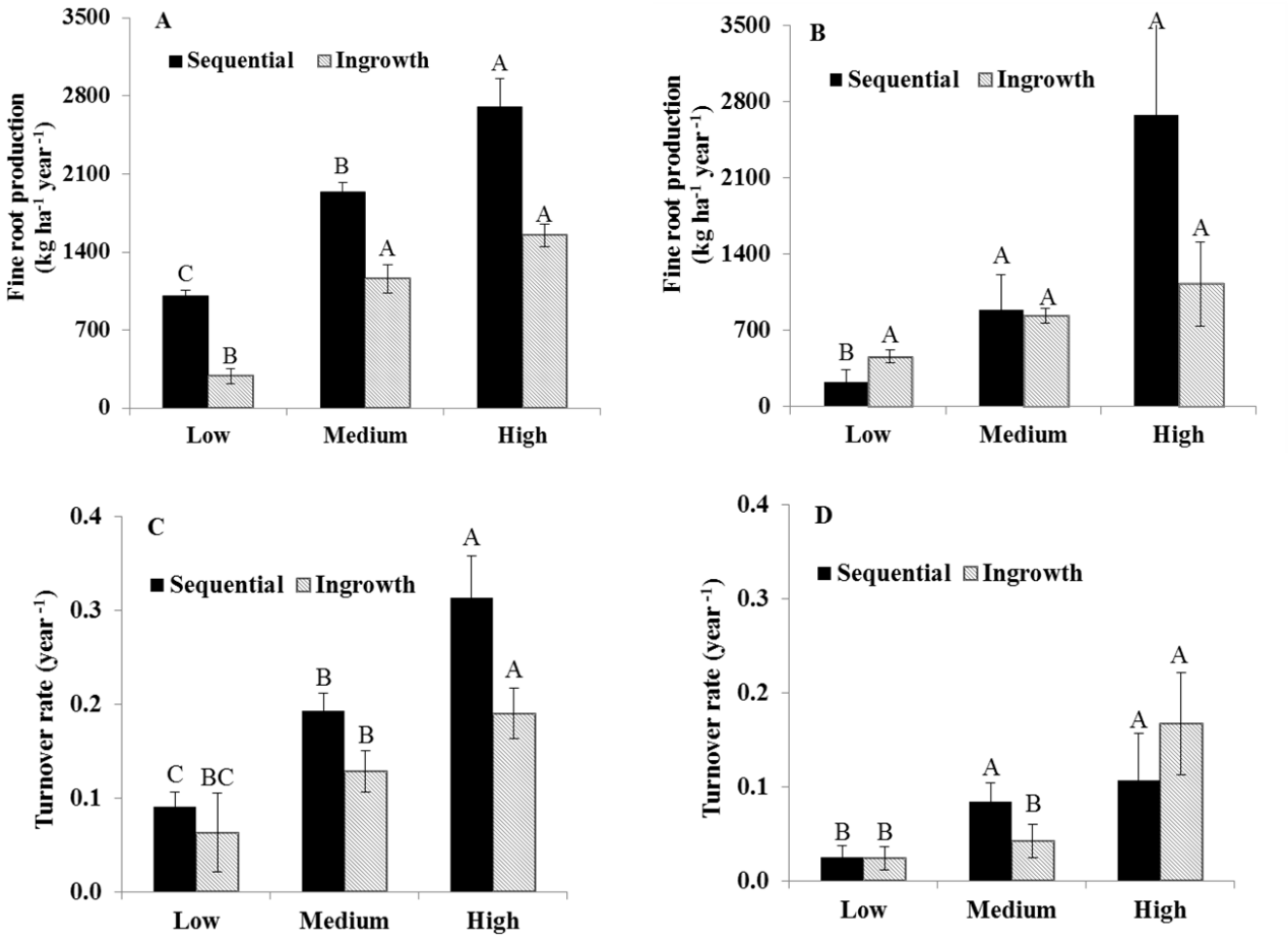

Figure 5-1 Fine root production in (A) lodgepole pine and (B) white spruce, and turnover rate in (C) lodgepole pine and (D) white spruce stands along a stand productivity gradient in oil sands reclamation measured by sequential and ingrowth core methods. Values are means SE (n = 24). Means across the productivity gradient within each method with the same uppercase letter are not significantly different at $P < 0.05$

CHAPTER 6 SYNTHESIS, CONCLUSIONS AND FUTURE RESEARCH

1. Research Overview

This research contributed to developing effective reclamation practices for disturbed oil sands areas by contributing to understanding the soil biogeochemical processes and fine root dynamics of vegetation in LFH mineral soil mix (LFH-MS) and peat mineral soil mix (PMM) organic capping materials used in oil sands reclamation. Four studies, including two field experiments and two laboratory experiments, were conducted. Nitrogen (N) availability and associated enzyme activities affected by organic capping materials types were evaluated for two years from June to October in 2011 and 2012. The effects of organic substrate type (glucose, acetic acid and alanine) and their diversity on microbial processes in two organic capping materials were evaluated in laboratory incubations for two months. The effects of organic to mineral soil ratios on soil biogeochemical processes were evaluated in the laboratory. Fine root properties, such as fine root length density (FRLD), and the surface area, production, decomposition and turnover rates of fine roots along productivity gradients of stands of white spruce and lodgepole pine planted on PMM over tailings sand (TS) and PMM over overburden (OB), respectively, were assessed between 2011 and 2012. Sequential coring and ingrowth core methods were used to measure fine root production and turnover rates. The percent mass remaining from the decomposing fine roots and the decomposition constant (k-value) were calculated with the use of the mesh bag technique.

2. Summary

2.1 Organic capping type affected nitrogen availability and associated enzyme activities in reconstructed oil sands soils

Organic capping materials, sampling depth and time had significant interactions that affected microbial biomass carbon (MBC) and nitrogen (MBN) and enzyme activities, including those of N-acetyl glucosaminidase (NAGase), arylamidase, protease and urease. The greater MBC, MBN and enzyme activities in LFH-MS than in PMM were attributed to the narrower carbon to nitrogen (C to N) ratio in LFH-MS than in PMM. The greater microbial activity in LFH-MS relative to PMM was due to the higher degree of organic matter (OM) decomposition and increased N availability in LFH-MS. The greater MBC, MBN and enzyme activities in the 0–10 cm of soil depth than in the 10–20 cm were likely

due to variations in oxygen (O_2) concentration, nutrient status, water content and pH (Ekenler and Tabatabai, 2004; Eilers et al., 2012). However, pH was a key contributing factor in this study in that it was associated with differences in measured soil processes with soil depth. The greater enzyme activities and N availability in the fall than in the summer were likely due to the priming effect from fresh organic input in the form of litter fall and root exudates.

2.2 Organic substrate type and diversity altered microbial processes in organic capping materials used in oil sands reclamation

Principal component analysis indicated that segregation of community level physiological profiles (CLPPs) with substrates capped with LFH-MS and PMM relative to the control was likely associated with the type of organic substrate added (Orwin et al., 2006) and organic substrate composition (Hernandez and Hobbie, 2010), which altered the C to N ratio, pH and dissolved organic carbon (DOC) concentration. Consequently, the microbial community composition changed. The effect of organic substrate diversity on CLPPs was more prominent in PMM than in LFH-MS likely because of the change in OM quality and microbial community composition in PMM. The change in LFH-MS in CLPPs was due to a change in DOC concentration. The significant increase in enzyme activities through organic substrate diversity was related to changes in CLPP and a reduction in C to N ratio in LFH-MS and PMM, which subsequently increased decomposition of OM and increased ammonium (NH_4^+) and nitrate (NO_3^-) N concentrations. This was evident in the greater expected values of enzyme activities and NH_4^+-N and NO_3^-N concentrations than the measured values in the presence of a mixture of two or three organic substrates.

2.3 Organic to mineral soil ratio altered the biogeochemical processes of organic capping materials

The organic to mineral soil ratio significantly altered carbon dioxide (CO_2) emission, DOC, dissolved organic nitrogen (DON), activities of β-glucosidase, cellobiohydrolase, phenol oxidase and leucine amino peptidase (LAP), and available N concentrations in LFH and peat. The differences in measured properties along the organic to mineral soil ratio were associated with changes in C to N ratio and pH. The CO_2 emission, and DOC, DON and available N concentrations increased with the amount of organic soil in LFH and peat. The differences in measured properties along the ratio were greater in LFH than in peat, and were likely associated with the lower C to N ratio and pH in LFH-MS than PMM (Jamro et al., 2014; Kwak et al., 2015a). The β-glucosidase, cellobiohydrolase, phenol oxidase and LAP

activities changed along the ratio in LFH and peat without a systematic pattern. In general, a greater enzyme activity was measured in LFH-100 (LFH to mineral soil ratio: 100:0) or peat-100 (peat to mineral soil ratio: 100:0) than in LFH-0 (LFH to mineral soil ratio: 0:100) or peat-0(peat to mineral soil ratio: 0:100)

2.4 Fine root dynamics in lodgepole pine and white spruce stands along productivity gradients in reclaimed oil sands sites

Fine root length density, production and turnover rates were greater in high productivity than in low productivity sites in lodgepole pine stands. However, the fine root surface area only increased from low to high productivity sites in white spruce stands. These fine root properties were positively correlated with tree height and diameter at breast height (DBH). Soil salinity and compaction limited fine root growth and dynamics of these factors in pine and spruce sites, respectively, as evident in the negative correlations with measured fine root properties. Sampling time significantly affected FRLD in pine and spruce stands and fine root biomass (FRB) in pine stands. A declining trend was observed in FRLD and FRB in pine, whereas FRLD only decreased in spruce from June to September regardless of the productivity level. These variations were likely due to differences in water content and soil temperature during the sampling months. The percent mass remaining of fine roots and k-values were not affected by productivity level in pine and spruce stands. These differences were caused by differences in root characteristics between tree species (Lazorko and Van Rees, 2012; Lilles et al., 2012, Khasa et al., 2005) and TS and OB substrate properties (Jung et al., 2014; Duan et al., 2015).

3. Synthesis

Current oil sands reclamation practices involve the use of PMM and LFH-MS as organic materials to cap the sub-soil and substrate materials such as TS and OB substrates. The PMM is predominantly used as a source of OM due to its high availability at the mining site. However, this material has been criticized in reclamation due to its inactive microbial community (Beasse et al., 2015), nutrient limitation (Duan et al., 2015) due to wider C to N ratio (Jamro et al., 2014; Kwak et al., 2015a) and it is considered less supportive to native vegetation (Brown and Naeth, 2014). The use of LFH-MS in oil sands reclamation is used as a source of OM and is also a rich source of native seed bank propagules (Mackenzie and Naeth, 2010). Availability of LFH-MS is limited at mining sites and its role in microbial

community establishment, soil enzyme activities and nutrient cycling at a wide scale in the oil sands region has not been evaluated (Naeth et al., 2013). The inherent properties of substrate materials such as high soluble salt concentrations, poor drainage, heavy compaction and nutrient limitation (Jung et al., 2014) due their direct and indirect effects on tree (Duan et al., 2014) and fine root growth (Jamro et al., 2015) have raised concern for sustainability of ecosystem re-establishment in the oil sands region. In view of the issues associated with reclamation materials, this study was conducted to compare PMM and LFH-MS effects on biogeochemical processes and fine root dynamics of pine and spruce planted on PMM over TS and OB substrates for improving and developing effective reclamation practices for disturbed oil sands areas.

The synthesis of research findings (Figure 6-1) showed that the organic capping materials had contrasting soil properties (chapter 2). The LFH-MS was a better soil quality material relative to PMM due to its greater N availability and associated enzyme activities. These differences were due to more decomposed OM and organic substrate availability in LFH-MS than in PMM due to its lower C to N ratio than PMM (Jamro et al., 2014; Kwak et al., 2015a). The positive and stronger relationships between N availability and enzyme activities in LFH-MS than PMM suggest that N fertilizer can be avoided or reduced if LFH-MS was applied as an organic capping material. Careful planning is required for salvaging LFH due to its limited availability (Naeth, et al., 2013). The greater N availability in the fall than in the summer in both materials with greater changes in LFH-MS than PMM were likely due to greater vegetation cover (Brown and Naeth, 2014) which likely added greater fresh labile C inputs through litterfall and root exudates in fall that provided greater organic substrate in LFH-MS than in PMM for greater enzyme activities and led to greater N mineralization (Kwak et al., 2015b).

Based on the findings of the first study, two lab incubation studies were conducted to address issues such as limited organic substrate availability and microbial activity in PMM and the limited availability of LFH-MS. In the first laboratory study, organic substrate type and diversity effects on microbial processes (chapter 3) indicated that the effect of organic substrates addition and their diversity was more pronounced in PMM than in LFH-MS due to greater change in OM quality measured as C to N ratio and microbial community composition as CLPPs. Whereas, the CLPPs changes in LFH-MS were due to change in DOC concentration. This supports the field study (chapter 2) results that the addition of organic substrate in the form of litterfall from vegetation could increase the organic substrate availability for microbial activities (Corre et al., 2002). As a result, they may enhance the OM

decomposition and increase N availability, which can eliminate the potential N limitation in PMM material and increase the forest stand productivity in oil sands reclamation (Duan et al., 2015). In the second laboratory study (chapter 4), increasing organic (LFH or peat) to MS ratio led to increased CO_2 emission rate, enzyme activities and available N. Therefore, it is clear from the findings that organic to MS ratio influences biogeochemical processes. Results showed that a 50:50 ratio of either LFH or peat to MS can provide enough available N for vegetation establishment which would help to maximize the future reclamation with available LFH material.

The understanding of fine root dynamics is also a key indicator for the evaluation of success oil sands reclamation (Lazorko and Van Rees, 2012; Jung et al., 2014) since they contribute largely in nutrient pool in soil than litterfall (Aerts et al., 1992) and changes in organic substrates availability for microbial activity in the form of root exudates (Corre et al., 2002). They play key roles in resource (water and nutrient) capture (West et al., 2004) and exploitation (Gilroy and Jones, 2000) from the resources limited environment and affect forest stand productivity in the oil sands region (Jung et al., 2014; Duan et al., 2015). Differences in fine root dynamics of pine and spruce along productivity levels were associated with inherited properties of TS and OB substrates (chapter 5). The EC and soil penetration resistance affected fine root dynamics in pine and spruce stands, respectively. The negative relationship of EC and soil penetration resistance with N availability indirectly reduced the fine root processes in both pine and spruce. Thus, substrate materials properties could affect the forest stand productivity (Jung et al., 2014; Duan et al., 2015) and oil sands reclamation process.

The LFH-MS is a better soil quality material than PMM due to N availability and enzyme activities; however, the availability of LFH is limited but optimizing the LFH-MS ratio such as 50:50 can help to take advantage of available LFH material. In addition, mixing LFH-MS with PMM can overcome the limitations of both materials, as indicated from substrate diversity and organic to mineral soil ratios in this research. An evaluation of the effects of substrate properties on fine root properties of vegetation can help to further understanding and improving the current oil reclamation practices.

4. Conclusions

The LFH-MS and PMM had contrasting soil properties and LFH-MS was a soil material with a better quality than PMM because of its greater N availability and enzyme

activities. The strong and positive relationships between N availability and enzyme activities suggest less dependency on N fertilization and less likelihood of N deficiency in sites reclaimed with LFH-MS material. They also indicate the need for careful planning of LFH-MS salvaging so that LFH could be utilized properly.

The study on organic substrate type and diversity effects on microbial processes showed that organic substrate addition in the form of organic inputs from vegetation could significantly affect soil processes in oil sands reclamation. The changes in microbial processes in organic materials by organic substrate addition suggest the need to extend this kind of research to field conditions. The greater change in CLPPs by organic substrate diversity in PMM than in LFH-MS indicate the need to augment PMM with LFH-MS as organic capping material to overcome potential limitations, such as slow low nutrient availabilities that affect forest stand productivity (Duan et al., 2015).

The results of this study showed that the organic to mineral soil ratio altered biogeochemical processes in materials used in oil sands reclamation. The changes in CO_2 emission, enzyme activities and available N were associated with changes in C to N ratio and pH along organic to mineral soil ratio. The findings of this study emphasize the importance of the ratios of materials for optimization, particularly of LFH, and require further evaluation under field conditions.

Fine root parameters, which were not changed in spruce stands, were changed along the productivity gradient in pine stands. The differences in fine root dynamics of the tree species were linked to inherit characteristics of TS and OB substrates. The electrical conductivity (EC) affected fine root dynamics in pine stands, whereas soil penetration resistance influenced those in spruce stands. The negative relationship of EC and soil compaction with N availability affected fine root dynamics indirectly in both species.

5. Future Research

5.1 Effects of C to N ratio on soil biogeochemistry

The C to N ratio is commonly used to measure OM decomposition rate and organic substrate availability (Mohanty et al., 2013). In this study, the differences in N availability and enzyme activity in LFH-MS and PMM were related to the lower C to N ratio in LFH-MS than in PMM. The differences in C to N ratio in LFH-MS and PMM were associated with the type of organic material added (Hemstock et al., 2010; Naeth et al., 2013) and the vegetation types materials grown (Forsch, 2014). The mineral and organic soil ratio altered the C to N

ratio because of a change in OM quality, which consequently affected biogeochemistry. Therefore, understanding how tdifferences of the C to N ratio alter biogeochemistry, which is required to improve current reclamation practices, is essential.

5.2 Role of mycorrhizae in changes in biogeochemistry

In field study, greater enzyme activities in LFH-MS than in PMM were associated with greater mycorrhizal biomass in LFH-MS than in PMM (Brown and Naeth, 2014). Mycorrhizal fungi produce several extracellular enzymes involved in OM decomposition (Smith and Read, 2008), thus mycorrhizae affect N availability (Burke et al., 2011). However, the interaction of mycorrhizae and N availability in oil sands reclamation is unknown. Differences in mycorrhizal biomass between LFH-MS and PMM were due to differences in tree species grown (Brown and Naeth, 2014) because trees are the hosts of mycorrhizal associations (Smith and Read, 2008). Evaluating the role of tree species in establishment of mycorrhizae and their subsequent effects on soil biogeochemistry would help improve oil sands reclamation practices.

5.3 The N fertilizer management in LFH-MS organic capping material

The positive relationships of N availability with enzyme activity in this study suggest the need to minimize N fertilizer application in developing ecosystems through LFH-MS material. At a small scale, fertilizer application is not recommended in ecosystems established by LFH-MS (Snively, 2014; Errington and Pinno, 2015). However, evaluating fertilizer application at a rigorous and large scale along with LFH-MS material would help utilize LFH material economically in oil sands reclamation.

5.4 Effects of LFH and PMM mixture on biogeochemistry

Peat mineral soil mix is commonly used as an organic capping material in oil sands reclamation because of its large availability at mining sites, but its current use in reclamation is criticized because of its slow decomposition (Hemstock et al., 2010)). Relative to LFH-MS, PMM fails to establish a microbial community similar to that in a natural forest. LFH-MS has proved to be a soil material with a better quality (Brown and Naeth, 2014; Jamro et al., 2014; Béasse et al., 2015; Kwak et al., 2015a) than PMM, but its availability in mining areas is limited. In this study, organic substrate addition altered the CLPPs and OM decomposition. An alternative approach, such as the mixing of LFH-MS and PMM, is therefore needed to overcome issues with future use of LFH-MS and PMM in reclamation.

Effects of LFH-MS and PMM mixture on biogeochemistry will help develop a new reclamation practices.

5.5 Measurement of in-situ priming effects

The greater enzyme activities and N availability in the fall than in the summer in this study suggested that in-situ priming effects of litter fall and rhizo-deposits need to be evaluated. The amount of litter fall in reclaimed sites needs to be calculated for proper estimation of priming effects in oil sands reclamation

5.6 Establishment of the biogeochemical interface

The organic to mineral soil ratio plays an important role in establishing a biogeochemical interface, which may be used to assess interactions between soil components (Pronk et al., 2012) in land reclamation. The regulation of the OM decomposition, nutrient cycling, enzyme activities and the microbial community composition is mainly affected by the organo-mineral interactions (Kögel-Knabner et al., 2008). A field evaluation of the effects of the organic to mineral soil ratio on the biogeochemistry in reclaimed oil sands mined areas is thus recommended.

5.7 Role of C to N ratio and pH regulation in biogeochemical interactions

In a laboratory study, it indicated that, C to N ratio and pH altered the biogeochemistry with increasing organic to mineral soil ratio. Thus the role of role of C to N ratio and soil pH in regulation of organic-mineral-biogeochemical interaction in oil sands reclamation needs to evaluate for developing the guidelines for ecosystem models for future reclamation practices in oil sands reclamation

5.8 Inherent substrate properties and fine root dynamics

The inherent properties of OB and TS, such as a high pH, EC and soil penetration resistance, have raised serious concerns for their use in current oil sands reclamation practices (Jung et al., 2014). In this study, fine root properties were negatively affected by EC and soil penetration resistance in pine and spruce stands, respectively. Research focused on effects of EC and soil penetration resistance on fine root dynamics using the minirhizotron method will help improve the use of TS and OB materials below organic capping materials.

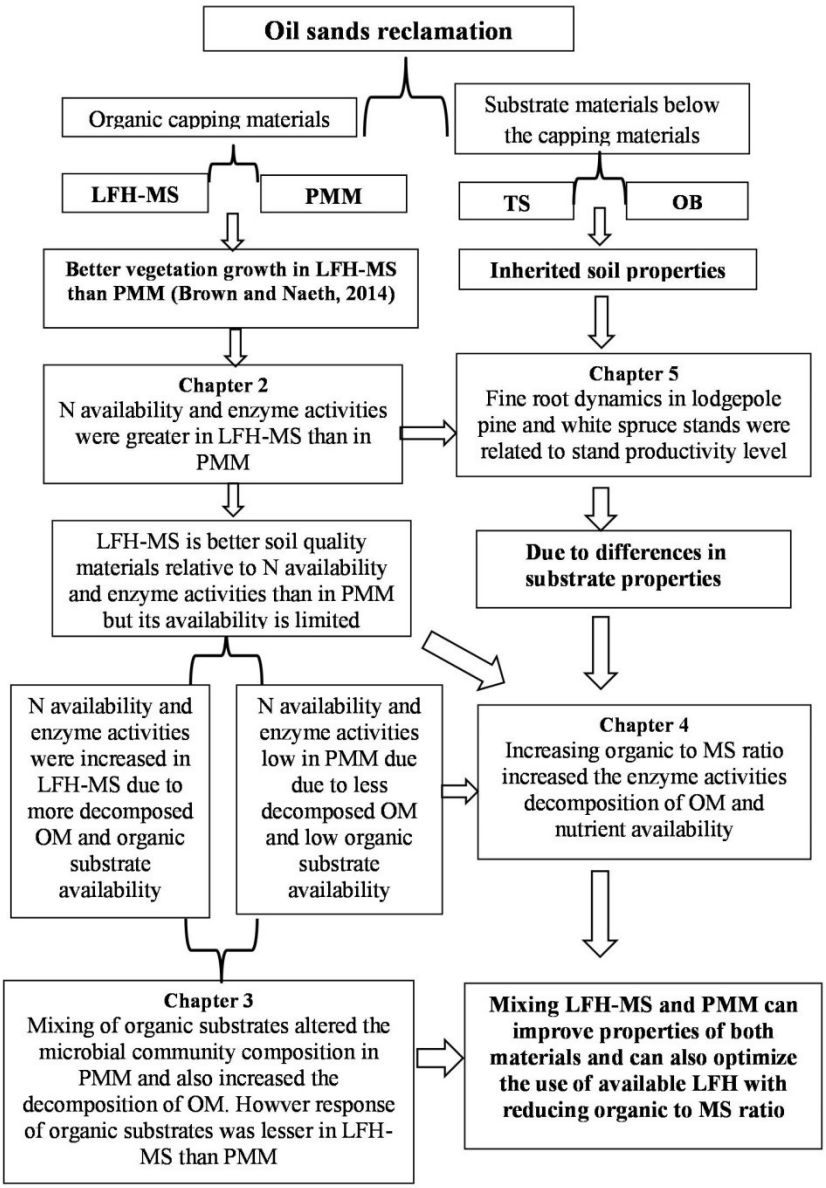

Figure 6-1 Flow chart of key findings of thesis research

*Abbreviations: LFH-MS= LFH-mineral soil, PMM= peat mineral soil mix, OB= overburden, TS= tailings sand, OM= organic matter, N= nitrogen

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Appendix A Repeated Measures ANOVA of fine root paramters of lodgepole pine and white spruce stands along stand productivity gradients in oil sands reclamation

Root parameter [a]	Stand productivity level		Sampling month		Stand productivity levelx sampling month	
	F value	*p* value	F value	*p* value	F value	*p* value
	Lodgepole pine					
Root surface area	1.46	0.274	0.73	0.556	2.06	0.142
Fine root length density	9.34	0.004	7.87	0.004	2.25	0.116
Fine root biomass	1.78	0.214	4.37	0.030	0.24	0.540
	White spruce					
Root surface area	5.86	0.019	29.01	<0.001	8.09	0.002
Fine root length density	0.52	0.607	27.29	<0.001	0.540	0.772
Fine root biomass	0.21	0.813	1.90	0.188	0.91	0.521