COARSE WOODY DEBRIS EFFECTS ON BIOGEOCHEMISTRY IN TWO RECONSTRUCTED SOILS IN THE ATHABASCA OIL SANDS REGION IN ALBERTA, CANADA

by

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ABSTRACT

Forest floor mineral soil mix (FMM) and peat mineral soil mix (PMM) are cover soils commonly used for land reclamation post open pit oil sands mining in northern Alberta, Canada. While in such land reclamation practices the organic matter comes from the peat material salvaged before land disturbance, coarse woody debris (CWD) can be used as an additional organic matter amendment for land reclamation. Effects of cover soil type (FMM vs PMM) and CWD (near vs away from CWD) on microbial community level physiological profile, extracellular enzyme activities, greenhouse gas emission rates and nitrogen (N) transformation rates were determined between 4 and 7 years after reclamation to assess whether applying CWD can facilitate land reclamation.

Soil sampling and analyses were conducted and greenhouse gas emission rates were measured monthly during growing seasons within 5 cm from CWD and more than 100 cm away from CWD. Monthly in situ soil incubation was conducted and plant root simulators were incubated to assess net N transformation rates and N supply rates, respectively. A laboratory incubation experiment using ^{15}N isotopic dilution was conducted to evaluate the effect of CWD leachate on gross and net N transformation rates.

The soil microbial community level physiological profile was changed by CWD in FMM ($p<0.01$) but not in PMM. The CWD increased ($p<0.05$) metabolic microbial community function (averaged well color development during incubation of Biolog Ecoplates) by 10-30% in both cover soils. Microbial biomass ($p<0.1$) and enzyme activities ($p<0.05$) were 22-84 and 16-181%, respectively, greater in FMM than in PMM and CWD increased ($p<0.1$) microbial biomass by 2-58% in both cover soils but not enzyme activities.

Soil respiration ($p<0.05$) and methane uptake ($p<0.01$) rates were greater in FMM than in PMM regardless of the distance from CWD. Coarse woody debris increased soil respiration ($p<0.05$) and methane uptake rates ($p<0.1$) by 22-33 and 13-34%, respectively, in FMM but not in PMM. Gross and net nitrification rates were 1.8 and 2.1 times, respectively, greater ($p<0.01$) in FMM than in PMM due to the greater microbial and enzyme activities in FMM. Net N mineralization rates were 2.1 times greater ($p<0.01$) in FMM than in PMM in laboratory and field incubation experiments due to the greater ($p<0.01$) N immobilization rates in PMM. The CWD increased ($p<0.05$) gross nitrification rates associated with increased microbial activities and function near CWD (field condition); however, addition of CWD extract also increased ($p<0.05$) N immobilization rates (laboratory incubation experiment) resulting in similar N transformation rates between near CWD and away from CWD. Nitrogen supply rates and inorganic N concentrations were not affected by both cover soil type and CWD due to the greater N uptake in FMM than in PMM and greater N immobilization near CWD than away from CWD. Applying CWD for land reclamation may increase N immobilization; however, it increases microbial activity and function thereby increasing organic matter decomposition. Effects of CWD on soil biogeochemistry differed depending on cover soil type and such effects were more significant in FMM than in PMM.

Cover soils had contrasting properties and FMM is a better cover soil relative to PMM for oil sands reclamation with greater microbial biomass, microbial and enzyme activities and N transformation rates in FMM. Application of CWD enhanced microbial activities and function that would increase nutrient cycling and organic matter decomposition; therefore, CWD application should benefit early ecosystem development in upland reclamation.

PREFACE

This thesis is an original work by Jin-Hyeob Kwak. Chapter 2 of this thesis has been published as Kwak, J.H., Chang, S.X., Naeth, M.A., Schaaf, W., "Coarse woody debris extract decreases nitrogen availability in two reclaimed oil sands soils in Canada." Ecological Engineering, vol. 84, 13-21 (2015). I was responsible for the performance of the experiment, data collection and analysis as well as manuscript writing. Chang, S.X. was the corresponding author and contributed to the design of the research and edited the manuscript. Naeth, M.A. and Schaaf, W. were co-supervisory authors and involved with concept formation and edited the manuscript.

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$\delta^{15}N$: nitrogen isotope composition

AOSR: Athabasca oil sands region

C:N: carbon to nitrogen ratio

CWD: coarse woody debris

DOC: dissolved organic carbon

DON: dissolved organic nitrogen

DOC:DON: DOC to DON ratio

FMM: forest floor mineral soil mix

MBC: microbial biomass carbon

MBN: microbial biomass nitrogen

N/IA ratio: ratio of gross nitrification rates to gross NH_4^+ immobilization rates

PMM: peat mineral soil mix

CHAPTER 1. GENERAL INTRODUCTION

1. Background

Oil sands mining in northern Alberta, Canada has disturbed large areas of boreal forest and such disturbed land must be returned to the pre-disturbance ecosystem with equivalent land capability according to provincial regulatory requirements (Province of Alberta, 2014). Forest floor mineral soil mix (hereafter FMM) and peat mineral soil mix (hereafter PMM) are common cover soils used for land reclamation to improve soil organic matter content and fertility and to provide plant propagules and microorganisms.

Coarse woody debris (CWD) can play important roles in natural forest ecosystems but the ecological value of CWD has often been underestimated. Applying CWD in land reclamation is a relatively novel practice which can increase spatial heterogeneity, increase soil water holding capacity, reduce soil erosion, regulate soil temperature and increase soil microbial activity and nutrient cycling. However, effects of CWD on biogeochemistry in reclaimed oil sands soils have not been well studied. This research was conducted to determine whether applying CWD for land reclamation after oil sands mining would improve soil microbial and enzyme activities, microbial community function and nitrogen (N) transformation rates, thereby accelerating early ecosystem development.

1.1 Oil sands mining and reclamation

Oil sands deposits in northern Alberta, Canada are considered the world's third largest reservoir of recoverable oil with an estimated 170 billion barrels of bitumen (Alberta Government, 2015). Main deposits are divided into Peace River, Athabasca Lake and Cold Lake regions and occupy approximately 140,200 km^2 of boreal forest. Oil sands are a mixture of bitumen (low quality heavy crude petroleum), quartz sand, silt, clay and water, which must be extracted and upgraded into synthetic crude oil for further refinement (CAPP, 2014). Open pit mining is a common practice to recover oil sands deposits within 75 m of the surface; approximately 4,800 km^2 of area can be extracted by open pit mining (Alberta Government, 2014). Open pit mining has disturbed more than 900 km^2 of boreal forest up to 2015 (Alberta Government, 2015) which must be returned to pre-disturbance equivalent

land capability (Province of Alberta, 2014). Equivalent land capability is defined as "the ability of the reclaimed land to support land uses similar to what existed prior to an activity, but the land uses will not necessarily be the same" (Government of Alberta, 2014). Only 104 ha of disturbed land by oil sands mining has been certified and approximately 77 km^2 is in the process of being reclaimed in 2015 (Alberta Government, 2015).

A common land reclamation practice after oil sands mining is returning disturbed land to upland forests. The general phases of land reclamation to upland forests are: surveying pre-disturbed soils and tree clearing, salvaging and storing reclamation materials, recontouring disturbed land, placing reclamation material, revegetation, maintaining and monitoring and certifying the reclaimed land (Fung and Macyk, 2000; Naeth et al., 2013). One of the most important steps of land reclamation is restoring soil functions, with topsoiling, or reapplication of salvaged soil to spoiled land, being a common practice used for land reclamation (DePuit, 1984; Sydnor and Redente, 2002). Applying cover soils is beneficial for increasing organic matter content and above ground biomass, improving soil fertility and water holding capacity and providing sources of plant propagules and soil microorganisms (DePuit, 1984; Sydnor and Redente, 2002; Mackenzie and Naeth, 2010).

Overburden material, which is geological material between surface soil and oil sands, and tailings sand, which is one main byproduct of the extraction process, are commonly used as land reclamation substrates. The FMM and PMM are common cover soils used for land reclamation in the Athabasca oil sands region. These organic materials are usually salvaged before surface mining and may be directly applied or stockpiled and used later (McMillan et al., 2007; Mackenzie and Naeth, 2010). The PMM is a mixture of approximately 1 m of organic peat horizon and 0.4 m of underlying mineral soil (Oil Sands Revegetation Reclamation Committee, 1998). The PMM has been used because of its high organic matter content and availability in the area. The FMM is a mix of upland forest floor, or LFH layer, with underlying mineral soil, mainly A horizon and sometimes including B horizon (Singh, 2007). Upland forest floor is organic soil horizons developed from the accumulation of leaves, twigs and woody materials (Soil Classification Working Group, 1988). Availability of FMM is limited and its long term effects, including decomposition and nutrient cycling, have not been well documented (Naeth et al., 2013). The PMM is thought to have a longer lasting effect than FMM due to its high organic matter content and

slow decomposition rate (Land Resources Network, 1993).

1.2 Coarse woody debris

Coarse woody debris is dead woody materials including snags, logs, chunks of wood, stumps, large branches and dead coarse roots (Harmon et al., 1986; Stevens, 1997). Large amounts of CWD have been produced during clearing of boreal forest before oil sands mining. This CWD was usually burned or buried as mandated by the Forest and Prairie Protection Act to limit fire risks (Province of Alberta, 2000). Using CWD for land reclamation is currently recommended for its ecological values (2010 Reclamation Criteria for Wellsites and Associated Facilities on Forested Lands permit). However, as applying CWD for land reclamation is novel practice, application rates of CWD for oil sands reclamation are limited to company specific best management practices and a review of the literature on natural inputs to forest ecosystems.

Amounts of CWD in natural forest ecosystems in Canada and the United States varied with disturbance type and study area (Table 1-1). Most studies reported the amount of CWD on a mass basis and some on a volumetric basis. To compare CWD amounts among natural ecosystems, regression equations were developed to convert biomass and volume of CWD to cover area using CWD amount data (n=18) in Harmon et al. (1986), where Area (%) = 0.156 × biomass (Mg ha^{-1}) (R^2 = 0.77) and Area (%) = 0.034 × volume (m^3 ha^{-1}) (R^2 = 0.94). Overall projected cover area of CWD ranged from 2 to 62% of the land surface, with a mean of 13.1%. The amount of CWD associated with pine beetle infestations was less than that associated with other types of disturbance because dead trees stayed as snags rather than downed logs. Amounts of CWD in temperate forest ecosystems were similar. For example, Harmon et al. (1986) summarized 20 studies published between 1973 and 1984 and found that projected cover area of CWD ranged from 1 to 53% and 1 to 6% for coniferous and deciduous forests, respectively. Recommended application rates according to historical CWD inputs in natural boreal forests are 60 to 100 m^3 ha^{-1} or 10 to 25% coverage for upland reclaimed sites and 30 to 50 m^3 ha^{-1} for lowland sites (Vinge and Pyper, 2013).

Size of CWD varies with the purpose of the studies (Harmon et al., 1986; Yan et al., 2006). Harmon et al. (1986) defined CWD as that with diameters greater than 2.5 cm, while others used diameters greater than 7.5 or 9.5 cm (Yan et al., 2006). The USDA Forest

Service and Long Term Ecological Research (Harmon and Sexton, 1996; Harmon et al., 2009) defined CWD as that with a diameter greater than 10 cm and a length longer than 1 m.

Coarse woody debris is usually categorized by 5 decay classes (Maser 1979, 1988; Brunner and Kimmins, 2003; Yan et al., 2006; Stevens, 2007). Class 1 CWD is freshly fallen and branches and bark are present; class 5 CWD is almost completely decayed with the original shape unrecognizable (Table 1-2). Wood density, volume and nutrient concentrations are correlated to decay class (Harmon et al., 1986; Laiho and Prescott, 2004). Most macro nutrient and base cation concentrations, such as N, phosphorus, potassium, calcium and magnesium, in CWD usually increase during decay (Laiho and Prescott, 2004). Decomposition rates vary with tree species; broad leaved trees decompose faster than coniferous trees due to their higher concentrations of sugar, amylum and protein (Zhou et al., 2007).

1.3 Biogeochemistry of reclaimed oil sands soils

Understanding and characterizing biogeochemistry, such as N cycling, microbial community function and microbial and enzyme activities, in reclaimed oil sands soils are important to accelerate early ecosystem development with land reclamation. Nitrogen is the most likely limiting factor for tree growth in the boreal forest ecosystem and reclaimed oil sands soils, and N turnover rate is closely related to plant growth and forest productivity (Gundersen et al., 2009; Yan et al., 2012; Duan et al., 2015).

Rates of N cycling in the soil can vary widely depending on numerous environmental factors and biotic factors. Soil water content and soil pH are important factors controlling microbial activity, and thus strongly influence soil N transformation rates (Cheng et al., 2012, 2013). Carbon (C) to N ratio (C:N) is a widely known factor that controls N mineralization and immobilization rates (Manzoni et al., 2008, 2010). Dissolved organic C and N (DON) are key organic matter pools determining N cycling (Cookson and Murphy, 2004; Jones et al., 2004). The dissolved organic C to dissolved organic N ratio is an important indicator of N transformation rates (Cookson et al., 2007), with higher ratios increasing N immobilization rates in soils, and decreasing N mineralization rates and N availability (Cookson et al., 2007). Applying cover soils on substrates, such as overburden or trailings sand, is essential to provide organic matter content and nutrients for plant

growth. However, slow organic matter decomposition and N cycling in reclaimed oil sands soils due to small microbial population sizes and reduced microbial activities may affect N availability (McMillan et al., 2007; Hemstock et al., 2010). Measuring and understanding N transformation rates and availability and their relationship with cover soil properties are important to accelerate revegetation during oil sands reclamation.

Soil microbial community and enzyme activity are important biological indicators of soil quality and net ecosystem productivity in natural and reclaimed ecosystems (Sinsabaugh et al., 1991; Sylvia et al., 1998; Alkorta et al., 2003; Dimitriu et al., 2010; Burns et al., 2013) and can be affected by substrate quality and quantity (Degens, 1998; Degens et al., 2000; Burns et al., 2013), plant community composition and productivity (Zak et al., 2003; Bach et al., 2008) and environmental factors (Dimitriu et al., 2010; Burns et al., 2013). Soil pH and water content are important determinants of microbial community (Will et al., 2010; Jin et al., 2011). Lower soil pH will increase the fungi to bacteria ratio because fungi growth is favoured by acidic soil pH while bacteria growth is promoted by alkaline or neutral soil pH (Will et al., 2010). Fungi are more resistant to water stress relative to bacteria (Jin et al., 2011). Water soluble C or dissolved organic C in soils is a most readily available C source for soil microorganisms (McGill et al., 1986; Wang et al., 2003) and increases in water soluble C or dissolved organic C concentration in soils may increase microbial activity, causing a positive priming effect (Kuzyakov et al., 2000). Size of microbial biomass is a key determinant for organic matter decomposition (van Veen and Kuikman, 1990; Ladd et al., 1995). Readily decomposable soil organic matter, such as water soluble organic matter or dissolved organic matter, is rapidly consumed by microorganisms, and then organic matter decomposition or nutrient cycling is regulated by turnover of the microbial biomass (van Veen and Kuikman, 1990; Ladd et al., 1995).

Plant litter is a primary source of energy and nutrients for soil organisms and the soil microbial community and activity are highly affected by plant species and productivity (Zak et al., 2003; Bach et al., 2008). Addition of organic C substrates (litter) to soils changes microbial C substrate utilization patterns (Degens, 1998; Degens et al., 2000). Soil microbial communities were associated with plant productivity and community composition in reclaimed oil sands soils (MacKenzie and Quideau, 2010; Hahn and Quideau, 2013). Mycorrhizal fungi, a symbiotic relationship of vascular plants and fungi, increase plant root

uptake of water and nutrients, especially in water and nutrient limited soils (Harmon et al., 1986; Dahlberg 2001). Ectomycorrhizae are common in tree species, and plant diversity and woody species abundance affect ecotomycorrhizae abundance and total fungal biomass (Vogt et al., 1995; Smith and Read, 2008).

Soil enzyme activities are affected by soil pH, microbial biomass and plant community composition (Hernández and Hobbie, 2010; Burns et al., 2013). Most enzyme activity is positively related to soil pH and microbial biomass (Burns et al., 2013). Fungi, including mycorrhizae, produce enzymes that degrade recalcitrant compounds when nutrients are limited (Lindahl et al., 2002; Smith and Read, 2008), with enzyme activities associated with soil fungal biomass (Andersson et al., 2004; Lucas and Casper, 2008; Muruganandam et al., 2009). Substrate quality and quantity are determinants of soil enzyme activity and are affected by plant community composition and productivity (Hernández and Hobbie, 2010; Burns et al., 2013).

1.4 Soil biogeochemistry in cover soils for oil sands reclamation

Properties of FMM and PMM were compared in many studies conducted in the Alberta Oil Sands Region (Mackenzie, 2006; McMillian et al., 2007; Mackenzie and Naeth, 2010; MacKenzie and Quideau, 2012; Brown and Naeth, 2014; Forsch, 2014; Jamro et al., 2014). The sources of organic matter of FMM and PMM are different, as FMM is salvaged from upland forests and PMM from wetlands. Thus properties of these two cover soils were affected by where and when they were salvaged and applied (Naeth et al., 2013). Bulk density was higher in FMM than in PMM (Mackenzie, 2006; Suncor Energy Inc., 2008) and water holding capacity was greater in PMM than in FMM (MacKenzie and Quideau, 2012) due to greater organic matter content in PMM.

The FMM had lower total C and N concentrations than PMM (McMillian et al., 2007; Mackenzie and Naeth, 2010; MacKenzie and Quideau, 2012; Brown and Naeth, 2014; Jamro et al., 2014), associated with higher organic matter content of PMM. The FMM is considered more decomposable than PMM because the C:N ratio was lower in FMM than in PMM (Suncor Energy Inc., 2008; Mackenzie and Naeth, 2010; Mackenzie, 2012; Jamro et al., 2014). Soil pH was higher in FMM and PMM than in natural forest floor due to the alkaline nature of salvaged mineral soils in the Alberta Oil Sands Region (Fung and Macyk,

2000). Soil pH was higher in PMM than in FMM (MacKenzie and Quideau, 2012; Brown and Naeth, 2014; Jamro et al., 2014).

The FMM contains a large native plant propagule bank (Mackenzie and Naeth, 2010) and FMM application was associated with greater plant abundance and diversity than with PMM (Brown and Naeth, 2014; Forsch, 2014). Microbial biomass was greater in FMM than PMM (McMillan et al., 2007; Brown, 2010; Hahn, 2012; Jamro et al., 2014) and increased over time since land reclamation (Hahn, 2012). Soil enzyme activity was greater in FMM than in PMM (Dimitriu et al., 2010; Jamro et al., 2014), associated with greater microbial biomass and vegetation cover in FMM. Microbial community composition of FMM and PMM was distinctively different based on phospholipid fatty acid analysis, with that of FMM more similar to the natural forests than PMM (Hahn and Quideau, 2013; Béasse et al., 2015).

1.5 Effects of coarse woody debris on soil biogeochemistry

Coarse woody debris plays important roles in forest ecosystems by providing habitats for microorganisms, insects and small animals (Harmon et al., 1986; Brant et al., 2006; Kappes et al., 2007), reducing soil erosion and nutrient leaching (Stevens, 1997; Debeljak, 2006), increasing nutrient and organic matter content (Harmon et al., 1986; Stevens, 1997; Lindsay and Cunningham, 2011; Goldin and Hutchinson, 2013) and increasing plant growth and productivity (Hofgaard, 1993; Brown and Naeth, 2014). However, ecological value of CWD has often been underestimated and applying CWD for land reclamation is a relatively novel practice. Several studies have assessed effects of CWD on vegetation cover and soil properties (Brown, 2010; Brown and Naeth, 2014; Forsch, 2014). Vegetation cover was greater with CWD than without CWD and was positively related to amount of CWD by the second year of reclamation (Brown, 2010; Brown and Naeth, 2014). However, differences in vegetation cover with CWD and without CWD became smaller 4 and 5 years after reclamation (Forsch, 2014). Applying CWD increased available phosphorus concentrations in soils while decreasing nitrate concentrations (Brown, 2010; Brown and Naeth, 2014).

Coarse woody debris affects soil properties in different ways. It can increase soil water content and decrease ranges of soil temperature fluctuation, providing more favourable habitats for microorganisms (Pyle and Brown, 1999; Kappes et al., 2007; Brown

and Naeth, 2014), and its leachate with low pH and high dissolved organic C and polyphenol content can affect microbial activity and nutrient cycling (Laiho and Prescott, 2004; Spears and Lajatha 2004; Hafner et al., 2005; Lajtha et al., 2005). Coarse woody debris increases spatial heterogeneity and microsites providing more favourable habitats for microorganism and detritivores (Kappes et al., 2007; Brown and Naeth, 2014), which would increase microbial activity and organic matter decomposition. Microbial biomass, soil respiration rates (Brant et al., 2006) and C and phosphorus degrading enzyme activities (Gonzalez-Polo et al., 2013) were greater under CWD than forest floor without CWD.

Increases in dissolved organic matter content through CWD leachate (Hafner et al., 2005; Lajtha et al., 2005) can increase microbial activity as dissolved organic matter is a readily available substrate for microorganisms (McGill et al., 1986; Wang et al., 2003). However, high C:N ratios of CWD leachate (Spears and Lajatha, 2004; Hafner et al., 2005) may increase N immobilization rates by decreasing N availability. Soil pH decreased under CWD due to the low pH of CWD leachate (Spears and Lajtha, 2004; Goldin and Hutchinson, 2013) and it suppressed activities of acid sensitive bacteria (DeBoer et al., 1989, 1990) and ammonium and nitrite oxidizing bacteria (Hunik et al., 1992; Boer and Kowalchuk, 2001), decreasing N transformation rates (Ste-Marie and Pare, 1999; Cheng et al., 2013).

Due to contrasting effects of CWD on soil properties, N transformation rates and N availability also vary (Spears et al., 2003; Hafner and Groffman, 2005; Metzger et al., 2008; Lindsay and Cunningham, 2011; Goldin and Hutchinson, 2013). Organic matter content, total N and nitrate concentrations were higher near CWD than those in the forest floor without CWD in woodlands in Australia (Lindsay and Cunningham, 2011; Goldin and Hutchinson, 2013). However, inorganic N concentrations decreased beneath CWD relative to forest floor without CWD in North America lodgepole pine forest (Busse, 1994) and mixed forest (Hafner and Groffman, 2005). Coarse woody debris contributes 1-18% of N (4 studies), 1-12% of phosphorus (4 studies), 18% of potassium (1 study) and 15% of calcium (1 study) of the annual above ground input in tree litter (Laiho and Prescott, 2004). Net N mineralization and nitrification rates were lower under CWD than in bare mineral soil in a post fire lodgepole pine forest (Metzger et al., 2008) and in mixed forest in New York State (Hafner and Groffman, 2005). Gross N mineralization rates were greater in control soils

than under CWD in Douglas fir forest in Oregon (Spears et al., 2003). Effects of CWD on N availability vary with tree species and climate conditions; however, no study has assessed effects of CWD on N transformation rates and N availability in reclaimed oil sands soils.

1.6 Greenhouse gas emission

Activities such as mining, transportation, extraction and upgrading in the Alberta Oil Sands Region emit large amounts of greenhouse gases to the atmosphere. Total greenhouse gas emissions were 46.8 Mt in 2010, accounting for 38.2 and 6.0 % of total emissions in Alberta and Canada, respectively (Government of Alberta, 2013). Reclamation can sequester atmospheric carbon dioxide by supporting plant growth. At the same time, it may increase carbon dioxide emissions by enhancing microbial decomposition of soil organic matter (Nilsson and Wiklund, 1995). Soil respiration is closely associated with size of microbial biomass and microbial community, affecting microbial activity and organic matter decomposition. Soil physical and chemical properties, such as pH, water content, temperature and organic matter content, will change microbial activity and soil respiration rates (Davidson et al., 1998; Raich and Schlesinger, 1992; Rustad et al., 2000; Lee et al., 2006). Soil respiration rates are also related to plant species composition and productivity through autotrophic respiration and amounts of litter (Raich and Schlesinger, 1992; Raich and Tufekcioglu, 2000).

Methane (CH_4) has 25 times greater global warming potential than carbon dioxide over a time scale of 100 years and is considered the second most common greenhouse gas emitted from soils after carbon dioxide (IPCC, 2007). Forest soils are the most active methane sinks in upland soils due to their higher methanotrophic activity relative to other ecosystems, such as grass lands or cultivated lands (Mer and Roger, 2001). Therefore, reclaiming disturbed land to upland boreal forest has high potential for oxidizing atmospheric methane and for mitigating global warming. Methane oxidation in upland soils is less intensively studied than its emission in wetlands. Soil water content, gas diffusivity and N fertilization are considered the main determinants for methane oxidation and uptake rates in soils (Steudler et al., 1989; Smith et al., 2000; Mer and Roger, 2001). Methanogens and methanotrophs can exist in the same physical areas and the net balance between two microbial groups can change with soil water content (Keller et al., 1983; Steudler et al.,

1989). Thus methane uptake rates can be negatively related to water filled pore space (Smith et al., 2000, 2003).

To date, soil respiration and methane uptake rates from reclaimed soils have not been adequately measured or estimated, and the effect of CWD on soil respiration and methane uptake rates have not been studied in reclaimed areas of the Alberta Oil Sands Region. The FMM and PMM have different soil properties and plant species composition and thus CWD would affect soil physical conditions and microbial activity, affecting greenhouse gas emission rates in these cover soils.

2. Thesis Structure

The overall objective of this study is to determine the effects of CWD on soil biogeochemistry such as N cycling, microbial community function, extracellular enzyme activity and greenhouse gas emission rates in reclaimed oil sands soils amended with FMM or PMM. Four experiments were conducted, including three field experiments and one laboratory incubation experiment, to test the following hypotheses.

- Soil microbial community level physiological profile in FMM and PMM cover soils will be different due to different sources of organic matter (forest floor and peat) and vegetation cover in FMM and PMM
- Soil enzyme and microbial activities will be greater if amended with FMM than with PMM due to greater microbial biomass and vegetation cover in FMM
- Nitrogen mineralization rates and soil respiration rates in soils will be greater in FMM than in PMM due to greater soil enzyme and microbial activities and lower C:N ratio in FMM.
- Applying CWD will increase soil enzyme and microbial activities and microbial community function by increasing soil water content and by decreasing soil temperature fluctuation ranges under CWD, which provide more favourable habitats for soil microorganisms.
- Increased soil enzyme and microbial activities and microbial community function under CWD will increase soil respiration, N mineralization and nitrification rates in both cover soils.

- Coarse woody debris extract will decrease gross and net N mineralization and nitrification rates and will increase N immobilization rates due to chemical properties of CWD extract such as low pH, high C:N ratio and high lignin content.
- Methane uptake rates will be greater in FMM and without CWD due to their associated drier soil conditions.

Application of CWD will change physical, chemical and biological properties of soils. These changes are closely related and will affect N cycling and greenhouse gas emission rates in soils. Nitrogen is one of the most important nutrients for tree growth and N availability will affect revegetation time of disturbed land. Thus, this study is important to determine whether use of CWD can improve soil properties and to evaluate whether CWD is a valuable organic matter for land reclamation.

This thesis consists of six chapters. Figure 1-1 provides a visual display of the studies. Chapter 1 provides background information and a study overview. Chapter 6 provides a summary of key findings, general conclusions and suggested future research. Each of the data chapters 2 to 5 constitutes a manuscript that is currently under review with a peer reviewed journal or will be submitted for publication shortly.

Chapter 2 focuses on chemical effects of CWD extract on gross and net N transformation rates. A manuscript entitled “Coarse woody debris extract decreases nitrogen availability in two reclaimed oil sands soils in Canada” was accepted with the journal Ecological Engineering.

Chapter 3 focuses on effects of CWD on soil microbial community level physiological profile and enzyme activity. A manuscript entitled “Coarse woody debris increases microbial community function but not enzyme activities in reclaimed oil sands soils” is under review with the journal PLOS ONE.

Chapter 4 focuses on effects of changed microbial activities by CWD application on greenhouse gas emission rates from cover soils. A manuscript entitled “Coarse woody debris effects on CO_2 emission and CH_4 uptake rates in a reclaimed oil sands soil depend on cover soil type” is under review with the journal Applied Soil Ecology.

Chapter 5 focuses on effects of CWD on N cycling and N availability in the field condition. A manuscript entitled “Nitrogen transformation rates and availability were

affected by cover soil type but not by coarse woody debris in a reclaimed oil sands soil" is being developed for submission for publication.

Table 1-1 Amounts of CWD (downed logs) in natural forest ecosystems.

Disturbance Type	Study Location	Forest Type	Biomass (Mg ha^{-1})	Volume (m^3 ha^{-1})	Projected Area (%)	Reference‡
Wildfire	BC, Canada	*Pinus contorta*	99	-	15	1
Wildfire	Wyoming, USA	*Pinus contorta*	41-284	-	6-44	2
Wildfire	Oregon and Washington, USA	*Pseudotsuga menziesii*	52-123	250-534	8-19	3
Pine beetle	BC, Canada	*Pinus contorta, Pseudotsuga menziesii*	16-42	-	2-7	4
Bark beetle	Yellowstone, USA	*Pseudotsuga menziesii*	15-62	-	2-10	5
Bark beetle	Yellowstone, USA	*Pinus contorta*	18-90	-	3-14	5
Wind	Washington, USA	*Picea sitchensis/ Tsuga heterophylla*	120-161	-	19-25	6
Harvesting	BC, Canada	*Pinus contorta*	18-28	-	3-4	1
Harvesting	Wyoming, USA	*Pinus contorta*	52-123	-	8-19	2
Undisturbed	Washington, USA	*Abies amabilis*	80-232	-	12-36	7
Undisturbed	California, USA	*Mixed conifer*	29-400	-	5-62	8
Undisturbed	Colorado, USA	*Picea engelmannii/ Abies lasiocarpa*	71	-	11	9
Undisturbed	Wyoming, USA	*Pinus contorta*	29-121	-	5-19	2
Undisturbed	Alberta, Canada	*Populus tremuloides*	-	94-112	3-4	10

‡ [1]Wei et al., 1997; [2]Tinker and Knight, 2000; [3]Spies et al., 1988; [4]Dordel et al., 2008; [5]Donato et al., 2013; [6]Graham and Gromack, 1982; [7]Grier et al., 1981; [8]Harmon et al., 1987; [9]Arthur and Fahey, 1990; [10]Lee et al., 1997

Table 1-2 Classification of decay class for coarse woody debris.

Characteristics	Decay Classes				
	1	2	3	4	5
Structural integrity	Sound	Sapwood slightly rotting, heartwood sound	Sapwood missing, heartwood mostly sound	Heartwood decayed	Soft
Leaves	Present	Absent	Absent	Absent	Absent
Branches	All twigs present	Larger twigs present	Larger branches present	Branch stubs present	Absent
Bark	Intact	Intact	Trace	Absent	Absent
Texture	Intact	Intact to partly soft	Hard, large pieces	Small, soft blocky pieces	Soft and powdery
Bole shape	Round	Round	Round	Round to oval	Oval to flat
Color of wood	Original color	Original color	Original color to faded	Light brown to reddish brown	Red brown to dark brown
Portion of tree on ground	Tree elevated on support	Tree elevated on support	Tree sagging near ground	All of tree on ground	All of tree on ground
Invaded by roots	No	No	Sapwood area	Throughout	Throughout
Vegetation growing	No	Little vegetation growing	Few shrubs, seedlings and mosses	Shrubs, mossed and trees	Shrubs, mossed and trees
Indirect measure	Cambium still fresh, dies < 1year	Cambium decayed, knife blade penetrates a few mm	Knife blade penetrates < 2 cm	Knife blade penetrates 2 to 5 cm	Knife blade penetrates all the way

Adapted from Maser et al., 1979 and Yan et al., 2006.

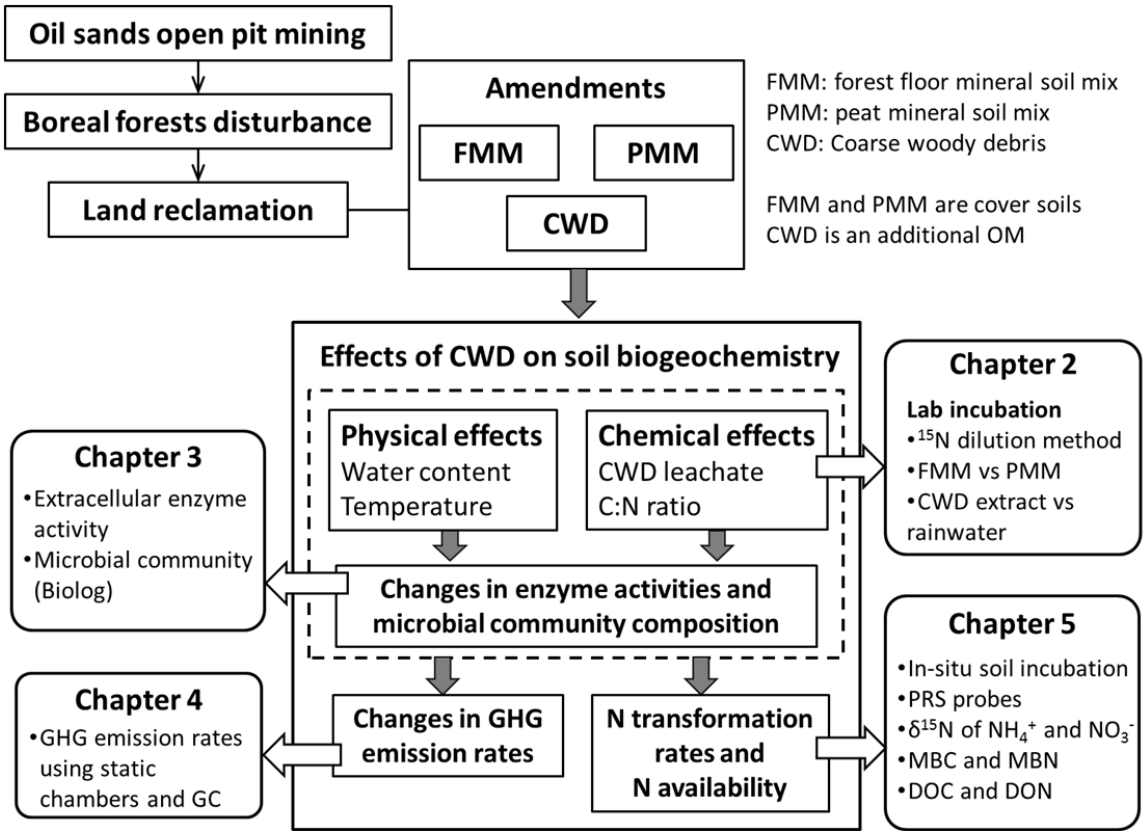

Figure 1-1 Flow chart of the study. Each box indicates treatments or changes of CWD application. Gray arrows indicate effects of treatments or changes on other parameters. Open (white) arrows indicate each experiment.

CHAPTER 2. COARSE WOODY DEBRIS EXTRACT DECREASES NITROGEN AVAILABILITY IN TWO RECLAIMED OIL SANDS SOILS IN CANADA

1. Introduction

Open pit mining in the Athabasca oil sands region (AOSR) has disturbed 767 km^2 of boreal mixedwood forests by 2013 in Alberta, Canada and 4,800 km^2 of land could be open-pit mined (Government of Alberta, 2013). Such disturbed land must be returned to ecosystems with land capability equivalent to pre-disturbance levels (Province of Alberta 2014). Applying forest floor mineral soil mix (FMM) or peat mineral soil mix (PMM) as cover soils is the most common practice for land reclamation after oil sands mining (Depuit, 1984; Sydnor and Redente, 2002) and is beneficial for increasing organic matter (OM) content, improving soil fertility and water holding capacity (WHC) and providing a source of propagules and soil microorganisms (Depuit, 1984; Sydnor and Redente, 2002; Mackenzie and Naeth, 2010). The PMM has high OM content and WHC and is readily available in northern Alberta (Fung and Macyk, 2000). The FMM has a large propagule bank and applying it in land reclamation increases native plant abundance and diversity (Mackenzie and Naeth, 2010). The FMM is considered more readily decomposable than PMM because of its lower carbon to nitrogen (C:N) ratios (Mackenzie and Naeth, 2010), greater microbial biomass (McMillian et al., 2007; Brown, 2010; Hahn and Quideau, 2013) and greater enzyme activities (Brown, 2010; Jamro et al., 2014).

Coarse woody debris (CWD), which is dead woody material, including standing dead trees, downed boles, large branches and dead coarse roots (Harmon et al., 1986), plays an important role in forest ecosystems by providing habitat for planted tree seedlings, microorganisms and small animals, reducing soil erosion and nutrient leaching and increasing nutrient and OM content (Harmon et al., 1986; Stevens, 1997). As CWD has important ecological values, applying it for land reclamation after oil sands mining can help

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rebuild disturbed ecosystems. However, applying CWD in reclamation of disturbed oil sands land is a relatively new practice with few data on its benefit. The application of CWD for reclamation can reduce soil erosion (Whisenant, 2005) and increase vegetation cover and woody plant density in oil sands mining reclamation by increasing microsites (Brown and Naeth, 2014). No study has been conducted on effects of CWD on soil properties in reclaimed oil sands soils and most studies have been conducted in natural forests with CWD produced by natural disturbances. Nutrient cycling and enzyme activities under CWD were tested in forests in North America (Spears et al., 2003; Laiho and Prescott 2004; Hafner and Groffman, 2005), Australia (Lindsay and Cunningham, 2011; Goldin and Hutchinson, 2013) and Argentina (Gonzalez-Polo et al., 2013) but all these studies were in natural ecosystems.

Available nitrogen (N) in soils is the most likely limiting factor for tree growth in the boreal forest ecosystem and N turnover rate is closely related to plant growth and forest productivity (Gundersen et al., 2009; Yan et al., 2012; Duan et al., 2015). Coarse woody debris will affect N cycling mainly in two ways: 1) it will change the physical environment, such as soil temperature and water content, resulting in changes in microbial and enzyme activities and N cycling and 2) CWD leachate will change soil chemical properties such as C:N ratio, dissolved organ C (DOC) and N (DON) concentrations; such changes will affect microbial processed and N cycling. Due to these different pathways, effects of CWD on N transformation rates or availability can be quite variable. For example, total N and nitrate (NO_3^-) concentrations increased under CWD in woodlands in Australia by greater mineralization associated with increased moisture availability under CWD (Lindsay and Cunningham, 2011; Goldin and Hutchinson, 2013). However, CWD application decreased N concentrations in forests in North America (Busse, 1994; Hafner and Groffman, 2005), N mineralization and nitrification rates in mixed forest in New York State (Hafner and Groffman, 2005) and gross N mineralization rates in an old-growth mixed coniferous forest in Oregon, USA (Spears et al., 2003) mainly due to increased CWD leachate with a high C:N ratio and intercept of litterfall by CWD.

Measuring gross N transformation rates using the ^{15}N isotopic pool dilution method can provide more detailed information on N cycling processes (Hart et al., 1994; Murphy et al., 2003; Booth et al., 2005). However, most studies measured net N transformation rates, which may underestimate gross N transformation rates and confound several simultaneous

processes (Davidson et al., 1991; Murphy et al., 2003). The ^{15}N isotopic pool dilution method permits independent estimation of each N transformation process including microbial assimilation of ammonium (NH_4^+) or NO_3^- (Davidson et al., 1991, 1992; Stark and Hart, 1997). Furthermore, a laboratory incubation experiment allows us to test only the effect of the CWD extract without confounding effects of changing soil temperature and water content in the field. This study focused on direct effects of CWD leachate on gross N transformation rates in reclaimed soils and will help to interpret N cycling under CWD in the field.

A laboratory incubation experiment using ^{15}N isotopic dilution was conducted to evaluate the effect of CWD leachate on N transformation rates in reclaimed oil sands soils such as FMM and PMM types of cover soils and to determine whether using CWD for land reclamation is beneficial for improving N availability. This study was specifically designed to improve our understanding of the effects of CWD leachate chemistry on soil processes. I hypothesized that 1) gross N mineralization and nitrification rates in FMM would be greater than that in PMM due to its higher microbial and enzyme activities and lower C:N ratio in FMM and 2) the CWD extract addition will increase N immobilization rates due to its high C:N ratio and would decrease nitrification rates and net N mineralization rates.

2. Materials and Methods

2.1 Site description and soil sampling

The research site is located on an oil sands company lease, about 24 km north of Fort McMurray, Alberta, Canada. Average annual temperature and precipitation from 1981 to 2010 were 1.0 °C and 418.6 mm, respectively (Environment Canada, 2014). A total of 36 randomly located 10 × 30 m^2 plots were established between November 2007 and February 2008. Half of the plots were covered with FMM and the other half with PMM. The FMM was salvaged from a mesic aspen-white spruce mixed forest and applied at a depth of 20 cm, over 30 cm of B and C horizon mixed subsoil. The PMM was applied at a depth of 30 cm over 100 cm clean overburden. A detailed description of the research site and experimental plots is provided in Brown (2010) and Brown and Naeth (2014).

Soil samples from 0 to 10 cm depth were collected from six FMM and six PMM plots

with an auger in summer 2012. In the laboratory, soil samples were air dried and passed through a 2 mm sieve to remove plant roots and CWD, then combined to form a composite sample. Parts of samples were used for chemical analyses.

To simulate CWD leachate in the field, ground CWD was extracted with rainwater collected in the field. Aspen woody debris, of decay class 1 or 2 according to the classification system described in Master et al. (1988) and British Columbia Ministry of Environment and British Columbia Ministry of Forest (2010), was collected in a mixedwood forest near the study site and oven dried at 60 °C. Newly formed CWD was used for the experiment to simulate CWD initially applied for reclamation at the study site. Whole woody debris including bark, sapwood and heartwood was ground and passed through a 0.84 mm sieve. Rainwater was collected on site in summer 2012 using 1 L bottles and funnels with a screen (Jung et al., 2011) and then frozen until used for the experiment. Ground woody debris was extracted with the rainwater at a ratio of 1:10 (w:v) and shaken at 250 rpm on a mechanical shaker for 1 hour, then filtered through Whatman No. 42 filter papers.

2.2 Laboratory procedures

For the laboratory incubation experiment, 250 mL Nalgene HDPE bottles [2 (FMM vs PMM) × 2 (CWD extract vs rainwater) × 2 ($^{15}NH_4NO_3$ vs $NH_4{}^{15}NO_3$) × 3 replications × 5 samplings = 120 bottles] were prepared. A portion of an air dried soil (30 g, oven dry weight basis) was placed in a bottle and incubated for 7 days under 40% WHC. After 7 days, 2 mL $^{15}NH_4NO_3$ solution (10 atom %) or $NH_4{}^{15}NO_3$ solution (10 atom %) was added to the soil in each bottle, with an equivalent application rate of 20 mg N kg^{-1} soil. Half of the $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ bottles received 2 mL of CWD extract and the other half received 2 mL of rainwater; the added CWD extract and rainwater are referred to as added solutions. Final water content was adjusted to 60% WHC with rainwater and the total weight was recorded on each bottle. Bottles were incubated at 25 °C for 4 days and sampled every day to determine N transformation rates in each day. For gross N transformation rate measurements, $(^{15}NH_4)_2SO_4$ and $K^{15}NO_3$ have been commonly used rather than $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ (Davidson et al., 1991; Hart et al., 1994). However, applying different forms of N can cause changes in the partitioning among N transformation processes (Mary et al.,

1998; Murphy et al., 2003). Applying $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ enables all gross N fluxes to be consistently measured among all treatments thus permitting more robust comparisons between treatments (Murphy et al., 2003).

Bottles were covered with aluminum foil with tiny holes to allow aeration but to minimize water loss through evaporation. Rainwater was added every day to maintain water content and tiny weed sprouts were removed with tweezers before adding rainwater to minimize loss of N by plant uptake. Twenty four samples [2 (FMM vs PMM) × 2 (CWD extract vs rainwater) × 2 ($^{15}NH_4NO_3$ vs $NH_4{}^{15}NO_3$) × 3 replications] were destructively collected at 30 min, 1, 2, 3 and 4 days after ^{15}N addition.

Soil samples were extracted with 2 mol L^{-1} KCl solution at a 1:5 (w:v) soil-to-extractant ratio and shaken at 250 rpm on a mechanical shaker for 1 hour, then filtered through Whatman No. 42 filter papers. An aliquot of 50 mL of each extract was steam distilled using MgO and Devarda's alloy sequentially on a steam distillation system (Vapodest 20, C. Gerhardt, Königswinter, Germany) to determine NH_4^+ and NO_3^- concentrations, respectively. The liberated NH_3 was collected in 0.005 mol L^{-1} H_2SO_4 solutions (Keeney and Nelson, 1982). To prevent isotopic cross contamination among samples, 25 mL reagent grade ethanol was added to distillation tubes and steam distilled for 3 min between each sample distillation (Hauck, 1982). Ammonium and nitrate concentrations were determined by titration with 0.01 mol L^{-1} NaOH potentiometrically using an auto titrator (719 S Titrino, Brinkmann Metrohm, USA). The distillates containing NH_4^+ were dried at 60 °C after acidifying to pH 3 with 0.05 mol L^{-1} H_2SO_4 (Feast and Dennis, 1996) and analyzed for ^{15}N using a stable isotope ratio mass spectrometer (Optima-EA; Micromass, Crewe, UK) linked to a CN analyzer (NA series 2, CE instruments, Italy).

Soil pH was measured in a 1:5 (w:v) soil-to-water ratio using a pH meter (DMP-2 mV, Thermo Orion, USA). A portion of the soil and CWD samples were ground with a ball mill and passed through a 0.15 mm sieve and analyzed for total N (TN) and total C (TC) concentrations using a Carlo Erba NA 1500 elemental analyzer (Carlo Erba Instruments, Milano, Italy). To analyze DOC and DON concentrations, air dried soil samples (5 g, oven dry weight basis) were weighed, then extracted with 50 mL of distilled water. After filtration, concentrations of C and N in the filtrate were measured with a TOC/TN analyzer (TOC-V_{CSN}, Shimadzu, Kyoto, Japan). Total C and N concentrations in the CWD extract

and rainwater were measured using the TOC/TN analyzer.

2.3 Calculations and statistical analyses

Gross and net N transformation rates were calculated for each incubation interval (1 day) and the entire incubation period (time weighted average for 4 days). Nitrogen transformation rates in the cover soils added with the rainwater were used to compare N transformations in FMM and PMM. Net N mineralization and nitrification rates were calculated as differences between initial and final inorganic N ($NH_4^+ + NO_3^-$) or NO_3^- pool sizes, respectively, divided by incubation period. Gross N transformation rates such as N mineralization, nitrification, NH_4^+ consumption and NO_3^- consumption rates were calculated following Kirkham and Bartholomew (1954) and Hart et al. (1994). Gross NH_4^+ immobilization rates were calculated as the difference between gross NH_4^+ consumption and gross nitrification rates. Gross NO_3^- consumption rates are the sum of microbial immobilization and possibly denitrification. However, denitrification rates are assumed to be negligible under 60% WHC in the short term incubation conducted in this study. Therefore, NO_3^- consumption is often reported directly as NO_3^- immobilization rates (Murphy et al., 2003).

Two-way ANOVAs were used to evaluate effects of cover soil and added solution types and their interaction on gross and net N transformation rates. All pair wise comparisons among treatments were done with Tukey's multiple comparison test. Before performing the ANOVA, normality of distribution and homogeneity of variance were checked with Kolmogorov-Smirnov and Levene's tests and data were log-transformed if necessary. All data were analyzed using the SAS 9.2 software (SAS Institute Inc., Cary, NC) and an α value of 0.05 was chosen to indicate statistical significance.

3. Results

3.1 Changes in concentrations and ^{15}N enrichments of mineral N

The NH_4^+ concentrations decreased during the incubation regardless of cover soil and added solution type (Figure 2-1a), whereas NO_3^- concentrations significantly increased in FMM and remained unchanged in PMM ($p>0.05$; Figure 2-1b). The NH_4^+ concentrations sharply decreased with the CWD extract addition in the first day of incubation in both FMM and

PMM and decreased gradually thereafter (Figure 2-1a and b). In contrast, NO_3^- concentrations in the soil with the CWD extract addition decreased slightly over time in the first two days and increased over time thereafter; NO_3^- concentrations in both FMM and PMM were lower in the CWD extract than in the rainwater treatment. The NO_3^- concentrations increased more sharply in FMM than in PMM and with the rainwater than with the CWD extract addition treatment, indicating that nitrification rates were greater in FMM than in PMM and in the rainwater than in the CWD extract treatment. Significant effects of cover soil ($p<0.001$), added solution type ($p<0.001$) and their interaction ($p=0.027$) on NH_4^+ and NO_3^- concentrations were observed (Table 2-2).

In the $^{15}NH_4NO_3$ labeled samples, the ^{15}N enrichment of the NH_4^+ pool decreased and that of the NO_3^- pool correspondingly increased regardless of cover soil or added solution type (Figure 2-2a and c). The ^{15}N enrichment of the NH_4^+ pool in PMM with CWD extract addition decreased sharply during the first day of incubation (Figure 2-2a) and then remained stable from day 1 to day 4, indicating that the abundance of the added $^{15}NH_4NO_3$ was not adequate for the incubation between day 1 and 4. The ^{15}N isotopic excess in NO_3^- increased over time in all treatments due to nitrification of ^{15}N labeled NH_4^+ (Figure 2-2c) and that it increased faster with the rainwater than with the CWD extract treatment, indicating that nitrification rates in the rainwater treatment were greater than that in the CWD extract treatment. In $NH_4{}^{15}NO_3$ labeled samples, ^{15}N enrichment of the NO_3^- pool continuously decreased during the 4 day incubation in all treatments (Figure 2-2d), likely due to dilution of $^{15}NO_3^-$ by the influx of NO_3^- from the nitrification of unlabeled NH_4^+. The ^{15}N enrichment of NH_4^+ decreased or remained relatively stable over time (Figure 2-2b), suggesting that there was no re-mineralization of immobilized $^{15}NO_3^-$ during the incubation.

3.2 N transformation rates

Gross N mineralization rates in PMM were greater than those in FMM but the differences were not statistically significant. Addition of the CWD extract significantly decreased gross N mineralization rates ($p<0.01$, Figure 2-3a) in both FMM and PMM. Gross N mineralization rates were greater in the CWD extract than in the rainwater addition treatment in the first day of incubation ($p<0.001$); rates were 1.6 and 5.7 times greater in the CWD extract than in the rainwater addition treatment for FMM and PMM, respectively

(Figure 2-3a). However, the CWD extract addition decreased gross N mineralization rates in the last three days of incubation ($p<0.001$); rates were 2.1 and 8.0 times greater in the rainwater than that in the CWD extract addition treatment for FMM and PMM, respectively (Figure 2-3a). Net N mineralization rates were positive and were greater in FMM than in PMM with the rainwater addition or were less negative in FMM than in PMM with the CWD extract addition (Figure 2-3c). Net N mineralization rates were greater with the rainwater addition than those with the CWD extract addition during incubation regardless of cover soil type ($p<0.001$) (Figure 2-3c). There were significant effects of cover soil ($p<0.001$) and added solution ($p<0.001$) type on net N mineralization rates, but there were no significant interactions between cover soil and added solution types (Table 2-2).

Gross nitrification rate was the highest in FMM with rainwater addition, followed by PMM with rainwater addition, FMM with CWD extract addition, and PMM with CWD extract addition (Figure 2-3b), with the rates significantly affected by cover soil ($p<0.001$), added solution ($p<0.001$) type and their interaction ($p<0.001$). Gross and net nitrification rates were 1.8 and 2.1 times greater, respectively, in FMM than in PMM ($p<0.001$). Addition of the CWD extract decreased gross nitrification rates in FMM and PMM by 62 and 45%, respectively, during incubation.

The NH_4^+ immobilization rates were significantly greater in PMM than in FMM, greater in the CWD extract than in the rainwater addition treatment (Figure 2-3e) and highest in the first day of incubation due to the increased NH_4^+ availability by mineralization. There were significant effects of cover soil ($p<0.001$) and added solution ($p<0.001$) types on NH_4^+ immobilization rates but there was no interaction between cover soil and added solution types. The NO_3^- immobilization rates showed similar patterns with gross nitrification rates (Figure 2-3f) indicating produced NO_3^- by nitrification were immobilized by microorganisms rather than immobilization of existing NO_3^-. The NO_3^- immobilization rates were lower than gross nitrification rates and cover soil ($p<0.001$) and added solution type ($p<0.001$) significantly affected NO_3^- immobilization rates.

4. Discussion

4.1 Greater N availability in FMM than in PMM

Effects of soil properties such as soil pH (Cookson et al., 2007; Cheng et al., 2013), C:N ratio (Manzoni et al., 2008, 2010), soil temperature (Lang et al., 2010) and water content (Chen et al., 2011; Cheng et al., 2012) on N transformation rates are well understood. Soil pH is positively correlated with gross N mineralization rates between pH 3.5 and 7 (Cookson et al., 2007; Cheng et al., 2013). In this study, pH was greater in PMM than in FMM, which may cause greater gross N mineralization rates in PMM. However, pH of FMM and PMM were within the optimum range for N mineralization (pH 6-8; Paul and Clark, 1989) and the effect of soil pH on gross N mineralization in FMM and PMM would be small. The C:N ratio of substrates is an index of quality of substrate and determines net mineralization and net immobilization rates (Manzoni et al., 2008, 2010). The C:N ratio of PMM is usually much higher than that of FMM (Brown, 2010; Mackenzie and Naeth, 2010) during the first several years after application of the cover soils. Nitrogen mineralization rate might be greater in FMM than in PMM at the initial stage of reclamation; however, that has not been tested. Cover soil samples in this study were collected four years after establishment of the reclamation plots and C:N ratios of FMM and PMM were similar. Similar substrate qualities such as pH and C:N ratio provided the same potential for the heterotrophic microbial community to mineralize organic N (McMillan et al., 2007) resulting in lack of differences in gross N mineralization rates between FMM and PMM. This indicates that gross N mineralization rates became similar between PMM and FMM as time since reclamation increased and soil properties in the reclamation sites converged.

I expected that N immobilization rates would be similar between the two cover soils because of the similar C:N ratios of the two cover soils. However, NH_4^+ immobilization rates were significantly greater in PMM likely due to greater DOC:DON ratio in PMM (Table 2-1). The DOC and DON are key OM pools determining N cycling (Cookson and Murphy, 2004; Jones et al., 2004) and DOC:DON ratio is an important indicator of N transformation rates (Cookson et al., 2007). Greater DOC:DON ratio in PMM could induce greater NH_4^+ immobilization rate in PMM than in FMM.

One study conducted in the AOSR found that gross nitrification rates were lower in PMM than in FMM (McMillian et al., 2007) and results from this study agree with that study. Activity of nitrifying bacteria and amount of substrate (NH_4^+) for nitrification are main determinants for gross nitrification rates (Davidson et al., 1992; Ste-Marie and Pare,

1999; Cheng et al., 2013) and can be affected by abiotic factors such as soil pH, temperature and water content. The FMM had higher microbial biomass (McMillan et al., 2007; Brown, 2010; Hahn and Quideau, 2013; Jamro et al., 2014) and enzyme activity (Brown, 2010; Jamro et al., 2014) than those in PMM in the AOSR. Overall greater microbial biomass and enzyme activity could cause greater gross and net nitrification rates in FMM than in PMM. In this study, soil water content and temperature were kept constant during incubation. Nitrification rate was generally positively related to soil pH (DeBoer et al., 1996; Ste-Marie and Pare, 1999; Cheng et al., 2013) and was suppressed at low pH, for example, below 4.5 (Hunik et al., 1992; Boer and Kowalchuk, 2001). Soil pH of FMM and PMM were similar and close to neutral leading to the lack of difference in nitrification rates between the two cover soils. Therefore, greater gross and net nitrification rates in FMM were mainly derived from inherently greater microbial and enzyme activities in FMM.

The ratio of gross nitrification and gross NH_4^+ immobilization rates (N/IA) has been used to quantify competition of the two microbial NH_4^+ consumption processes, such as gross nitrification and gross immobilization and to determine the potential risk of NO_3^- loss (Booth et al., 2005; Hoyle et al., 2006). The greater N/IA ratio in FMM than in PMM (Figure 2-4) indicates that greater NH_4^+ immobilization rates in PMM decreased NH_4^+ availability for nitrifying bacteria and lowered nitrification rates in PMM than in FMM. Increases in gross nitrification rates over time during incubation in FMM could be attributed to increasing nitrifying bacteria population over time. The amount of NH_4^+ produced by mineralization was kept relatively stable over time and other conditions such as pH, temperature and water content were stable. However, gross nitrification rates in PMM seem to be affected by NH_4^+ availability rather than nitrifying bacteria activity, showing similar patterns of gross N mineralization and gross nitrification during incubation (Figure 2-3). Microbial heterotrophs are strong competitors for NH_4^+ relative to autotrophic nitrifiers (Vitousek et al., 1982; Hart et al., 1994) and this competition would have been stronger in PMM than in FMM due to higher DOC:DON ratios in PMM. Also, heterotrophic assimilation of NH_4^+ could dominate over nitrification because of rapid growing heterotrophic microbial population as compared to autotrophic nitrifiers (Hart et al., 1994). Applying FMM for land reclamation after oil sands mining can be more beneficial for N availability showing greater nitrification and less N immobilization rates in FMM although

gross N mineralization rates between FMM and PMM were similar several years after their application.

4.2 Decreases in gross N mineralization and nitrification rates by the CWD extract addition

Addition of the CWD extract surprisingly increased gross N mineralization rates in the first day of incubation in both FMM and PMM mainly due to the priming effect of added CWD extract. Addition of fresh C in the CWD extract stimulated microbial activity and then accelerated decomposition of soil OM (Kuzyakov et al., 2000; Fontaine et al., 2003) in both cover soils. Availability of DOC and DON is one determinant of N cycling in soils (Cookson and Murphy, 2004; Jones et al., 2004). The amounts of DOC and DON of soils in this study were similar, indicating amounts of mineralizable OM in the short term incubation were similar. Markedly high gross N mineralization rates in the first day of incubation may consume a large amount of mineralizable OM; gross N mineralization in the first day of incubation accounts for 45 and 80% in FMM and PMM, respectively, of whole gross N mineralization during entire incubation (Figure 2-3) and thereafter, deplete the substrate for decomposition, which could decrease gross mineralization rates. A negative relationship between C:N ratio of substrates and gross N mineralization rates (Hart et al., 1994) and high C:N ratio of added CWD extract could also decrease gross N mineralization rates from the second day of incubation. A study conducted in a mixed coniferous forest in Oregon showed greater gross N mineralization rates in mineral soils collected from an area without CWD than those under CWD (Spears et al., 2003) mainly due to its high C:N ratio of CWD leachate.

Gross N mineralization rates varied depending on forest type and detritus C quality was a key determinant of N turnover rates (Campbell and Gower, 2000). High C:N ratio of substrates increases N immobilization rates and decreases N availability (Hart et al., 1994; Manzoni et al., 2008, 2010; Cheng et al., 2013). Nitrogen concentrations decreased under CWD in forests in northern America (Busse, 1994; Hafner and Groffman, 2005) and CWD application decreased NO_3^- concentrations in reclaimed oil sands soils (Brown and Naeth, 2014). Addition of labile forms of C source caused greater N immobilization than addition of recalcitrant C substrate (Magill and Aber, 2000). The CWD extract used in this study is

water soluble labile C and N and it increased N immobilization rates in both FMM and PMM. Increases in the amount of CWD leachate in the field would increase N immobilization rates and decrease N availability.

Soil pH is a major determinant of nitrification rates (DeBoer et al., 1996; Ste-Marie and Pare, 1999; Cheng et al., 2013). The activity of acid sensitive bacteria (DeBoer et al., 1989, 1990) and ammonium oxidizing and nitrite oxidizing bacteria (Hunik et al., 1992; Boer and Kowalchuk, 2001) were suppressed under low pH resulting in decreased gross and net nitrification rates (Ste-Marie and Pare, 1999; Cheng et al., 2013). In this study, pH of added CWD extract is low compared to FMM, PMM, or rainwater. Although adding 2 mL of CWD extract would not markedly change soil pH, the pH of the CWD extract was lower than that of rainwater, indicating that acid sensitive nitrifying bacteria could be suppressed by the CWD extract addition as compared to rainwater addition. Another potential reason for the low gross and net nitrification rates with the CWD extract addition may be the high C:N ratio in the added CWD extract. High C:N ratio of substrates can increase NH_4^+ immobilization rates by decreasing NH_4^+ availability for nitrifying bacteria, resulting in low gross nitrification rate (Christenson et al., 2009; Cheng et al., 2011). Microbial heterotrophs (immobilization) are generally more competitive for NH_4^+ consumption than autotrophic nitrifies (nitrification) (Vitousek et al., 1982; Tietema and Wessel, 1992). Although gross N mineralization rates in cover soils with the CWD extract addition in the first day of incubation were higher than that with the rainwater addition, gross nitrification rates were greater in the rainwater addition than in the CWD extract addition treatment in both FMM and PMM (Figure 2-3b). This is mainly due to high NH_4^+ immobilization rates in the first day of incubation in the CWD extract addition treatment (Figure 2-3e). The N/IA ratios were lower with CWD extract addition than those with the rainwater addition in both FMM and PMM during the entire incubation (Figure 2-4), indicating that the CWD extract stimulated NH_4^+ immobilization rates and decreased nitrification rates.

4.3 Quality of CWD, ^{15}N abundance and incubation period affected N transformation rates

The quality of CWD represented by its C:N ratio, lignin to N ratio and cellulose and lignin concentration varies with decay class and tree species (Harmon et al., 1986; Means et al.,

1992). Nitrogen concentration in CWD usually increases during decomposition, decreasing the C:N ratio (Laiho and Prescott, 2004). Cellulose and hemicellulose decay faster than lignin (Harmon et al., 1986; Means et al., 1985), leading to greater lignin/cellulose and lignin/N ratios. Coniferous species generally have higher lignin content than deciduous species (Harmon et al., 1986). Lignin content, lignin:N ratio and C:N ratio are important OM quality indicators that affect OM decomposition rates (Taylor et al., 1989). I used relatively fresh aspen CWD in this study; the effects of CWD of different decay classes and different species on N transformation rates need to be studied to understand long-term effects of CWD on N transformation rates in cover soils. Furthermore, CWD extract used in this study may have different chemical composition with CWD leachate produced in the field. Although chemical composition is different between artificially and naturally produced CWD leachate, overall effects on N transformation in soils will be similar if tree species and decay class are same between artificial and natural CWD leachate.

Gross N mineralization rates in PMM with the CWD extract addition could be underestimated because the abundance of the added $^{15}NH_4NO_3$ (10 atom %) was not adequate according to changes in ^{15}N abundance of NH_4^+. Gross N mineralization rates from day 1 to day 4 could not be determined as the added $^{15}NH_4$ was all consumed the first day of incubation. Therefore, using highly enriched ^{15}N is recommended to maintain sufficient enrichment during the entire incubation period (Murphy et al., 2003).

The length of incubation and sampling interval are also important factors for interpreting the results. If I conducted a 24- or 48-hour incubation experiment of most common practices, I could conclude that gross N mineralization rates were greater with the CWD extract addition; rates were 1.6 and 5.7 times greater with the CWD extract addition than with the rainwater addition in FMM and PMM, respectively, in a 24-hour incubation, and 1.9 and 2.3 times greater, respectively, in a 48-hour incubation (Figure 2-3a). However, results of 4-day incubation showed similar gross N mineralization rates among treatments and the 1-day sampling interval helped to reveal detailed N transformation rates. Although gross N mineralization rates in PMM with the CWD extract addition could be underestimated, I could find similar patterns of N transformation rates in FMM and PMM with the CWD extract addition with 1-day sampling interval. Four-day incubation still shows a relative initial stage of N transformation processes. Long-term incubation

experiments need to be conducted to evaluate the long-term effects of CWD extract addition on N transformation rates.

5. Conclusions

The FMM was a better cover soil than PMM for its greater N availability. Gross nitrification rates were greater in FMM than in PMM, associated with greater microbial biomass and enzyme activity in FMM, while gross NH_4^+ immobilization rates were greater in PMM than in FMM, due to greater DOC:DON ratio in PMM. Fresh aspen CWD extract addition to cover soils decreased N availability by decreasing net N mineralization and nitrification rates and by increasing gross NH_4^+ immobilization rates in both cover soils, mainly due to the high C:N ratio and low pH of the CWD extract. Although chemical properties of CWD extract used in this study may be different with CWD leachate produced in the fields and actual N transformation rates could differ between this study and field conditions, results from this study provides information of effects of CWD leachate on N transformation processes over time. Results from this study suggest that using FMM as a cover soil for land reclamation can increase N availability relative to using PMM as a cover soil and CWD application can decrease N availability through production of leachates from CWD. However, CWD application may still benefit land reclamation by controlling soil erosion, providing microsites, increasing plant species diversity and soil moisture availability, and regulating soil temperature changes. More research is needed to examine long-term effects of cover soil type and CWD on N cycling in the field and to design strategies for using CWD for land reclamation. The policy implication based on this study is that the application of CWD should be encouraged, where feasible, to improve land reclamation practices and to promote early ecosystem development.

Table 2-1 Chemical properties of forest floor mineral soil mix (FMM), peat mineral soil mix (PMM), CWD extract and rainwater used in the incubation experiment.

Properties	pH	NH_4^+-N	NO_3^--N	DOC	DON	DOC: DON	TC	TN	C:N
		(mg kg^{-1})					(g kg^{-1})		
FMM	6.13 (0.26)	18.1 (1.2)	21.2 (1.5)	409.4 (15.9)	27.9 (0.8)	15 (0.1)	47.5 (2.5)	2.1 (0.1)	22 (0.4)
PMM	6.97 (0.24)	2.4 (0.3)	16.0 (0.3)	453.1 (31.2)	19.8 (1.1)	23 (0.6)	88.5 (9.6)	3.4 (0.4)	26 (0.1)
		(mg L^{-1})					(mg L^{-1})		
CWD extract	4.56 (0.01)	0.49 (0.06)	0.00 (0.00)	2571.6 (4.5)	13.5 (0.4)	190 (5.2)	2571.6 (4.5)	14.0 (0.6)	184 (4.6)
Rainwater	5.61 (0.01)	0.34 (0.04)	0.00 (0.00)	18.7 (0.1)	0.3 (0.0)	59 (0.6)	18.7 (0.1)	0.6 (0.0)	32 (0.5)

Means are followed by SEs in brackets (n=3).
DOC= dissolved organic carbon, DON= dissolved organic nitrogen, TC= total carbon and TN= total nitrogen

Table 2-2 ANOVA results (p values) for the effects of cover soil type (FMM and PMM), added solution type (CWD extract and rainwater) and incubation interval on NH_4^+-N, NO_3^--N, atom% excess of NH_4^+-N (APE-A) and NO_3^--N (APE-N), gross N mineralization (m), nitrification (n), NH_4^+ immobilization (*i*a), NO_3^- immobilization (*i*n), net N mineralization (net-m) and net nitrification (net-n) rates.

Factor	NH_4^+-N	NO_3^--N	APE-A	APE-N	m	n	*i*a	*i*n	net-m	net-n
Transformation rates at each incubation interval										
Cover soil (S)	<0.001	<0.001	0.006	0.049	<0.001	<0.001	<0.001	<0.001	0.011	<0.001
Added solution (A)	<0.001	<0.001	0.232	0.010	0.065	<0.001	<0.001	<0.001	<0.001	<0.001
Incubation interval (I)	<0.001	<0.001	<0.001	0.048	<0.001	0.0001	<0.001	<0.001	<0.001	<0.001
S×A[a]	<0.001	0.027	0.758	0.005	0.001	0.006	0.335	0.010	0.695	0.606
S×I	<0.001	<0.001	<0.001	0.991	<0.001	<0.001	<0.001	<0.001	0.130	0.042
A×I	<0.001	<0.001	0.012	0.919	<0.001	0.001	<0.001	0.001	<0.001	<0.001
S×A×I	<0.001	0.004	0.136	0.828	<0.001	0.199	<0.001	0.212	0.249	0.001
Time weighted average transformation rates (0-4d)										
Cover soil	NA	NA	NA	NA	0.071	<0.001	<0.001	<0.001	<0.001	<0.001
Added solution	NA	NA	NA	NA	0.005	<0.001	<0.001	<0.001	<0.001	<0.001
S×A	NA	NA	NA	NA	0.008	<0.001	<0.001	<0.001	0.332	0.291

NA: not applicable

[a]S×A, S×I, A×I and S×A×I indicate cover soil × added solution, cover soil × incubation interval, added solution × incubation interval and cover soil × added solution × incubation interval interactions, respectively.

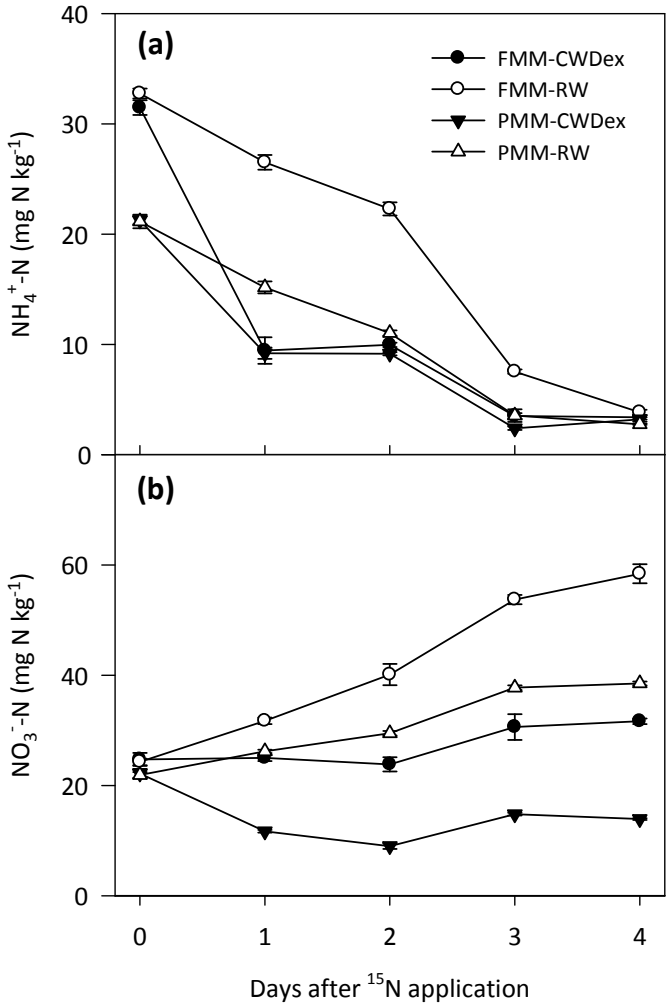

Figure 2-1 Changes in a) NH_4^+-N concentrations (mg N kg^{-1} soil) and b) NO_3^--N concentrations over a 4-day period incubated with CWD extract (CWDex) and rainwater (RW) in forest floor mineral soil mix (FMM) and peat mineral soil mix (PMM). As changes in NH_4^+ and NO_3^- concentrations over time followed the same trends for both the $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ addition treatments, averaged NH_4^+ and NO_3^- concentrations of the $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ used. Vertical bars are standard error of the mean (n=6).

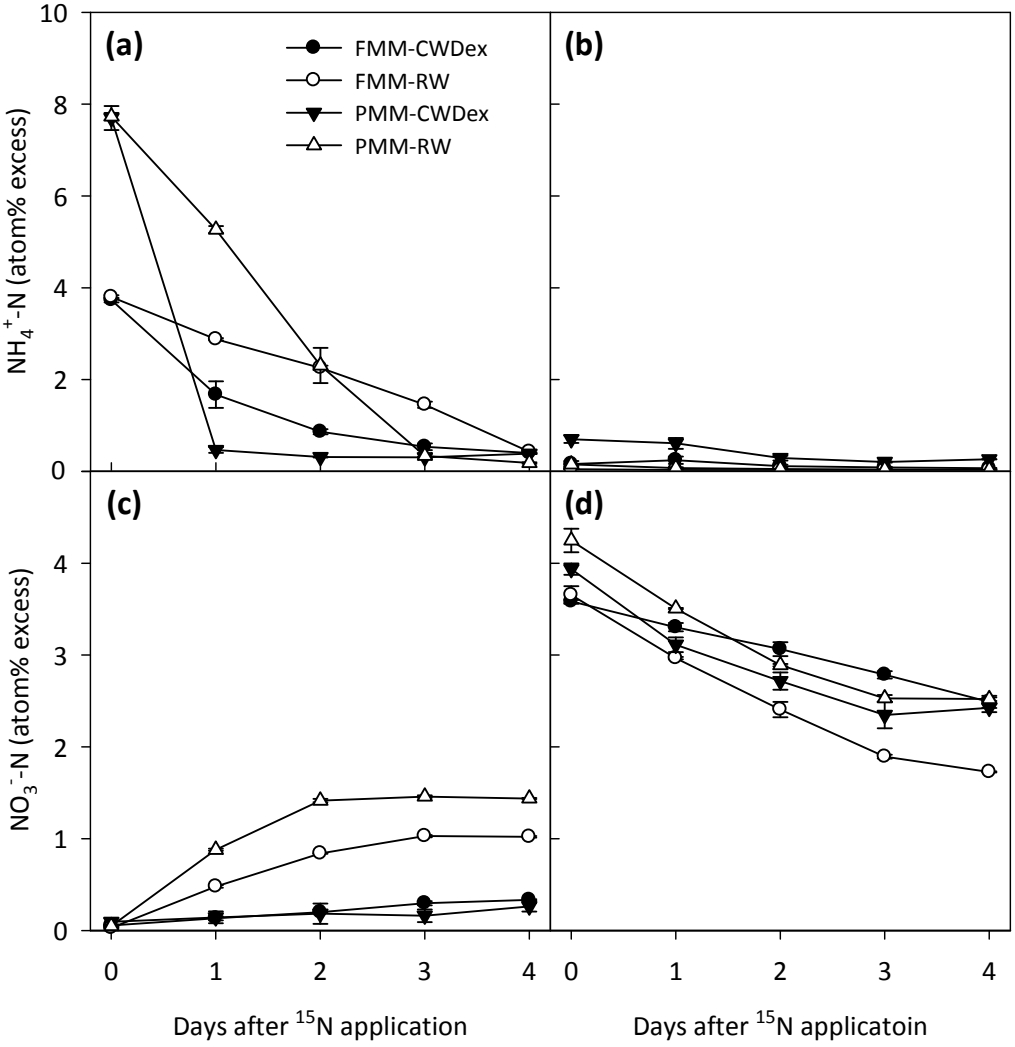

Figure 2-2 Changes in ^{15}N abundance (atom% excess) of NH_4^+-N in the a) $^{15}NH_4NO_3$ and b) $NH_4{}^{15}NO_3$ and ^{15}N abundance of NO_3^--N in the c) $^{15}NH_4NO_3$ and d) $NH_4{}^{15}NO_3$ over a 4-day period incubated with CWD extract (CWDex) and rainwater (RW) in forest floor mineral soil mix (FMM) and peat mineral soil mix (PMM). Vertical bars are standard error of the mean (n=3).

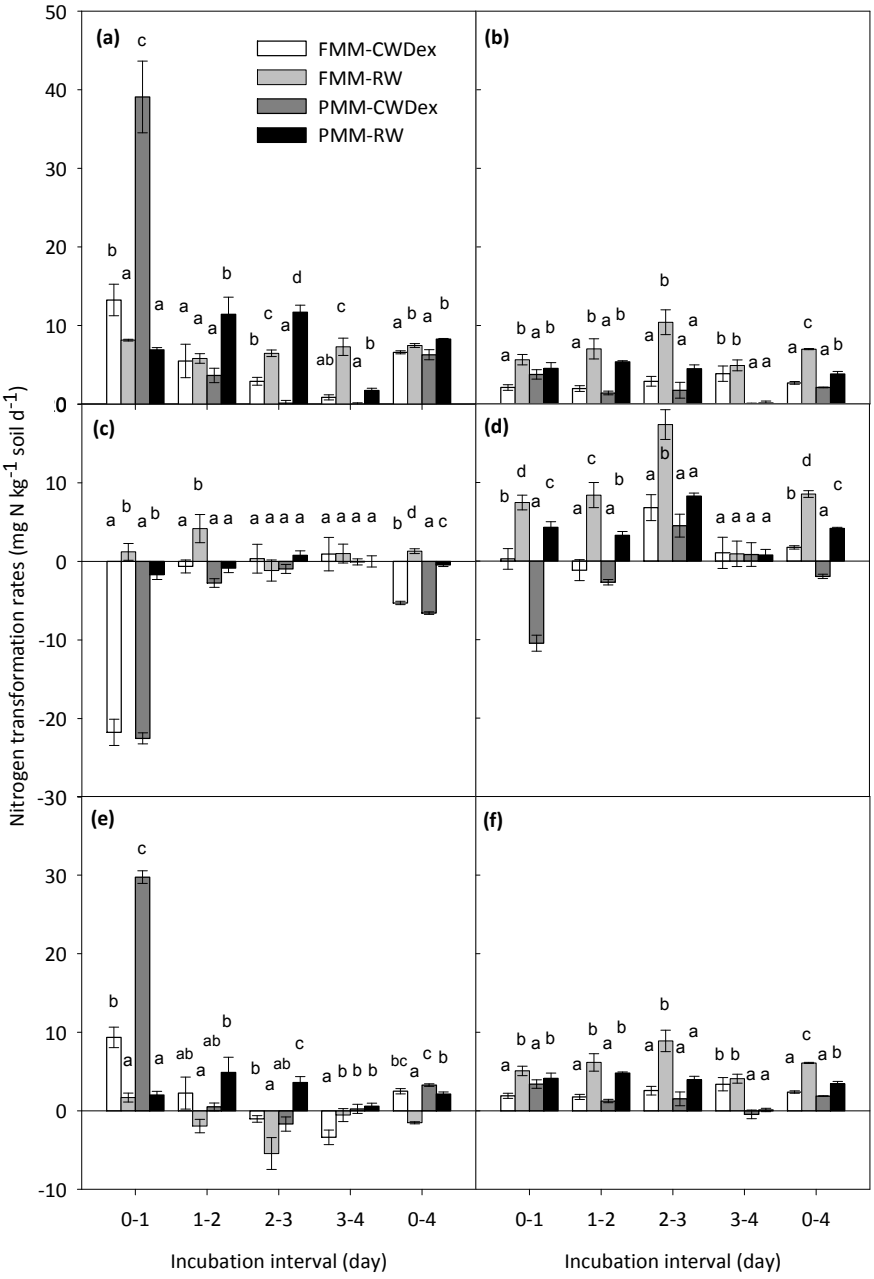

Figure 2-3 Changes in a) gross N mineralization, b) gross nitrification, c) net N mineralization, d) net nitrification, e) NH_4^+ immobilization and f) NO_3^- immobilization rates (mg N kg^{-1} d^{-1}) for each incubation interval (0-1, 1-2, 2-3 and 3-4 d) and time weighted average for 4 days (0-4 d) over a 4-day period incubated with CWD extract (CWDex) and rainwater (RW) in forest floor mineral soil mix (FMM) and peat mineral soil mix (PMM). Different letters represent significant differences between treatments within each incubation interval at $p<0.05$. Vertical bars are standard error of the mean (n=3).

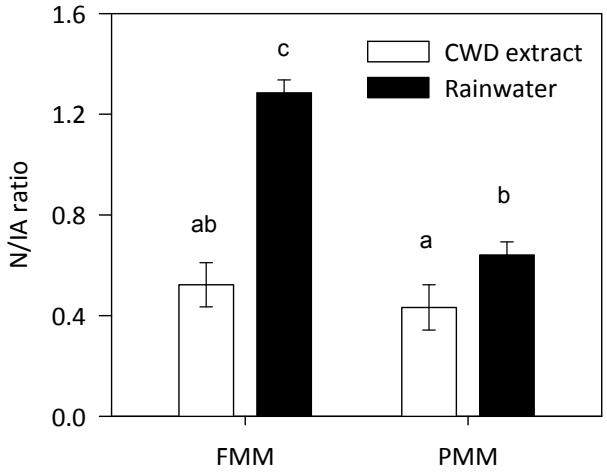

Figure 2-4 Ratio of gross nitrification rates to gross NH_4^+ immobilization rates (N/IA ratio) over a 4-day period incubated with CWD extract and rainwater in forest floor mineral soil mix (FMM) and peat mineral soil mix (PMM). Different letters represent significant differences between treatments at $p<0.05$. Vertical bars are standard error of the mean (n=3).

CHAPTER 3. COARSE WOODY DEBRIS INCREASES MICROBIAL COMMUNITY FUNCTIONAL DIVERSITY BUT NOT ENZYME ACTIVITIES IN RECLAIMED OIL SANDS SOILS

1. Introduction

The Athabasca oil sands region in northern Alberta, Canada, is the largest single oil sands deposit in the world with an estimated 1.6 trillion barrels of bitumen, a low quality crude oil mixed with sands and water (Alberta Government, 2013). Open-pit mining, one of the most common practices to recover oil from the oil sands in this region (Alberta Government, 2013), has disturbed large areas of mixedwood boreal forests. Oil sands companies are required to reclaim such disturbed lands to equivalent land capability that existed before open-pit mining (Province of Alberta, 2014).

A common reclamation practice in this region is returning disturbed land to upland boreal forests. To support plant growth, approximately 30 cm of cover soils are applied over substrates such as geological overburdens or tailing sands due to a lack of nutrients and microorganisms, high salinity, and high concentrations of toxic materials including naphthenic acids, polycyclic aromatic hydrocarbons, phenolic compounds and trace metals in the substrate (Depuit, 1984; Sydnor and Redente, 2002). Soil microbial community and enzymes are essential for organic matter decomposition, nutrient, and thus plant growth and revegetation (van Veen and Kuikman, 1990; Sinsabaugh et al., 1991; Ladd et al., 1995; Sylvia et al., 1998; Burns et al., 2013). Readily decomposable soil organic matter is rapidly consumed by microorganisms, and then decomposition is dominated by turnover of the microbial biomass (van Veen and Kuikman, 1990; Ladd et al., 1995). Microbial community and enzyme activity are important biological indicators of soil quality and net ecosystem productivity in natural and reclaimed ecosystems (Sinsabaugh et al., 1991; Sylvia et al., 1998; Alkorta et al., 2003; Dimitriu et al., 2010; Burns et al., 2013). Characterizing and understanding microbial community and enzyme activity are important for successful land

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reclamation after oil sands mining.

Soil microbial community and enzyme activities are affected by substrate quality and quantity (Degens, 1998; Degens et al., 2000; Burns et al., 2013), plant community composition and productivity (Zak et al., 2003; Bach et al., 2008), and environmental factors (Dimitriu et al., 2010; Burns et al., 2013). Several studies have assessed microbial community structure using phospholipid fatty acids (PLFA) analysis in reclaimed oil sands soils in northern Alberta (MacKenzie and Quideau, 2010; Dimitriu et al., 2010; Hahn and Quideau, 2013; Béasse et al., 2015), and this method mainly concerns taxonomic diversity. In addition to taxonomic diversity, knowledge of microbial community function and functional diversity is also important to understand the role of the microbial community in different soils (Garland, 1997; Preston-Mafham et al., 2002). Assessing the soil microbial community level physiological profile (CLPP) is a relatively fast and reliable method for detecting overall changes in microbial community function and structure and a Biolog Ecoplate is commonly used to determine microbial CLPP (Garland, 1997; Preston-Mafham et al., 2002). Potential metabolic activity of microbial community (microbial community functional diversity) is indicated from average well color development (AWCD) of Biolog plates and community structure comes from the characteristic pattern of substrate utilization with multivariate statistical analysis (ie, clustering, principal component analysis or canonical correspondence analysis (Garland, 1997; Preston-Mafham et al., 2002)). However, the technique has several drawbacks such as culture dependence and the possibility of microbial community growth and change during the incubation (Garland, 1997; Preston-Mafham et al., 2002).

Forest floor (litter, fragmented litter, and humus) mineral soil mix (hereafter FMM) and peat mineral soil mix (hereafter PMM) are cover soils commonly used for oil sands reclamation in northern Alberta. The PMM is readily available in northern Alberta and has been used for oil sands reclamation while availability of FMM is limited. Applying FMM for oil sands reclamation has several advantages relative to PMM and FMM has been used for oil sands reclamation recently. Properties of cover soils, such as FMM and PMM, used for oil sands reclamation in northern Alberta have been compared (McMillan et al., 2007; Mackenzie and Naeth, 2010; Jamro et al., 2014; Brown and Naeth, 2014). The FMM is considered more decomposable with lower carbon to nitrogen (C:N) ratios (Mackenzie and

Naeth, 2010). Soil water retention is greater in PMM than in FMM due to the higher organic matter content in PMM (MacKenzie and Quideau, 2010). As FMM contains more propagules and seeds in seed banks (Mackenzie and Naeth, 2010), vegetation cover and woody species abundance were greater in FMM than in PMM when the materials were used for land reclamation (Mackenzie and Naeth, 2010; Brown, 2010; Brown and Naeth, 2014; Forsch, 2014). As soil properties and vegetation covers differ between FMM and PMM when these were applied for reclamation, microbial community and enzyme activities were different in reclaimed oil sands soils (MacKenzie and Quideau, 2010; Hahn and Quideau, 2013; Jamro et al., 2014).

Coarse woody debris (CWD), including large branches, logs, standing dead trees, and dead coarse roots, plays important ecological roles in forest ecosystems (Harmon et al., 1986; Stevens, 1997). Large amounts of CWD are produced during oil sands mining and they are usually burnt or buried on site. Reclaimed areas tend to be directly exposed to climatic changes due to a relatively homogeneous landscape and lack of vegetation cover. Applying CWD during land reclamation will be beneficial for re-forestation by regulating soil temperature and water content, controlling soil erosion, increasing soil organic matter content, and increasing spatial heterogeneity and microsites to provide more favorable habitats for microorganisms (Harmon et al., 1986; Stevens, 1997; Pyle and Brown, 1999; Debeljak, 2006). However, only a few studies have evaluated ecological effects of CWD in a reclaimed oil sands landscape (Brown, 2010; Brown and Naeth, 2014; Forsch, 2014).

Many studies assessed the effect of CWD on microbial community and enzyme activities in natural forest ecosystems. Coarse woody debris increased fungal to bacterial ratio, soil respiration, and microbial biomass in temperate coniferous forests (Brant et al., 2006). Gonzalez-Polo et al. (2013) found that CWD application increased C and phosphorus degrading soil enzyme activities in an old growth beech forest in Argentina. However, no study has evaluated the effect of CWD on microbial community CLPP and enzyme activities in reclaimed oil sands soils.

This study was conducted to determine if applying CWD on reclaimed oil sands soils amended with FMM or PMM will affect microbial community functional diversity and soil enzyme activities. Hypotheses of this study are 1) soil microbial CLPP would be different between the two cover soils due to their contrasting properties, and microbial biomass

would be greater in FMM than in PMM, 2) enzyme activities would be greater in FMM than in PMM regardless of CWD application due to greater microbial biomass and vegetation cover in FMM, 3) CWD would change microbial CLPP due to increased labile C content coming from CWD leachate, and 4) CWD would enhance microbial biomass, AWCD and enzyme activities due to increased availability of microsites. To test these hypotheses, I conducted field experiments 6 and 7 years after land reclamation in an open pit oil sands mining area in the Athabasca oil sands region in northern Alberta.

2. Materials and Methods

2.1 Study site

This study was conducted at the Southeast Dump at Suncor Lease 86/17 (56° 58'N, 111° 22'W), approximately 24 km north of Fort McMurray, Alberta, Canada. The site was cleared in 1999 for open pit oil sands mining, and thereafter, was used as a saline-sodic overburden waste dump until 2004. The owner of the land (Suncor Energy Inc.) gave permission to conduct the study on this site. A detailed description of the research site and experimental plots is provided in Brown (2010) and Brown and Naeth (2014).

For the study area, average annual temperature from 1981 to 2010 was 1.0 °C and average annual precipitation was 418.6 mm, with 316.3 mm as rainfall and 133.8 cm as snowfall (Environment Canada, 2014). The mean average temperature between July and September was 16.0 and 15.1 °C for 2013 and 2014, respectively, and the total precipitation during the sampling period, from July to September, was 120.0 mm in 2013 and 154.8 mm in 2014 (Figure 3-1).

2.2 Experimental design and plot establishment

A factorial experiment consisted of 2 cover soil types (FMM vs PMM) and 2 sampling distances from CWD (near vs away from CWD) with 6 replications was designed for this research. Study plots 10 × 30 m in size were established between November 2007 and February 2008. Six plots were covered with FMM and six plots were covered with PMM. The FMM was applied at a depth of 20 cm, over 30 cm of B and C horizon mixed subsoil, over 100 cm of clean overburden. The PMM was applied at a depth of 30 cm over 100 cm of clean overburden (Brown and Naeth, 2014). In February 2008, trembling aspen (*Populus*

tremuloides Michx.) CWD, with diameter bigger than 10 cm, was salvaged and applied on each plot. The CWD pieces did not overlap in the plot to provide maximum contact with the soil surface and covered 10 to 20% of each plot.

Plots were covered by naturally established forbs, grasses, shrubs, and mosses (Brown, 2010; Brown and Naeth, 2014). Overall vegetation cover and cover of woody species were greater in FMM than in PMM plots, 65.1 ± 2.1 and 33.9 ± 1.8% per available ground (area without CWD) for FMM and PMM, respectively, and were positively related to CWD cover in the second growing season (2009) after plot establishment (Brown, 2010; Brown and Naeth, 2014). Vegetation cover increased over time and remained greater in FMM than in PMM in the fourth (2011) and fifth (2012) years after plot establishment; vegetation cover was similar near CWD and away from CWD; total vegetation covers were 43.4 ± 2.7 and 45.2 ± 1.6% for near CWD and away from CWD, respectively, in FMM and 33.3 ± 3.0 and 30.5 ± 3.4%, respectively, in PMM in 2012 (Brown, 2010; Brown and Naeth, 2014).

2.3 Soil sampling and analysis

Soil was sampled in the 0 to 10 cm layer using an auger on July 26, August 26, and September 28 in 2013 and on July 8, August 8, and September 4 in 2014. Three soil samples were collected from each treatment, three within 5 cm from CWD and three more than 100 cm from CWD, in each plot and bulked to form a composite sample of each treatment; a total of 24 composite soil samples were collected [4 treatments (2 cover soils × 2 sampling distances from CWD) × 6 replications]. Soil samples were transported to the laboratory on ice packs in a cooler. Fresh soil samples were passed through a 2-mm sieve, and stored in a refrigerator at 4 °C until analysis. All analyses were completed within 4 days after sampling. A sub-sample of each soil sample was used to determine available N, microbial biomass C (MBC) and N (MBN), dissolved organic C (DOC) and N (DON), soil microbial community level physiological profile, and extracellular enzyme activities. The remainder of each sample was air dried at room temperature and used to determine pH and electrical conductivity (EC). A portion of the air dried sample was ground into fine powder using a ball mill (MM200, REtsch GmbH, Haan, Germany) and used for total C and total N analyses.

Soil pH was determined using a pH meter (Orion, Thermo Fisher Scientific Inc.,

Beverly, MA, USA) and EC using an AP75 portable waterproof conductivity/TDS meter (Thermo Fisher Scientific Inc., Waltham, MA, USA) at a 1:5 soil weight to deionized water volume ratio. Available N including ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were determined via steam distillation (Keeney and Nelson, 1982). Soil MBC and MBN were determined by chloroform fumigation extraction (Jenkinson and Ladd, 1981). Fresh soil samples were fumigated with ethanol free chloroform for 24 hours in an evacuated desiccator. Fumigated and unfumigated samples were extracted with 0.5 mol L^{-1} potassium sulfate solutions at a 1:10 soil weight to potassium sulfate solution volume ratio and filtered using Whatman No. 42 filter papers. Extractable C and N were determined using a TOC-V_{CSN} analyzer (Shimadzu, Kyoto, Japan). To determine DOC and DON concentrations, 5 g of fresh soil samples were extracted with 50 mL of deionized water and filtered using Whatman No. 42 filter papers. Concentrations of C and N in the filtrate were determined using the TOC-V_{CSN} analyzer. Extractable C was used to represent DOC and the difference between extractable N and available N (NH_4^++NO_3^-) was used to represent DON. Total C and N were determined using an automated elemental analyzer (NA-1500 series, Carlo Erba, Milan, Italy).

2.4 Soil microbial community level physiological profile

Soil microbial CLPP was determined using a Biolog Ecoplate (Biolog Inc., Hayward, CA, USA), which contains 31 C substrates and one control with 3 replications in a 96 well microplate. One gram of each fresh soil sample was put into a sterile flask with a 100 mL of 0.87% sterilized sodium chloride solution, shaken for 30 min and diluted 1,000 times with 0.87% sterilized sodium chloride solution. A 150 µL aliquot of each soil suspension was inoculated into each well of the Ecoplate. Ecoplates were incubated for 168 hours at 25 ℃ in the dark. The optical density of each well was read at 590 nm every 24 hours using a microplate reader (Emax, Biolog Microstation, CA, USA).

To describe the soil microbial community functional diversity, averaged well color development (AWCD) and area under the curve (*A*) were calculated with optical density values measured every 24 hours. The AWCD data were calculated according to Garland and Mills (1991);

$$\text{AWCD} = \sum (C - R)/n$$

where C is color production within each well, R is absorbance of the control well, and n is the number of C sources used in the Ecoplate.

The area under the curve A_{ik} for substrate i and plate k was calculated by joining the color levels at successive time point of *t(1), t(2),…, t(n)* by straight lines, and summing the areas corresponding to each segment between successive time points by the trapezium rule (Hackett and Griffiths, 1997);

$$A = \frac{1}{2}\sum_{j=1}^{n-1}[t(j+1) - t(j)][C_{ikt(j+1)} - C_{ikt(j)}]$$

where $C_{ikt(j)}$ is the color development of substrate i for plate k at time *t(j)*.

2.5 Soil enzyme activities

Extracellular enzyme activities involved in C and N cycling, including β-1,4-N-acetylglucosaminidase (NAGase, enzyme classification (EC) number 3.2.1.14), β-1,4-glucosidase (EC 3.2.1.21), cellobiohydrolase (EC 3.2.1.91), and peroxidase (EC 1.11.1.x) were measured according to Sinsabaugh et al. (2003). Soil sample suspensions were prepared by placing one gram of fresh soil in a 250 mL Nalgene HDPE bottle, adding 125 mL of sodium acetate buffer (50 mmol L^{-1}, pH 5), and homogenizing on an end-over-end shaker for 30 min at room temperature. Then soil suspensions were transferred into 96 well plates that were continuously stirred on a stir plate less than 2 minutes to keep soil suspensions homogenized.

For NAGase, β-1,4-glucosidase, and cellobiohydrolase activities, 200 µL of soil suspension and 50 µL of 200 µmol L^{-1} of each substrate were pipetted into black 96 well plates. Reference standards and quench controls were added to each reference and quench well in each plate. The plates were incubated at 20 °C in the dark for 3, 3, and 7 hours for NAGase, β-1,4-glucosidase, and cellobiohydrolase, respectively. After the incubation, a 20 µL of 0.5 mol L^{-1} sodium hydroxide solution was added to each well to stop the enzyme

reaction. Fluorescence was measured at 360 nm excitation and 460 nm emissions using a multi-detection microplate reader (Synergy HT, Bio-Tek Instruments, Winooski, VT, USA).

For peroxidase activity, 200 µL of soil suspension and 50 µL of 200 µmol L^{-1} of substrate (3,4-dihydroxy-L-phenylalanine) were pipetted onto clean transparent 96 well plates. A 10 µL 0.3% hydrogen peroxide solution was added to each well after the substrate. The plates were incubated at 20 °C in the dark for 5 hours. After incubation, absorbance was measured at 460 nm using the multi-detection microplate reader.

2.6 Statistical analyses

Two-way analysis of variance (ANOVA) was conducted to determine the differences in soil properties and each enzyme activity using the SAS software (SAS Institute Inc., NC, USA). Before performing the ANOVA, the normality of distribution and homogeneity of variance were checked with Kolmogorov-Smirnov and Levene's tests. A repeated measures ANOVA was conducted to assess cover soil type and distance from CWD effects over time on MBC, MBN, each enzyme activity, and AWCD using the PROC MIXED model using the SAS 9.3 software. Distance from CWD was used as a split-plot factor and the month of each sampling was considered a repeated measures variable for determining seasonal variations in 2013 and 2014. In this analysis, the output statistics passed tests for compound symmetry. Tucek's HSD test was used to determine the significant differences between cover soil type, distance from CWD, month of sampling and their interactions. Pearson correlation and multiple regression analyses were used to determine which soil parameters have strong relationships with MBC, MBN, enzyme activities, and AWCD. Slope of changes of AWCD over time was compared using an analysis of covariance.

Color development in each Ecoplate well followed a sigmoidal curve with time and the response of each substrate was different with time; some had short response times while others had longer lags (Preston-Mafham et al., 2002). There was no well leached saturation level before 168-hour incubation and AWCD measured at 168-hour was used for statistical analyses.

The A_{ik} data were analyzed with a principal component analysis (PCA) to test changes in microbial CLPP as affected by cover soil type and distance from CWD by permutation tests with the Vegan Package of the R software (R Development Core Team, 2012).

Statistical differences of microbial CLPP between treatments were assessed using multiple-response permutation procedures (MRPP) (Mielke and Berry, 2007) with the R software. The significant level was set at α=0.05 for all statistical analyses.

3. Results

3.1 Properties of cover soils

Soil pH, total C, and total N were significantly affected by cover soil type but not by distance from CWD or their interactions, and they were higher in PMM than in FMM (Table 3-1). Soil EC was significantly greater in PMM than in FMM and greater near CWD than away from CWD in PMM.

Soil DOC concentrations in 2013 were not affected by cover soil type, distance from CWD, or their interactions, except for September 2013 (Table 3-2). Soil DOC concentrations were significantly greater in PMM than in FMM in July and August 2014. Applying CWD increased DOC concentrations in FMM in September 2014. However, soil DON concentrations were not affected by cover soil type or distance from CWD in 2013 and 2014. Gravimetric soil water contents were significantly higher in PMM than in FMM in July and August but were not affected by distance from CWD in both 2013 and 2014 (Table 3-2).

3.2 Microbial biomass C and N

Soil MBC was greater in FMM than in PMM and CWD increased MBC in cover soils; however, effects of cover soils and CWD were variable among sampling months (Table 3-2). Soil MBC was significantly greater in FMM than in PMM in July and August 2013 and in July 2014, and was greater near CWD than away from CWD in July 2013 and in July and August 2014. Soil MBC was positively related to DOC, DON, MBN, and soil water content in FMM in 2013 and 2014, to DOC in 2013 and 2014 and soil water content in 2013 in PMM (Table 3-4).

Soil MBN showed a similar pattern to that of MBC and was significantly increased by CWD in most samplings, except for September 2014 (Table 3-2). Soil MBN was significantly greater in FMM than in PMM in July 2013 and in July and September 2014 but was not affected by interaction of cover soil type and distance from CWD. Soil MBN

was positively related to DOC, DON, and soil water content in 2013 and to DOC and soil water content in 2014 in both FMM and PMM (Table 3-4).

3.3 Soil microbial community level physiological profile and enzyme activities

The AWCD was significantly affected by cover soil type, distance from CWD, and sampling time (Table 3-3). The AWCD was significantly greater in PMM than in FMM in each sampling regardless of distance from CWD in all sampling times in 2014 (Figure 3-2). The CWD application enhanced AWCD in August ($p<0.001$ and $p=0.157$ for FMM and PMM, respectively) and September ($p<0.001$ for both cover soils) samplings. The AWCD was positively related to DOC, MBC, and soil water content in FMM and PMM (Table 3-4). Soil water content and DOC concentrations were the main factors determining the AWCD among treatments, with AWCD=0.02 × soil water content + 0.001× DOC + 0.814; R^2=0.77; $p<0.001$; β (standardized coefficient)=0.71 and 0.15 for soil water content and DOC, respectively.

Soil microbial CLPP was different between FMM and PMM (Figure 3-3, MRPP; $p<0.001$). The CWD application changed microbial CLPP in FMM (MRPP; $p=0.046$) but not in PMM (MRPP; $p=0.124$). The first (PC1) and second principal components (PC2) explained 21.4 and 11.5% of the total variation in C substrate utilization profiles, respectively (Figure 3-3).

Cover soil type and sampling time significantly affected enzyme activities, including β-1,4-glucosidase, cellobiohydrolase and peroxidase in both 2013 and 2014; however, NAGase activities were not affected by cover soil type (Table 3-3, Figure 3-4). There was no significant effect of CWD or interaction between cover soil type and distance from CWD on enzyme activities, except β-1,4-glucosidase activity in 2014. Soil enzyme activities in 2014 were significantly greater than those in 2013. Soil enzyme activities were positively related to DOC, DON, MBC and MBN in both cover soils without any relationship of peroxidase activity with other soil properties (Table 3-4).

4. Discussion

4.1 Soil microbial community level physiological profile and microbial biomass

Soil microbial CLPP in the studied cover soils were significantly different and microbial

biomass in FMM was greater than that in PMM in three out of six samplings, supporting part of the first hypothesis. The cover soils FMM and PMM used for oil sands reclamation in northern Alberta had contrasting soil properties due to the different sources of organic matter (McMillan et al., 2007; Mackenzie and Naeth, 2010; Jamro et al., 2014; Brown and Naeth, 2014). Forest floor is salvaged from upland forests while peat is salvaged from wetlands, causing distinctive microbial community and microbial biomass between FMM and PMM (Hahn and Quideau, 2013; Béasse et al., 2015). Soil MBC was 4 times greater in FMM than in PMM in the year of plot establishment at the same research plot (Brown and Naeth, 2014). Although differences in microbial biomass between FMM and PMM were decreased 6 and 7 years after plot establishment, microbial biomass was still generally greater in FMM than in PMM. In addition to initial differences of microbial biomass between cover soils, lower soil pH and soil water content in FMM than in PMM may cause greater fungal biomass and greater overall microbial biomass in FMM as (Will et al., 2010) fungi is favored in acidic soil pH and bacteria prefer alkaline or neutral soil pH, fungi are more resistant to water stress than bacteria (Jin et al., 2011). Greater MBC and MBN but lower AWCD in FMM than in PMM indicates that bacterial biomass would be greater in PMM than in FMM while fungal biomass is greater in FMM than in PMM. Only some of bacteria are culturable in the Ecoplate and AWCD represents function of bacteria only, not fungi (Garland, 1997; Preston-Mafham et al., 2002).

Both above- and belowground litter and root exudates are primary energy and nutrient sources for soil microorganisms (Griffiths et al., 1999; Yang and Crowley, 2000; Zak et al., 2003; Bach et al., 2008) and greater plant litter input from more diverse vegetation cover in FMM (Brown and Naeth, 2014; Forsch 2014) likely contributed to the greater microbial biomass in FMM (Degens et al., 2000). Greater MBC and MBN in FMM than in PMM in July 2013 and 2014 is likely attributed to greater vegetation cover and root exudate in FMM. However, decreases in vegetation productivity and litter input in the later growing season probably caused no differences of microbial biomass in September samplings. Greater diversity of plant species in FMM than in PMM (Brown and Naeth, 2014; Forsch, 2014) and contrasting soil properties between two cover soils may cause distinctive microbial CLPP in two cover soils (Degens, 1998; Zak et al., 2003). It would be helpful to know changes in soil microbial community structure using culture-independent approaches, and

soil microbial community structure was closely related to plant productivity and species composition according to PLFA analysis (Zak et al., 2003; Bach et al., 2008; Hahn and Quideau, 2013). For example, microbial biomass and fungal abundance increased with the higher levels of plant productivity associated with greater plant species diversity in seven years of plant diversity manipulation experiment (Zak et al., 2003). Soil microbial community structure was closely associated with plant productivity and community in a Norway spruce (*Picea abies* (L.) Karst.) dominant forest (Bach et al., 2008) and in reclaimed oil sands soils (MacKenzie and Quideau, 2010; Hahn and Quideau, 2013). In addition, greater mycorrhizal (part of the total fungal community) biomass in FMM than in PMM (Brown, 2010) was associated with higher woody species cover in FMM; such changes may affect fungal to bacterial ratio and soil microbial CLPP.

Applying CWD for land reclamation changed microbial CLPP in FMM, but not in PMM, which supported part of the third hypothesis. Increased DOC concentration through CWD leachate in August and September 2014, especially in FMM, which has distinct C composition compared to cover soils (Lajtha et al., 2005), may change microbial C utilization pattern and microbial CLPP in FMM (Degens, 1998; Degens et al., 2000) although CWD had little affect DOC concentrations in cover soils. However, CWD did not increase DOC concentrations in PMM without any changes of microbial CLPP. Increases in DOC and MBN in 2014 by CWD, especially in FMM, and positive relationship of AWCD with DOC and MBN indicates that increased labile C availability by CWD application increased microbial community functional diversity. Increased AWCD in PMM is probably attributed to greater MBC near CWD than away from CWD in 2014 although there was no difference in MBC in September. Fungi are responsible for the degradation of recalcitrant substrates such as lignin, and fungal biomass increased near CWD (Brant et al., 2006; Kappes et al., 2007), increasing overall microbial biomass and changing microbial CLPP. Applying CWD increases microsites (Pyle and Brown, 1999; Debeljak, 2006) and provides more favorable habitats for microorganisms and detritivores (Harmon et al., 1986; Kappes et al., 2007). Increases in detritivore abundance near CWD increases litter fragmentation and can provide more labile substrates for microorganism. Water soluble C or DOC in soils are considered the most readily available C source for soil microorganisms (Mcgill et al., 1986; Wang et al., 2003) and increases in DOC concentration by CWD application may

cause positive priming effect (Kuzyakov et al., 2000) and increase microbial community functional diversity. Increased microbial community functional diversity by CWD application suggests that using CWD for oil sands reclamation would enhance nutrient cycling and improve early ecosystem development.

The AWCD was strongly affected by soil water content and greater AWCD in July than August and September is related to highest precipitation and soil water content in July. Furthermore, greater soil water content in PMM than in FMM supported more bacterial community resulting in greater AWCD in PMM. Soil water content is a key factor for microbial community function (Sylvia et al., 1998). Bacteria are more susceptible to changing water content (Jin et al., 2011) and such phenomenon is more significant in reclaimed oil sands soils than in natural soils (Hahn and Quideau, 2013).

4.2 Soil enzyme activities

The greater soil enzyme activities in FMM than in PMM support the second hypothesis. Soil enzyme activity is affected by soil pH, microbial population size, microbial community such as the size of the fungal population size, and plant community composition and productivity (Hernandez and Hobbie, 2010; Burns et al., 2013; Jamro et al., 2014). Lower soil pH and greater woody plant abundance in FMM than in PMM (Brown and Naeth, 2014; Forsch, 2014) increase fungal biomass and mycorrhizal biomass in FMM (Sylvia et al., 1998; Brown, 2010; Jin et al., 2011). Fungi are responsible for extracellular enzyme production to decompose recalcitrant substrate (Andersson et al., 2004; Muruganandam et al., 2009), and greater fungal and mycorrhizal biomass in FMM (Brown, 2010) may be linked with greater enzyme activities in FMM. Soil enzyme activities in this study were positively correlated with MBC and MBN, consistent with an earlier study on reclaimed oil sands soils (Jamro et al., 2014). Higher amounts of litter and root exudates in FMM (Forsch, 2014) would increase microbial biomass and enzyme activities (Hernandez and Hobbie, 2010). The greater overall enzyme activities in 2014 than in 2013 could also be attributed to increased vegetation cover and litter amount over time (Brown and Naeth, 2014; Forsch, 2014). Decreases in enzyme activities from August to September in 2014 were probably affected by decreasing vegetation productivity due to decreasing air temperature. Activities of C degrading enzymes such as β-1,4-glucosidase, cellobiohydrolase, and peroxidase, were

significantly greater in FMM than in PMM, indicating that organic matter decomposition would be greater in FMM than in PMM. The NAGase activity had strong relationship with N availability (Andersson et al., 2004) and similar NAGase activity in FMM and PMM indicates that N availability would be similar in FMM than in PMM.

I had expected that CWD application would increase soil enzyme activities because of the effect of CWD on vegetation cover and microsites (Brown and Naeth, 2014). However, CWD did not affect soil enzyme activities, rejecting part of the fourth hypothesis. Similar soil properties and vegetation cover between near CWD and away from CWD would cause similar enzyme activities between the two locations. As the overall vegetation cover increased over the year in each plot, differences in vegetation cover became smaller between near CWD and away from CWD in later years (Forsch, 2014). The lack of CWD effects on soil enzyme activities were further supported by similar soil properties and vegetation cover between near CWD and away from CWD. A study conducted in an old-growth beech forest showed that C and phosphorus degrading enzyme activities were increased under CWD as compared to soils not under the influence of CWD (Gonzalez-Polo et al., 2013). In their study, increases in enzyme activities under CWD have been attributed to higher soil moisture content and DOC, and thus increased microbial biomass.

5. Conclusions

The two cover soils (FMM and PMM) commonly used for oil sands reclamation had different microbial CLPP, microbial biomass and enzyme activities associated with contrasting soil properties and vegetation cover. The FMM is a more favorable cover soil for land reclamation due to its greater organic matter decomposition and nutrient supply rates, microbial biomass, and enzyme activities relative to PMM. The CWD changed soil microbial CLPP in FMM and increased microbial biomass in most samplings and microbial community functional diversity (AWCD) in both FMM and PMM associated with increased microsites. The CWD did not affect enzyme activities due to similar sol properties and vegetation cover between near CWD and away from CWD. Effects of CWD on microbial CLPP and microbial biomass depended on cover soil type and sampling time; CWD had more significant effects when applied on FMM. Increased microbial community functional diversity and microbial biomass by CWD in the studied cover soils suggest that applying

CWD for land reclamation would benefit early ecosystem development by increasing organic matter decomposition and nutrient cycling. Applying CWD for land reclamation is strongly recommended in terms of not only accelerating upland reclamation but also recycling natural resources.

Table 3-1 Chemical properties of forest floor mineral soil mix (FMM) and peat mineral soil mix (PMM) collected from near CWD and away from CWD locations in a reclamation experiment.

Treatment		pH	EC[a] (dS m^{-1})	Total C (g kg^{-1})	Total N (g kg^{-1})	C:N
Cover soil	Distance from CWD					
FMM	Near	5.90 (0.12)	0.18 (0.01)	39.7 (4.9)	1.4 (0.2)	30.1 (3.7)
	Away	6.05 (0.10)	0.21 (0.02)	40.8 (5.6)	1.4 (0.3)	32.8 (2.9)
PMM	Near	7.06 (0.03)	0.37 (0.02)	68.4 (8.2)	2.4 (0.3)	28.6 (1.6)
	Away	7.18 (0.04)	0.45 (0.03)	72.7 (10.3)	2.4 (0.4)	31.3 (1.3)
Two-way ANOVA						
Cover soil (S)		***	*	*	*	ns
Distance from CWD (D)		ns	*	ns	ns	ns
S x D		ns	ns	ns	ns	ns

Values are means with SE (n=6);
* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant;
EC= electrical conductivity and C:N= carbon to nitrogen ratio

Table 3-2 Dissolved organic carbon (DOC) and nitrogen (DON), gravimetric soil water content (SWC), and microbial biomass C (MBC) and N (MBN) in cover soils in 2013 and 2014 and effects of cover soil type (FMM vs PMM) and distance from CWD (near vs away) on soil properties in cover soils used for oil sands reclamation.

Treatment		DOC (mg kg^{-1})			DON (mg kg^{-1})			SWC (%)			MBC (mg kg^{-1})			MBN (mg kg^{-1})		
Cover soil	Distance from CWD	July	Aug.	Sept.	July	Aug.	Sept.	July	Aug.	Sept.	July	Aug.	Sept.	July	Aug.	Sept.
2013																
FMM	Near	243.5 (39.8)	302.3 (81.7)	259.8 (23.9)	9.3 (2.0)	8.7 (2.5)	5.4 (1.9)	29.9 (6.9)	25.7 (4.1)	20.6 (4.5)	400.3 (96.0)	382.7 (92.2)	299.7 (65.2)	87.0 (22.9)	65.4 (15.7)	57.8 (11.9)
	Away	220.8 (27.8)	227.4 (21.7)	298.1 (25.4)	7.1 (1.0)	5.7 (0.9)	5.0 (1.5)	26.5 (6.6)	25.0 (5.1)	20.9 (6.7)	267.3 (38.9)	286.6 (39.8)	248.4 (74.0)	55.1 (8.3)	45.7 (10.8)	40.7 (10.1)
PMM	Near	305.8 (26.4)	319.7 (36.0)	323.4 (27.3)	5.6 (1.0)	6.8 (1.1)	2.4 (0.7)	43.2 (6.0)	46.4 (7.2)	32.0 (3.3)	212.1 (44.2)	220.6 (45.5)	279.9 (50.7)	39.9 (7.2)	35.5 (8.2)	37.4 (4.6)
	Away	286.9 (19.1)	332.6 (52.4)	383.7 (38.1)	6.0 (0.9)	7.8 (1.3)	5.1 (1.2)	41.5 (4.6)	47.5 (8.3)	28.2 (4.7)	196.4 (24.6)	173.7 (51.1)	176.4 (35.5)	37.2 (7.0)	33.3 (8.1)	26.1 (6.0)
Two-way ANOVA																
Cover soil (S)		ns	ns	ns	ns	ns	ns	*	*	ns	*	*	ns	*	ns	ns
Distance from CWD (D)		ns	ns	*	ns	ns	ns	ns	ns	ns	*	ns	ns	*	*	**
S x D		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Values are mean with SE (n=18); * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant.

Table 3-2 (cont.) Dissolved organic carbon (DOC) and nitrogen (DON), gravimetric soil water content (SWC), and microbial biomass C (MBC) and N (MBN) in cover soils in 2013 and 2014 and effects of cover soil type (FMM vs PMM) and distance from CWD (near vs away) on soil properties in cover soils used for oil sands reclamation.

Treatment		DOC (mg kg^{-1})			DON (mg kg^{-1})			SWC (%)			MBC (mg kg^{-1})			MBN (mg kg^{-1})		
Cover soil	Distance from CWD	July	Aug.	Sept.	July	Aug.	Sept.	July	Aug.	Sept.	July	Aug.	Sept.	July	Aug.	Sept.
2014																
FMM	Near	158.7 (25.7)	181.4 (29.3)	150.2 (15.0)	8.6 (0.8)	11.3 (1.7)	8.8 (0.6)	26.1 (4.9)	11.8 (2.6)	20.3 (4.3)	251.8 (52.1)	279.3 (54.6)	347.6 (50.5)	58.2 (11.3)	42.6 (8.3)	59.6 (9.9)
	Away	132.0 (22.6)	156.3 (10.5)	118.0 (7.5)	8.1 (1.8)	10.9 (0.1)	6.8 (0.7)	20.5 (4.7)	10.7 (2.0)	22.0 (2.7)	200.8 (21.1)	250.8 (38.5)	309.9 (54.1)	44.9 (5.8)	34.8 (5.0)	53.2 (8.8)
PMM	Near	192.0 (26.0)	220.4 (21.6)	184.1 (22.6)	7.1 (0.8)	10.2 (1.8)	6.5 (1.0)	38.0 (6.2)	19.6 (2.8)	28.3 (4.6)	144.7 (38.2)	300.3 (57.9)	276.9 (46.3)	35.4 (7.6)	31.7 (6.1)	39.8 (6.9)
	Away	216.1 (23.6)	246.1 (30.1)	176.5 (19.4)	9.7 (1.4)	9.7 (1.1)	6.6 (0.7)	37.9 (5.9)	18.7 (2.2)	32.5 (4.4)	91.5 (28.4)	197.6 (35.1)	237.2 (43.4)	28.3 (6.5)	23.8 (3.5)	33.1 (6.2)
Two-way ANOVA																
Cover soil (S)		*	*	ns	ns	ns	ns	*	*	ns	*	ns	ns	*	ns	*
Distance from CWD (D)		ns	ns	*	ns	ns	ns	ns	ns	ns	*	**	ns	**	**	ns
S x D		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Values are mean with SE (n=18); * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant.

Table 3-3 Effects of cover soils, distance from the CWD, sampling time and their interactions on soil properties.

Soil properties[a]	Cover soils (S)		Distance from CWD (D)		Sampling time (T)		S × D		S × T		D × T		S × D × T	
	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
2013														
MBC	3.13	0.111	7.20	0.025	0.15	0.857	0.14	0.716	1.30	0.286	0.62	0.534	4.00	0.028
MBN	3.67	0.088	13.84	0.005	9.15	0.001	6.44	0.031	1.69	0.199	0.40	0.675	0.95	0.397
NAGase	2.45	0.152	1.41	0.264	77.80	<0.001	0.09	0.768	8.58	0.001	3.06	0.060	0.88	0.424
GLU	9.20	0.014	0.40	0.541	5.24	0.011	0.72	0.419	1.36	0.271	0.00	0.999	0.30	0.745
CEL	10.43	0.010	0.76	0.406	12.25	<0.001	0.05	0.836	2.74	0.078	0.78	0.467	0.72	0.492
PER	7.76	0.013	0.84	0.371	2.09	0.142	2.17	0.159	0.08	0.927	1.94	0.161	0.90	0.418
2014														
MBC	1.44	0.258	18.35	0.002	31.32	<0.001	1.47	0.253	5.16	0.010	0.39	0.681	0.75	0.480
MBN	3.15	0.106	15.03	0.003	16.87	<0.001	0.20	0.667	2.42	0.103	0.36	0.701	0.37	0.691
NAGase	3.16	0.106	4.59	0.056	32.66	<0.001	1.62	0.230	0.64	0.531	0.66	0.522	0.59	0.559
GLU	27.66	<0.001	28.17	0.001	49.51	<0.001	6.12	0.035	6.06	0.005	0.45	0.644	0.20	0.817
CEL	17.16	0.002	3.47	0.091	25.62	<0.001	0.61	0.452	2.25	0.119	0.53	0.592	2.53	0.093
PER	46.63	<0.001	0.10	0.765	50.06	<0.001	0.07	0.796	2.29	0.116	3.00	0.062	0.40	0.675
AWCD	6.51	0.029	8.18	0.016	19.49	<0.001	0.01	0.905	3.28	0.049	2.57	0.089	0.34	0.717

[a]Soil properties: MBC = microbial biomass C, MBN = microbial biomass N, NAGase = β-1,4-N-acetylglucosaminidase, GLU =β-1,4-glucosidase, CEL = cellobiohydrolase, PER = Peroxidase, and AWCD = average well color development measured after 168 hours of incubation.

Table 3-4 Pearson correlation coefficient (*r*-value) and significance* among soil variables in cover soils used for oil sands reclamation (n=36).

Variable[a]	DOC	DON	SWC	MBC	MBN	NAGase	GLU	CEL	PER
	FMM in 2013								
MBC	0.68**	0.58**	0.78**						
MBN	0.52**	0.60**	0.77**	0.92**					
NAGase	0.52**	-0.08	0.28	0.47*	0.26				
GLU	0.37	0.23	0.66**	0.51**	0.46*	0.53**			
CEL	0.42*	0.29	0.57**	0.58**	0.58**	0.40	0.43*		
PER	-0.11	-0.09	-0.01	0.18	0.15	-0.13	-0.42	-0.05	
	PMM in 2013								
MBC	0.47**	0.26	0.65**						
MBN	0.36*	0.50**	0.79**	0.79**					
NAGase	0.65**	0.26	0.35*	0.63**	0.48**				
GLU	0.45**	0.50**	0.72**	0.77**	0.79**	0.76**			
CEL	0.38*	0.31	0.29	0.49**	0.54**	0.40	0.54*		
PER	0.01	0.17	0.14	0.09	0.11	0.03	0.22	0.10	
	FMM in 2014								
MBC	0.68**	0.46**	0.54**						
MBN	0.57**	0.26	0.78**	0.88**					
NAGase	0.84**	0.75**	0.39*	0.64**	0.53**				
GLU	0.69**	0.61**	0.38*	0.48**	0.42*	0.82**			
CEL	0.76**	0.64**	0.60**	0.57**	0.59**	0.84**	0.90**		
PER	0.11	0.07	0.14	-0.12	0.11	0.04	0.24	0.31	
AWCD	0.33*	0.16	0.65**	0.33*	0.51**	0.42*	0.46**	0.54**	0.11
	PMM in 2014								
MBC	0.31*	0.27	0.28						
MBN	0.38*	0.27	0.72**	0.76**					
NAGase	0.71**	0.72**	0.29	0.47**	0.49**				
GLU	0.63**	0.64**	0.44**	0.44**	0.62**	0.94**			
CEL	0.65**	0.70**	0.30	0.55**	0.56**	0.91**	0.91**		
PER	0.05	0.10	0.26	-0.35	0.09	0.20	0.29	0.13	
AWCD	0.30*	0.27	0.75**	0.11	0.64**	0.45**	0.60**	0.49**	0.53**

[a]Variables: DOC=dissolved organic C, DON=dissolved organic N, MBC = microbial biomass C, MBN = microbial biomass N, SWC = gravimetric soil water content, NAGase = β-1,4-N-acetylglucosaminidase, GLU =β-1,4-glucosidase, CEL = cellobiohydrolase, PER = Peroxidase, and AWCD=average well color development measured after 168 hours of incubation;
Values are Pearson correlation coefficient; * = $p < 0.05$; ** = $p < 0.01$.

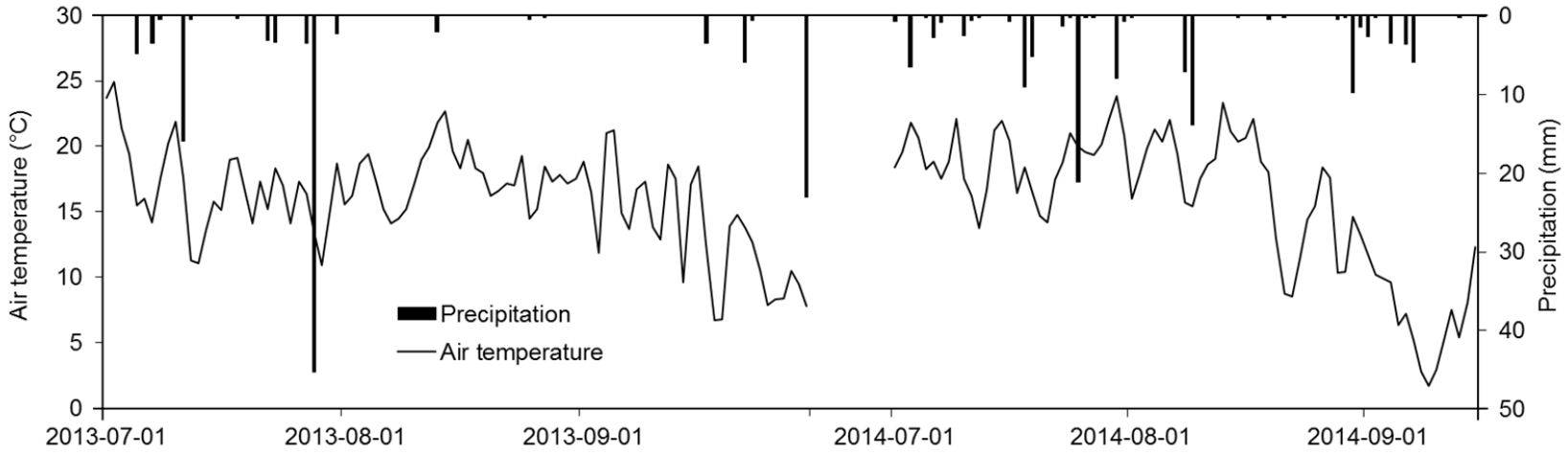

Figure 3-1 Daily precipitation (bar) and air temperature (line) during sampling periods.

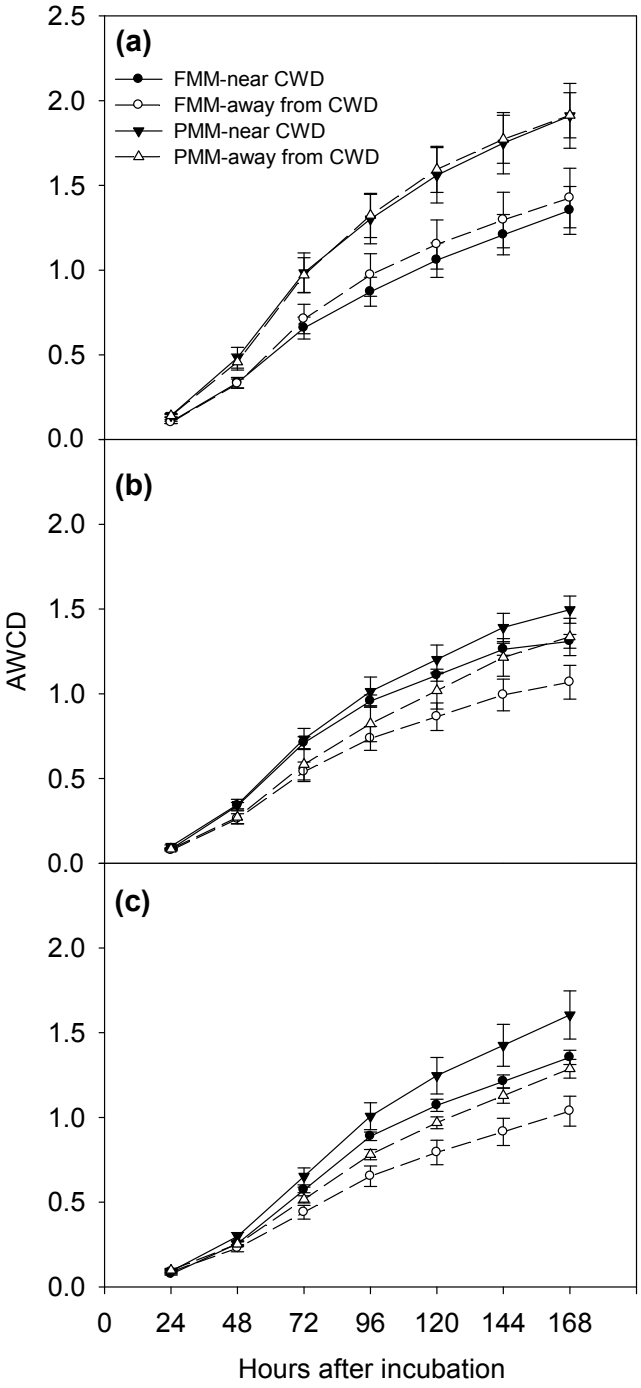

Figure 3-2 Changes in average well color development (AWCD) in cover soils used for oil sands reclamation; a) July, b) August, and c) September in 2014. Error bars indicate standard errors (n=6).

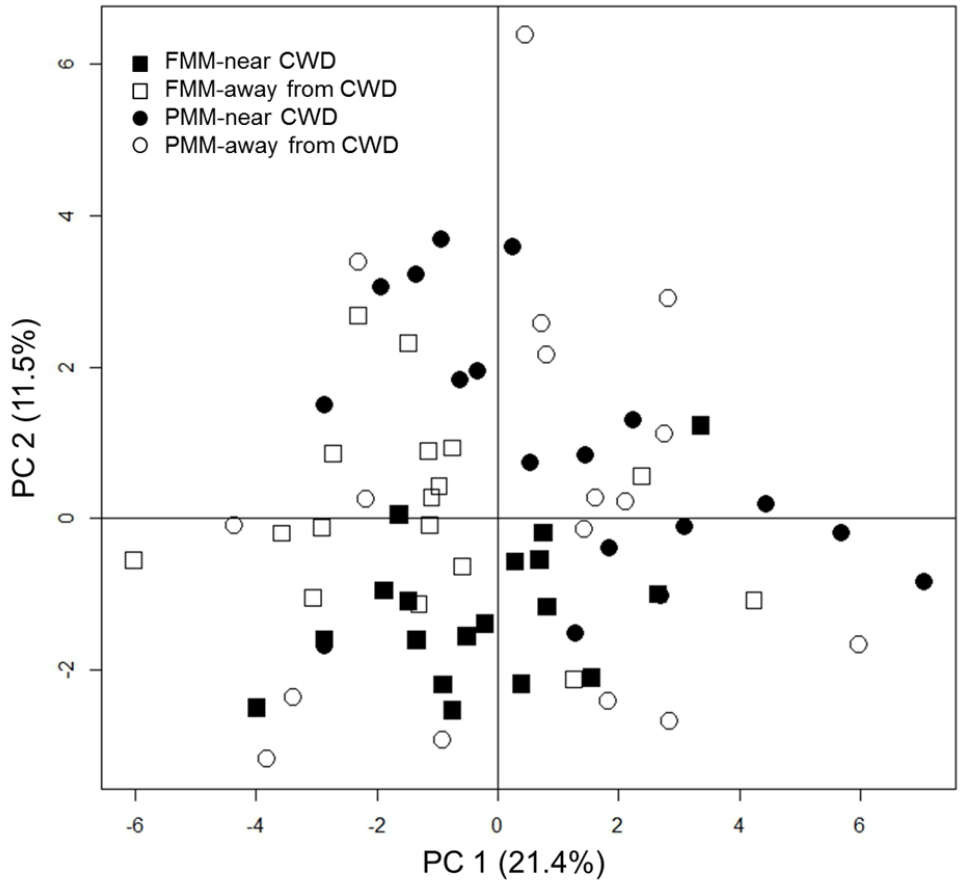

Figure 3-3 Changes in soil microbial community level physiological profile in cover soils used for soil sands reclamation. Principal component analysis (PCA) of the community level physiological profile in 2014 based on the area under the curve (*A*).

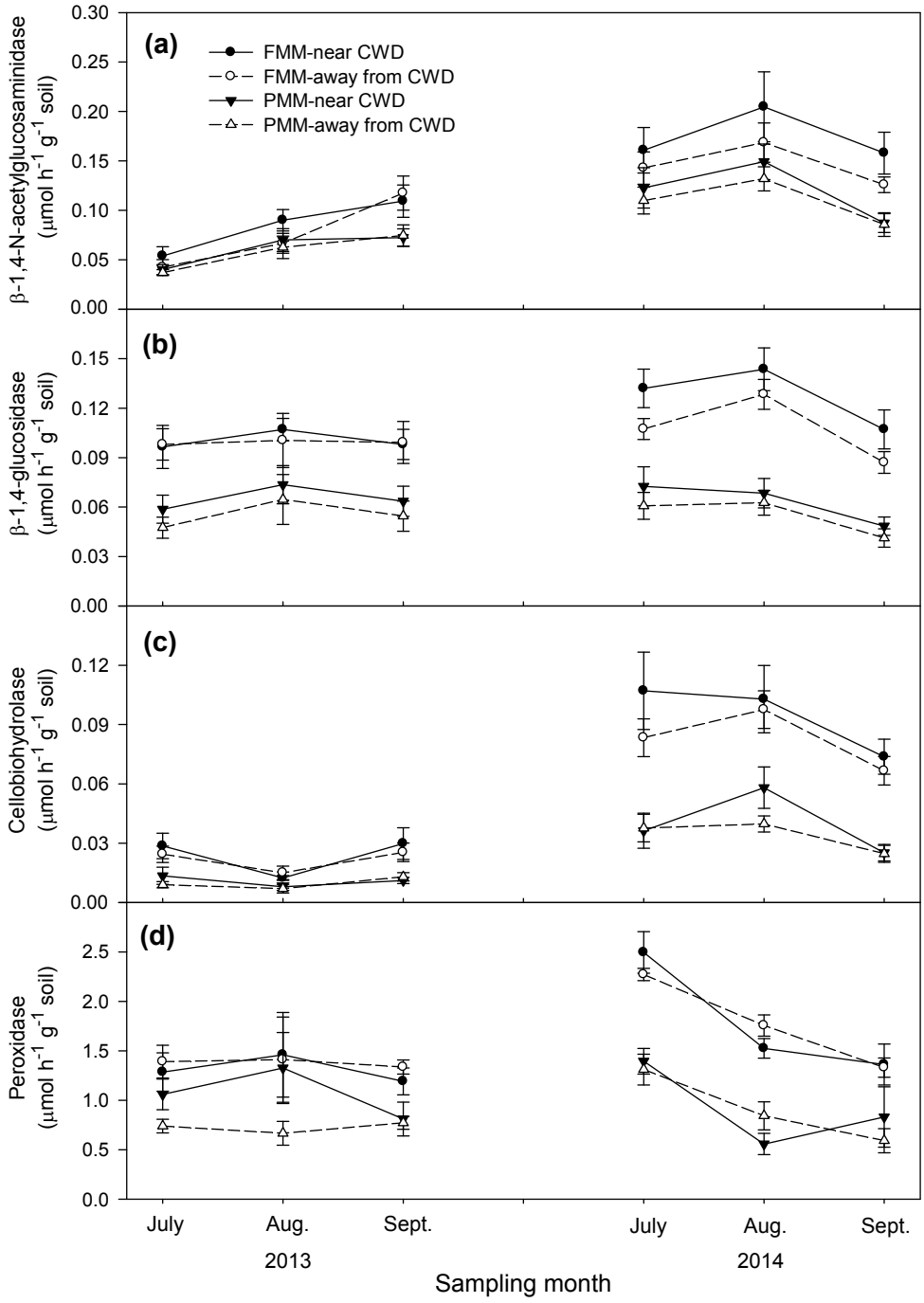

Figure 3-4 Changes in soil enzyme activities in cover soils in 2013 and 2014; a) β-1,4-N-acetylglucosaminidase, b) β-1,4-glucosidase, c) cellobiohydrolase, and (d) peroxidase activities. Error bars indicate standard errors (n=6).

CHAPTER 4. COARSE WOODY DEBRIS EFFECTS ON GREENHOUSE GAS EMISSION RATES IN A RECLAIMED OIL SANDS SOIL DEPEND ON COVER SOIL TYPE

1. Introduction

The Athabasca oil sands region (AOSR) in northern Alberta, Canada has the largest single bitumen deposit in the world (Alberta Government, 2014). Surface mining is one of the common practices for oil sands extraction and has disturbed 767 km^2 of boreal forest until 2013 (Alberta Government, 2014), thereby reducing the potential for atmospheric carbon dioxide (CO_2) sequestration and methane (CH_4) uptake. Total greenhouse gas emissions due to oil sands activities, such as mining, transportation, extraction and upgrading, were 55 Mt in 2011 and account for 23 and 8% of total greenhouse gas emissions in Alberta and Canada, respectively (Alberta Government, 2014). Land disturbed by oil sands operations is regulated to be returned to equivalent land capability of pre-disturbance levels (Province of Alberta, 2014).

Land reclamation can increase atmospheric CO_2 sequestration by supporting plant growth but can simultaneously promote CO_2 emission from cover soils by enhancing microbial decomposition of soil organic matter (Welham et al., 2012). Soil respiration rates are influenced by several factors, such as soil temperature and water content (Davidson et al., 1998; Davidson and Janssens, 2006; Lee et al., 2006; Raich and Schlesinger, 1992), vegetation and substrate quality (Raich and Tufekcioglu, 2000; Wang et al., 2003), net ecosystem productivity (Raich and Potter, 1995; Raich and Tufekcioglu, 2000), microbial population size and activities (Jenkinson et al., 1976; Rice et al., 1996; Shen et al., 1997), and land use and disturbance regimes (Rustad et al., 2000).

Methane has 25 times greater global warming potential than CO_2 over a time scale of 100 years (IPCC, 2007) and is considered the second most common greenhouse gas after CO_2 emitted from soils (IPCC, 2007). Forest soils are the most active CH_4 sinks in upland soils due to the higher methanotrophic activity relative to other ecosystems, such as grass

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land or cultivated land (Mer and Roger, 2001). Therefore, returning disturbed land to upland boreal forest has high potential for oxidizing atmospheric CH_4 and mitigating global warming. Methane oxidation in upland soils is less intensively studied than CH_4 emission in wetlands, and soil water content, gas diffusivity, and nitrogen fertilization are main determinants for CH_4 oxidation and uptake rates in soils (Steudler et al., 1989; Mer and Roger, 2001; Smith et al., 2000).

Nitrous oxide (N_2O) is also an important greenhouse gas emitted from soils; N_2O has 298 times greater global warming potential than CO_2 over a 100-year span (IPCC, 2007). Nitrification and denitrification are the main microbial processes that produce N_2O in soils. Soil water content and temperature are the most important determinants of N_2O emission as they regulate microbial processes and are positively related to N_2O emission (Keller et al., 1983; Smith et al., 2003).

Peat mineral soil mix (PMM) and forest floor mineral soil mix (FMM) are cover soils commonly used over substrates such as geological overburden or tailings sand to provide organic matter, improve soil fertility and water holding capacity, and provide a source of plant propagules and soil microorganisms for land reclamation in the AOSR (DePuit, 1984; Mackenzie and Naeth, 2010; Sydnor and Redente, 2002). Cover soils such as FMM and PMM used for land reclamation have different physical, chemical, and biological properties (Jamro et al., 2014; Mackenzie and Naeth, 2010; McMillan et al., 2007) mainly because organic matter in the PMM salvaged from wetlands is less decomposed than the FMM salvaged from upland forests. The FMM therefore contains more decomposable material because of its lower carbon to nitrogen (C:N) ratio (Mackenzie and Naeth, 2010), and it has higher nitrogen (N) availability and microbial and enzyme activities relative to PMM (Brown and Naeth, 2014; Dimitriu et al., 2010; Jamro et al., 2014; McMillian et al., 2007). The FMM also contained more native plant propagules (Mackenzie and Naeth, 2010) and vegetation cover was typically greater in FMM than in PMM (Brown and Naeth, 2014; Mackenzie and Naeth, 2010).

In the AOSR, coarse woody debris (CWD), which includes dead trees, downed boles, large branches, and dead coarse roots (Harmon et al., 1986; Stevens, 1997), has been applied to the soil surface as a novel land reclamation method to help increase the success of vegetation establishment in early ecosystem development (Brown and Naeth, 2014;

Forsch, 2014). Application of CWD increases soil organic matter content, creates microsites which increase germination and emergence of plant propagules, regulates soil temperature and water content, increases microbial and enzyme activities, and controls soil erosion (Brown and Naeth, 2014; Gonzalez-Polo et al., 2013; Harmon et al., 1986; Stevens, 1997). Large amounts of CWD are produced during clear-cutting of forest stands prior to open-pit mining. Application of CWD for land reclamation is a relatively new practice and its effect on greenhouse gas emission rates has not been studied.

The purpose of this study was to determine the effects of CWD on soil respiration rates and CH_4 uptake in reclaimed oil sands soils with FMM and PMM as cover soils. We hypothesized that 1) soil respiration rates in FMM would be greater than those in PMM regardless of CWD application due to greater microbial and enzyme activities and vegetation cover in FMM, 2) application of CWD would increase soil respiration rates in cover soils due to enhanced microbial activity resulting from increased soil water content and narrow soil temperature range, and the effects of CWD on soil respiration rates would be greater in FMM than in PMM as FMM is drier than PMM and changes in soil water content and microbial activity will be greater in FMM, 3) CH_4 uptake rates would be greater in FMM and without CWD due to drier conditions of FMM and without CWD and 4) N_2O emission rates would be greater in PMM and with CWD due to higher soil water contents in PMM and with CWD. To test these hypotheses, field experiments in the AOSR were conducted 5 and 6 years after land reclamation, which was completed after oil sands mining activities.

2. Materials and Methods

2.1. Study site

The research site is located on an oil sands company lease (56° 58'N, 111° 22'W), about 24 km north of Fort McMurray, Alberta, Canada. The site is located in the mixedwood boreal forest which consists of upland forest, wetlands, and rolling plains which were cleared in 1999 for open-pit oil sands mining. The site was thereafter used for a saline-sodic overburden waste dump until 2004. In the area of oil sands mining, upland forest is dominated by Gray Luvisolic soil with some Dystric and Eutric Brunisols and the wetland

is composed predominantly of Mesisols based on the Canadian system of soil classification (Soil Classification Working Group, 1998). A detailed description of the research site and experimental plots is provided in Brown (2010) and Brown and Naeth (2014).

Average annual temperature from 1981 to 2010 was 1.0 °C and average annual precipitation was 418.6 mm, with 316.3 mm as rain and 133.8 cm as snow (Environment Canada, 2014). The mean average temperature and total precipitation between July and September (sampling period) were 15.9 and 16.0 °C for 2012 and 2013, respectively, and 244.8 and 120.0 mm for 2012 and 2013, respectively (Environment Canada, 2014).

2.2. Experimental design and plot establishment

This experiment was conducted with a 2 (FMM vs PMM) × 2 (near vs away from CWD) factorial design with 6 replications. The study plots were established between November 2007 and February 2008 and were 10 × 30 m in size. Six plots were covered with FMM and six plots with PMM. The FMM was salvaged from a mesic aspen-white spruce mixed forest to a depth of 20 cm and applied at a 20 cm thickness, over 30 cm of B and C horizon mixed subsoil and 100 cm of clean overburden. The PMM, approximately 60% peat and 40% underlying mineral soil, was salvaged from a wetland on the open-pit mining site before mining and applied at a 30 cm thickness over 100 cm of clean overburden material (Brown and Naeth, 2014). Trembling aspen (*Populus tremuloides*) CWD with a minimum of 10 cm diameter was salvaged and applied on each plot in February 2008. The CWD was placed to provide maximum contact with the soil surface. The CWD pieces did not overlap or contact each other and covered approximately 10-20% of each plot. A mixed fertilizer ($N:P_2O_5:K_2O$) was applied as per standard reclamation practice at a rate of 300 kg ha^{-1} (23.5:25.0:8.0) in June 2008 and at 250 kg ha^{-1} (31.5:16.0:5.0) in August 2009 (Brown and Naeth, 2014). Plots were again aerially fertilized in June 2010 and 2011 using granular urea, monoammonium phosphate and muriate of potash at a rate of 250 kg ha^{-1} (Brown, 2010; Forsch, 2014).

Within each plot, four 1 × 1 m subplots were established for gas and soil sampling, two within 5 cm from the CWD and the other two more than 1 m away from the CWD. Near and away from CWD subplots represent areas that have been affected by CWD or not, respectively. Plots were covered by native forbs, grasses, shrubs, and mosses (Brown and

Naeth, 2014). Overall vegetation and woody species covers were greater in FMM than in PMM and near CWD than away from CWD and increased over time (Brown and Naeth, 2014; Forsch, 2014).

2.3. Gas sampling and gas efflux calculation

Gas efflux was measured using static chambers (Hutchinson and Mosier, 1981; headspace 10 cm height, 0.00104 m^3 volume) on 26 July, 29 August, and 26 September in 2012 and on 26 July, 26 August, and 28 September in 2013. Plastic soil collars fitted to the static chambers were placed in each subplot; in total 48 collars [(2 near CWD + 2 away from CWD) × (6 FMM + 6 PMM)] were installed. The collars (4.3 cm height, 10.9 cm inner diameter) were pushed approximately 3 cm into the soil at least 24 h prior to the first sampling and remained in place for the entire study period to avoid gas release caused by soil disruption, which occurs when placing the collars into the soil. Gas samples (20 mL) were collected using a gas tight syringe prior to placing the chamber over the collars (ambient condition, t = 0) and 10, 20, and 30 minutes after placing the chamber over the collars through a rubber septum. Samples were stored in pre-evacuated 10 mL soda glass Isomass Exetainers, to provide a positive pressure in the Exetainer. Gas samples were collected between 0800 and 1200 h to avoid potential impact of diurnal temperature changes on soil respiration measurements. Two plots (FMM and PMM) were paired and gas samples from the paired plots were collected at the same time to minimize the effect of time lag between plots on soil respiration rates. The CO_2, CH_4 and N_2O concentrations in the collected gas samples were analyzed using a Varian CP-3800 gas chromatograph (GC, Varian Canada, Mississauga, Canada) equipped with a thermal conductivity detector.

Soil respiration and CH_4 uptake rates were calculated based on CO_2 and CH_4 concentrations at 0, 10, 20, and 30 minute samplings using Eqs. (1) (Nakayama, 1990) and (2). A mole of an ideal gas at standard temperature (0 °C) and pressure (101.3 kPa) has a volume of 22.4 L, which can be converted to 44.6 moles m^{-3}. This ideal volume is then corrected for the actual air temperature at the time of sampling and used to calculate the efflux.

$$\text{Efflux} = \Delta C \times T \times V / \Delta t \times A = \Delta C \times T \times h / \Delta t \quad (1)$$

$$T = 44.6 \text{ mol } m^{-3} \times 273.15 / (273.15 + T_A) \quad (2)$$

where ΔC is the change in CO_2 and CH_4 concentration in the selected time interval (μmol mol^{-1}), T is temperature adjustment for molecular volume of gas (mol m^{-3}), V is the volume of the static gas chamber (m^3), A is the area of ground covered by the Hutchinson chamber (m^2), h is the height of the static chamber (m), Δt is the time interval between samplings (s), and T_A is actual air temperature (°C). Methane flux was converted to CO_2-equivalents based on a global warming potential of 25 over a 100-year time scale (IPCC, 2007) and the global warming potential of each treatment was calculated as the sum of CO_2, CH_4, and N_2O (CO_2-equivalent) fluxes.

2.4. Soil temperature and water content measurements

Soil temperature and volumetric water content were measured using volumetric smart soil water sensors (Decagon Devices Inc., Pullman, WA, USA) and 12-bit smart temperature sensors (Onset Computer Corporation, Bourne, MA, USA) connected to HOBO micro station data loggers (Onset Computer Corporation, Bourne, MA, USA). Three sensors were installed per treatment at 5 cm depth in FMM and PMM plots near the CWD and 1 m away from the CWD. Data were collected hourly during the study period and minimum, maximum, and mean monthly soil temperature and volumetric water content were calculated (Table 4-3).

2.5. Soil and CWD sampling and analyses

Soil samples were collected from the 0-10 cm layer using an auger on the same day gas sampling was conducted. Four soil samples were randomly collected from each subplot and bulked to form a composite sample. Soil samples were stored on ice packs in a cooler and transported to the laboratory. Fresh soil samples were homogenized, passed through a 2-mm sieve, and refrigerated at 4 °C until analysis. All analyses were completed within 7 days of sampling. A sub-sample of each sample was used to analyze ammonium (NH_4^+), nitrate (NO_3^-), microbial biomass C (MBC) and N (MBN), and dissolved organic C (DOC) and N (DON). The remainder of each sample was air-dried at room temperature and used for pH

and electrical conductivity (EC) analyses. A portion of the air-dried sample was ground using a ball mill (MM200, Retsch GmbH, Haan, Germany) and used for measurement of total C and total N as described below.

Soil pH and EC were analyzed with a pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, Ma, USA) and an AP75 portable waterproof conductivity/TDS meter (Thermo Fisher Scientific Inc., Waltham, MA), respectively, at a 1:5 soil weight-to-deionized water volume ratio. The concentrations of NH_4^+ and NO_3^- were analyzed via a steam distillation method using magnesium oxide and Devada's alloy sequentially after 2 mol L^{-1} potassium chloride extraction at a 1:5 soil weight-to-potassium chloride volume ratio (Keeney and Nelson, 1982). Soil MBC and MBN were analyzed using the chloroform fumigation extraction method (Jenkinson et al., 1981). Fresh soil samples were fumigated with ethanol-free chloroform for 24 hours in an evacuated desiccator. Fumigated and unfumigated samples were extracted with 0.5 mol L^{-1} potassium sulfate solutions at a 1:10 soil weight-to-potassium sulfate solution volume ratio and filtered using a Whatman No. 42 filter paper. Extractable C and N were analyzed using a TOC-V_{CSN} analyzer (Shimadzu, Kyoto, Japan). To analyze DOC and DON concentrations, 5 g of fresh soil samples were extracted with 50 mL of deionized water and filtered using a Whatman No. 42 filter paper. Concentrations of C and N in the filtrate were determined using the TOC-V_{CSN} analyzer. Extractable C was used to represent DOC and the difference between extractable N and available N (NH_4^++NO_3^-) was used to represent DON. Total C and N were analyzed using an automated elemental analyzer (NA-1500 series, Carlo Erba, Milan, Italy).

Coarse woody debris was collected in July 2012 and oven dried at 60 °C to constant weight. The woody debris that includes bark, sapwood and heartwood was ground and passed through a 0.84 mm sieve. Ground CWD was extracted with deionized water at a 1:10 soil weight-to-deionized water volume ratio, shaken at 250 rpm on a mechanical shaker for 1 hour, and filtered through Whatman No. 42 filter papers. The pH of the extracts was measured using the pH meter described above. A portion of the ground CWD was further ground to fine powder using the ball mill described above. Total C and N in the finely ground samples were analyzed using the automated elemental analyzer described above.

2.6. Statistical analyses

A repeated measures analysis of variance (ANOVA) was used to assess the effects of soil type and distance from CWD on greenhouse gas emission rates over time using the PROC MIXED model. The month of each sampling was considered a repeated measured variable for determining seasonal variation in 2012 and 2013. In this analysis, the output statistics passed tests for compound symmetry. Tukey's HSD test was used to test the significance of differences between soil type, distance from CWD, month of sampling and their interactions. Two-way ANOVAs were conducted to test the significance of differences in soil properties. Before performing the ANOVA, normality of distribution and homogeneity of variance were checked with Kolmogorov-Smirnov and Levene's tests, respectively. Linear regression was used to determine the relationship between soil parameter and greenhouse gas emission rates. Regression slopes between treatments were compared using an analysis of covariance. All analyses were performed using the SAS 9.3 software (SAS Institute Inc., NC, USA) and an α value of 0.05 was chosen to indicate statistical significance.

3. Results

3.1. Chemical and microbiological properties of cover soils and CWD

Soil pH, EC, total C, and total N were significantly affected by cover soil type but not by distance from CWD (Table 4-1); they were higher in PMM than in FMM regardless of the distance from CWD. The C:N ratio was highly variable among replications and did not differ between treatments. The pH of CWD was lower than that of cover soils. Total C concentrations and C:N ratio of CWD were higher than those of cover soils (Table 4-1).

Soil DOC concentrations were significantly affected by cover soil type but not by distance from CWD or their interactions (Table 4-2). Soil DOC concentrations were significantly greater in PMM than in FMM in 2012 and 2013; however, there were no significant differences in DON among treatments.

Soil MBC and MBN were not affected by cover soil type or distance from CWD in 2012 and were affected by distance from CWD but not by cover soil type or their interactions in 2013 (Table 4-2). Although MBC and MBN were greater in FMM than in PMM and near CWD than away from CWD in 2012, there was high variability among

replications and no significant difference between treatments. In 2013, CWD significantly increased MBC and MBN in cover soils.

3.2. Soil temperature and water content

Soil temperature was significantly affected by cover soil type, distance from CWD, and their interactions in July and August 2012 and 2013 and was affected by cover soil type but not by distance from CWD in September 2012 and 2013 (Table 4-3). Soil temperature in PMM was significantly higher than that in FMM in every month. The CWD decreased soil temperature in PMM ($p<0.05$) without significant effect in FMM during the study period. Soil temperature range (differences between maximum and minimum) was narrower in FMM than in PMM and near CWD than away from CWD in FMM and PMM ($p<0.05$); soil temperature ranges were 7.6 ± 0.8 (mean ± SE) and 8.9 ± 0.7 ºC near CWD and away from CWD, respectively, in FMM; and 9.9 ± 0.7 and 11.7 ± 0.8 ºC, respectively, in PMM.

Soil volumetric water content was significantly affected by cover soil type, distance from CWD, and their interactions (Table 4-3). Soil volumetric water content in PMM was significantly greater than that in FMM during the study period (Table 4-3). In FMM, the CWD increased volumetric water content in July and August 2012 ($p<0.05$); however, the effect was reversed in September 2012. In contrast, volumetric water contents were greater away from CWD than near CWD in PMM. The volumetric water content ranges were greater away from CWD than near CWD in FMM but the opposite was found in PMM ($p<0.05$). The ranges of volumetric water content were 0.17 ± 0.03 and 0.28 ± 0.04 m^{-3} m^{-3} for near CWD and away from CWD, respectively, in FMM, and 0.28 ± 0.04 and 0.21 ± 0.04 m^{-3} m^{-3}, respectively, in PMM.

3.3. Soil respiration rates

Soil respiration rates were significantly affected by cover soil type, distance from CWD, and sampling time but not by interaction between cover soil type and distance from CWD in 2012 and 2013 (Table 4-4). Soil respiration rates followed similar patterns between treatments and between 2012 and 2013. Soil respiration rates in FMM were significantly greater than those in PMM at each sampling time regardless of the distance from CWD in 2012 and 2013 (Figure 4-1); rates were 461.3-1148.2 and 293.0-677.2 mg CO_2 m^{-2} h^{-1} for

FMM and PMM, respectively, in 2012, and 355.3-1318.0 and 234.6-700.0 mg CO_2 m^{-2} h^{-1} for FMM and PMM, respectively, in 2013. The CWD increased soil respiration in FMM in July and August 2012 and 2013 ($p<0.05$) but not in PMM ($p>0.05$) except in July 2012. Soil respiration rates were positively related to MBC ($p=0.004$) and MBN ($p<0.001$, Figure 4-3). Soil respiration rates decreased from July to September in 2012 and 2013 regardless of treatments ($p<0.05$).

Soil respiration rates were positively correlated with mean soil temperature in all treatments in 2012 and 2013 ($p<0.05$, Figure 4-4). Regression slopes between soil temperature and soil respiration rates were greater in FMM than in PMM regardless of the distance from CWD ($p<0.001$). There were no differences between near CWD and away from CWD ($p=0.120$ and 0.209 for FMM and PMM, respectively) in 2012. Regression slopes in 2013 were greater in FMM than in PMM ($p<0.001$) and near CWD than away from CWD in FMM ($p=0.044$) and PMM ($p=0.003$).

3.4. Soil CH_4 and N_2O efflux

Methane efflux showed negative values in all treatments in 2012 and 2013 indicating that CH_4 uptake occurred in cover soils used for this experiment. Methane uptake rates were significantly affected by cover soil type but not by distance from CWD, sampling time, or any interactions in 2012 and 2013 (Table 4-4). Methane uptake rates were significantly greater in FMM (0.15-0.17 and 0.10-0.17 mg CH_4 m^{-2} h^{-1} for 2012 and 2013, respectively) than in PMM (0.07-0.11 and 0.07-0.12 mg CH_4 m^{-2} h^{-1} for 2012 and 2013, respectively) regardless of the distance from CWD (Figure 4-2). The CWD increased CH_4 uptake rates in FMM ($p<0.05$) but not in PMM ($p>0.05$) in 2012 samplings. There was no effect of the CWD on CH_4 uptake rates in 2013 except in PMM in August 2013. Methane uptake rates were negatively related to volumetric soil water content ($p<0.001$, Figure 4-5) with no relationship to soil temperature.

Concentrations of N_2O were under detection limit in most samplings and overall N_2O emission rates in cover soils were generally low, ranging from 0.001 to 0.016 mg N_2O m^{-2} h^{-1} (Figure 4-3). Emission rates of N_2O were not affected by cover soil type or CWD in each sampling and there was no clear temporal trend among samplings.

3.5. Global warming potential of CO_2, CH_4 and N_2O effluxes

The global warming potential of CO_2, CH_4 and N_2O effluxes was similar to soil respiration rates as CH_4 uptake and N_2O emission rates were very small compared to soil respiration rates (data not shown). Global warming potentials were greater in FMM than in PMM regardless of CWD application or not ($p<0.05$). Applying CWD increased global warming potentials in FMM ($p<0.05$) but not in PMM.

4. Discussion

4.1. Soil respiration rates

Soil respiration rates were significantly greater in FMM than in PMM in each sampling regardless whether CWD was applied or not, supporting the first hypothesis. Substrate supply and quality are important factors regulating soil respiration (Raich and Schlesinger, 1992; Wang et al., 2003). Water soluble C or DOC is considered the most readily available C source for microorganisms (McGill et al., 1986; Wang et al., 2003) and will affect microbial activities and CO_2 emission rates. In this study, DOC concentrations were greater in PMM than in FMM but soil respiration rates showed a reverse pattern with DOC concentration, indicating that microorganisms could not fully utilize DOC in PMM. Soil microbial biomass is the main driving force in organic matter decomposition, and MBC is one indicator for the potential rate of C flux (Franzluebbers et al., 1999; Rice et al., 1996) due to its high turnover rate. Several studies reported a close relationship between MBC and soil respiration rates (Jenkinson et al., 1976; Shen et al., 1997), and results from this study support the literature on the positive relationship between MBC and soil respiration. Previous studies conducted in the AOSR reported higher microbial biomass in FMM than those in PMM (Brown, 2010; Jamro et al., 2014; McMillan et al., 2007). McMillan et al. (2007) showed greater soil respiration rates in FMM than those in PMM coupled with greater MBC and MBN in FMM. Greater soil respiration in FMM in this study could be attributed to greater MBC in FMM than that in PMM.

Both size of microbial biomass and microbial community composition are important for organic matter decomposition. Microbial communities in cover soils are affected from where the materials were salvaged and microbial communities in FMM is more similar to upland forests (Dimitriu et al., 2010) and active for aerobic decomposition. Soil enzymes

play an important role in C and N cycling (Burns et al., 2013; Sinsabaugh et al., 1991) and activities of organic matter decomposition related enzymes, such as β-1,4-N-acetylglucosaminidase and β-1,4-glucosidase, were higher in FMM than in PMM (Dimitriu et al., 2010; Jamro et al., 2014), which would increase soil respiration rates.

Soil temperature and water content are important factors affecting soil respiration through their effects on soil microbial activities (Davidson et al., 1998; Lee et al., 2006; Raich and Schlesinger, 1992; Rustad et al., 2000) and soil respiration rates are generally positively correlated with soil temperature and soil water content in upland ecosystems (Raich and Schlesinger, 1992; Saurette et al., 2005). Soil respiration rates in PMM should be expected to be greater than those in FMM due to the higher soil temperature and volumetric water content in PMM; however, soil respiration rates were significantly greater in FMM than in PMM. Gas diffusivity is important for oxygen supply to microorganisms and organic matter decomposition, which is a major control on CO_2 emission (Bastida et al., 2009; Smith et al., 2003). Higher organic matter content and water content in PMM decreased aeration porosity in PMM (25.6 ± 1.3%) relative to FMM (51.7 ± 1.2%), decreasing oxygen supply to microorganisms and microbial respiration rates.

Applying CWD significantly increased soil respiration rates in FMM but not in PMM, supporting the second hypothesis. Early stages of reclaimed land are directly exposed to changing environmental conditions, such as temperature fluctuations and drying-wetting cycles, which are less favorable conditions for microorganisms. Applying CWD for land reclamation decreased soil temperature range and increased soil water content, especially in FMM, creating more microsites (Brown and Naeth, 2014). Such conditions can provide more favorable habitats for microorganisms, which may increase microbial activity and organic matter decomposition near CWD. Soil MBC and MBN were greater near CWD than away from CWD in this study, especially in FMM. Gonzalez-Polo et al. (2013) found that C-degrading soil enzyme activity such as β-1,4-glucosidase increased under CWD. Increased microsites and greater MBC, MBN, and enzyme activities near CWD increased soil respiration rates and therefore may accelerate organic matter decomposition in cover soils. Vegetation cover near CWD was greater than that away from CWD at the same research plots (Brown and Naeth et al., 2014) and results from this study support findings in earlier studies that vegetation development was linked to soil properties such as soil water

content, MBC, MBN and enzyme activities.

Soil respiration rates were positively related to soil temperature in this study. The effects of soil temperature on soil respiration were greater in FMM than in PMM regardless of the distance from CWD in 2012 and 2013 and were greater near CWD than away from CWD in both cover soils, especially in 2013. As MBC and MBN were greater in FMM than in PMM and near CWD than away from CWD and were positively related to soil respiration rates, soil respiration rates were more sensitive to changing soil temperature in FMM than in PMM and near CWD than away from CWD. Similarly, MBC and MBN were greater in 2013 than in 2012 and effects of soil temperature on soil respiration were greater in 2013 than in 2012 regardless of treatments.

Decreases in soil respiration rates from July to September in 2012 and 2013 regardless of the treatments were mainly affected by soil temperature. Soil temperatures decreased from July to September and decreased microbial activities in cover soils as described above. Temperature affects not only microbial activity but also root respiration (Davidson and Janssens, 2006; Raich and Schlesinger, 1992). Mean air temperature in the study area at the end of September was 10.5 and 8.3 ºC in 2012 and 2013, respectively (Environment Canada, 2014). Decreased vegetation cover at the end of the growing season caused by decreasing air temperature may also decrease soil respiration rates by decreasing root biomass and associated root respiration (Davidson and Janssens, 2006; Raich and Schlesinger, 1992).

Soil respiration rates are related to vegetation type and its net primary productivity by affecting autotrophic respiration and amounts of detritus (Raich and Schlesinger, 1992; Raich and Tufekcioglu, 2000). The FMM contains a denser native seed bank and propagules (Mackenzie and Naeth, 2010) and vegetation cover was significantly greater with FMM for oil sands reclamation in the AOSR than with PMM (Brown and Naeth, 2014, Forsch, 2014). The mean value of the contribution of root respiration to total soil respiration for forest vegetation from an intensive literature review was 46% (Hanson et al., 2000). Greater vegetation and root density caused by FMM application may induce greater root respiration and total soil respiration in FMM than in PMM, although I did not measure root respiration separately.

Root exudate and root detritus are readily available substrates and energy sources for

soil microorganisms, and increases in vegetation cover and root biomass enhance microbial activities and organic matter decomposition rates leading to increased microbial activities and positive priming effects (DeGrood et al., 2005; Kuzyakov et al., 2000; Ponder and Tadros, 2002). Greater vegetation cover in FMM than in PMM (Brown and Naeth, 2014; Forsch, 2014) might increase both autotrophic and heterotrophic respirations. Mycorrhizae fungi, part of the total fungal community, account for a large portion of total microbial biomass and accelerate organic matter decomposition by producing extracellular enzymes (Smith and Read, 2008). Mycorrhizal biomass was greater in FMM than in PMM, associated with greater woody plant cover in FMM (Brown and Naeth, 2014), resulting in greater soil respiration in FMM.

4.2. Soil CH_4 uptake and N_2O emission rates

Methane uptake rates were significantly greater in FMM than in PMM (Figure 4-2) and this supported part of our third hypothesis. Upland forests are one of the most active CH_4 sinks in terrestrial ecosystems (Mer and Roger, 2001; Steudler et al., 1989), and soil water content was the major factor affecting CH_4 uptake rates in aerobic soils (Keller et al., 1983; Smith et al., 2000; Steudler et al., 1989). Methane uptake rates in cover soils in our study were in the range of those measured in temperate (0.008-0.132 mg C m^{-2} h^{-1}) and boreal (0.008-0.115 mg C m^{-2} h^{-1}) forests (Steudler et al., 1989), indicating that oil sands reclamation has high potential as a CH_4 sink. Methanogens and methanotrophs exist in the same areas and the net balance between two microbial groups changes depending on soil water content (Keller et al., 1983; Steudler et al., 1989). Soil water content was significantly lower in FMM than in PMM (Table 4-3) and CH_4 uptake rates were negatively related to soil water content, consistent with previous studies (Keller et al., 1983; Smith et al., 2000; Steudler et al., 1989). The CH_4 uptake rates were negatively related to water filled pore space and positively related to gas diffusivity (Smith et al., 2000, 2003). Aeration porosity during the study period was greater in FMM than in PMM as described above, indicating that gas diffusivity was greater in FMM, resulting in greater CH_4 uptake rates.

Applying CWD did not change CH_4 uptake rates in PMM and it increased CH_4 uptake in FMM in July and August 2012. Therefore, we reject part of our third hypothesis that CH_4 uptake would be greater away from CWD than near CWD. Increases in CH_4 uptake rates by

CWD in FMM in 2012 might be attributed to increases in microsites and microbial activities near CWD rather than effects of soil water content. The CWD increased soil water content in FMM when CH_4 uptake rates were increased in July and August 2012 (Table 4-3). However, aeration porosities of near CWD and away from CWD were 55.3 ± 2.8 and 63.4 ± 1.6%, respectively, indicating that soils were still (net) aerobic. The CWD increased microsites (Brown and Naeth, 2014); therefore microbial activity, including that of methanotrophs, would be increased, resulting in greater CH_4 uptake near CWD.

Nitrous oxide emission rates were not affected by cover soil type or CWD and we therefore reject the fourth hypothesis. Nitrification and denitrification are the main microbial biological processes that produce N_2O in soils (Keller et al., 1989; Smith et al., 2003). Net nitrification rates in the same research plots were greater in FMM than in PMM in growing seasons (Kwak et al., 2015d), while denitrification potential was greater in PMM than in FMM due to its higher organic matter content and lower water filled pore space in PMM as described above. Such interactive effects may have caused the lack of significant effects of cover soil type on N_2O emission rates. Application of CWD increased microsites (Brown and Naeth, 2014) thereby increasing microbial activity and N_2O emission rates. However, there was no significant CWD effect on N_2O emission rates likely due to large variability among replications.

4.3. Global warming potential of CO_2, CH_4 and N_2O effluxes

The global warming potential showed a similar pattern to that of soil respiration rates as CH_4 uptake and N_2O emission rates were very small as compared to soil respiration rates (0.015-0.037 mg CH_4 m^{-1} h^{-1} and 0.001-0.016 mg N_2O m^{-1} h^{-1} vs 293.0-1148.2 mg CO_2 m^{-1} h^{-1}). Therefore, the global warming potential was greater in FMM than in PMM although CH_4 uptake rates were greater in FMM. Applying CWD increased the global warming potential in FMM due to greater soil respiration rates near CWD in FMM. However, CWD did not affect global warming potentials in PMM as there were no differences of soil respiration, CH_4 uptake and N_2O emission rates between near CWD and away from CWD. Although the global warming potential was greater in FMM than in PMM and near CWD than away from CWD, vegetation cover was greater in FMM and near CWD (Brown and Naeth, 2014; Forsch, 2014), indicating that CO_2 sequestration rates would be also greater in

FMM and near CWD. Further research that compare greenhouse gas emissions from cover soils and net primary productivity would be needed to understand the effects of cover soils and CWD on the overall global warming potential in the reclaimed landscape.

Results from our study do not represent the whole lifecycle of greenhouse gas emissions from cover soils because we measured greenhouse gas emission rates only between July and September. The late starting point for greenhouse gas emissions in this study was caused by site access restrictions. In addition, measuring greenhouse gas emission rates during winter is not possible in northern Alberta with thick show cover and freezing temperatures. Soil respiration and CH_4 uptake rates measured three times a year for two years showed the same pattern in each sampling. Although soil respiration and CH_4 uptake rates were variable because of soil heterogeneity and climate variability, our data show that applying FMM and CWD increased the global warming potential. Despite the limited number of samplings, comparisons among treatments still provide a valuable dataset.

5. Conclusions

The effects of CWD on soil respiration and CH_4 uptake rates were dependent on the cover soil type; CWD increased soil respiration and CH_4 uptake rates in FMM but not in PMM. Soil microbial biomass and temperature were the main determinants for soil respiration rates in the studied cover soils used for oil sands reclamation. Effects of changing soil temperature on soil respiration rates were greater in FMM than in PMM and near CWD than away from CWD due to the greater MBC and MBN in FMM and near CWD. Methane uptake rates were mainly affected by soil water content, and reclaimed oil sands soils act as CH_4 sinks. Nitrous oxide emission rates were not affected by cover soil type or CWD. However, contribution of CH_4 uptake and N_2O emission rates on the global warming potential was negligible compared to soil respiration rates in the studied cover soils. Applying CWD for oil sands reclamation will increase carbon storage in CWD biomass itself but will increase soil respiration rates and therefore organic matter mineralization in FMM. Results from this study provide support to findings in earlier studies that CWD application benefits vegetation establishment in land reclamation increasing soil water content, MBC, MBN and enzyme activities. Applying FMM and CWD for land reclamation post open-pit oil sands mining increased greenhouse gas emissions; however, at the same

time, it increased vegetation establishment, which can increase CO_2 sequestration rates. Further study, measuring net balance between greenhouse gas emissions from cover soils and net primary productivity, would be needed to understand the effects of cover soils and CWD on overall global warming potential in the reclaimed oil sands landscape.

Table 4-1 Chemical properties of forest floor mineral soil mix (FMM), peat mineral soil mix (PMM) and coarse woody debris (CWD) collected from a reclamation experiment.

Treatment		pH	EC[a] ($dS\ m^{-1}$)	Total C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	C:N
Cover soil	Distance from CWD					
FMM	Near	6.05 (0.12)	0.18 (0.01)	39.7 (4.9)	1.4 (0.2)	30.1 (3.7)
	Away	5.89 (0.10)	0.21 (0.02)	40.8 (5.6)	1.4 (0.3)	32.8 (2.9)
PMM	Near	6.86 (0.03)	0.40 (0.02)	68.4 (8.2)	2.4 (0.3)	28.6 (1.6)
	Away	6.86 (0.04)	0.48 (0.03)	72.7 (10.3)	2.4 (0.4)	31.3 (1.3)
Two-way ANOVA						
Cover soil		***	*	*	*	ns
Distance from CWD		ns	**	ns	Ns	ns
Soil×distance from CWD		ns	ns	ns	Ns	ns
CWD		4.56 (0.01)	ND	499.3 (0.3)	3.6 (0.2)	140.2 (0.8)

Values are means with SE (n=6);
* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant;
EC= electrical conductivity, C:N= carbon to nitrogen ratio and ND=not determined.

Table 4-2 Dissolved organic carbon (DOC) and nitrogen (DON) and microbial biomass carbon (MBC) and nitrogen (MBN) in cover soils in 2012 and 2013 and effects of cover soil type (FMM vs PMM) and distance from CWD (near vs away) on soil properties in cover soils used for oil sands reclamation.

Treatments		DOC (mg kg^{-1})		DON (mg kg^{-1})		MBC (mg kg^{-1})		MBN (mg kg^{-1})	
Cover soil	Distance	2012	2013	2012	2013	2012	2013	2012	2013
FMM	Near	189.1 (10.6)	231.1 (20.3)	12.8 (0.7)	6.7 (1.1)	270.3 (21.8)	277.1 (32.6)	36.3 (5.3)	49.0 (7.2)
	Away	181.9 (10.6)	252.1 (16.1)	12.3 (0.9)	5.9 (0.6)	217.7 (15.9)	233.8 (17.4)	31.5 (3.1)	36.4 (3.5)
PMM	Near	261.1 (16.5)	316.9 (17.1)	11.0 (1.0)	5.4 (0.6)	260.9 (36.2)	235.6 (27.2)	33.4 (5.1)	33.4 (4.1)
	Away	234.4 (9.0)	335.4 (23.9)	9.6 (0.8)	5.9 (0.5)	207.9 (29.1)	182.6 (19.7)	27.8 (4.3)	30.7 (3.9)
Two-way ANOVA									
Cover soil		*	*	ns	ns	ns	ns	ns	Ns
Distance from CWD		ns	Ns	ns	ns	*	*	ns	*
Soil×distance from CWD		ns	Ns	ns	ns	ns	ns	ns	Ns

Values are mean with SE (n=18); * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant;
DOC= dissolved organic carbon, DON= dissolve organic nitrogen, MBC=microbial biomass carbon, MBN=microbial biomass nitrogen.

Table 4-3 Minimum, maximum, and mean soil temperature and volumetric soil water content at 5 cm below the ground surface in cover soils used for land reclamation.

Treatment		Soil temperature (°C)									Volumetric soil water content (m^3 m^{-3})								
		July			August			September			July			August			September		
Cover soil	Dis-tance	Min.	Max.	Mean[†]	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
2012																			
FMM	Near	12.9	21.7	17.3	11.1	21.4	16.4	9.1	15.4	12.2	0.10	0.37	0.21	0.07	0.16	0.11	0.07	0.27	0.21
	Away	12.6	22.5	17.5	10.7	21.5	16.3	8.4	16.2	12.2	0.01	0.36	0.15	0.00	0.13	0.03	0.00	0.37	0.26
PMM	Near	13.1	24.1	18.5	11.4	22.6	17.2	8.1	17.4	12.4	0.24	0.62	0.40	0.21	0.42	0.28	0.21	0.59	0.49
	Away	13.0	26.0	19.2	11.3	23.9	17.7	7.9	18.0	12.6	0.29	0.58	0.43	0.27	0.41	0.33	0.27	0.59	0.50
Two-way ANOVA[‡]																			
Cover oil				***			***			***			***			***			***
Distance from CWD				***			**			ns			***			***			***
Soil×distance from CWD				***			***			ns			***			***			***
2013																			
FMM	Near	12.1	19.1	15.1	13.4	18.1	15.4	8.7	17.2	13.0	0.17	0.30	0.24	0.10	0.24	0.16	0.08	0.26	0.11
	Away	11.5	19.9	15.2	12.6	18.8	15.4	7.6	17.8	12.8	0.11	0.35	0.23	0.04	0.29	0.15	0.01	0.36	0.10
PMM	Near	12.1	22.3	16.4	13.4	20.1	16.4	8.2	19.2	13.6	0.37	0.56	0.47	0.26	0.48	0.36	0.21	0.51	0.28
	Away	12.0	25.0	17.6	13.6	22.2	17.4	7.6	20.6	13.8	0.49	0.61	0.54	0.41	0.55	0.49	0.33	0.58	0.41
Two-way ANOVA																			
Cover soil				***			***			***			***			***			***
Distance from CWD				***			***			ns			***			***			***
Soil×distance from CWD				***			***			**			***			***			***

[†]Standard errors of mean soil temperature and mean volumetric soil water content were not shown because they were less than 0.1 and 0.01 for soil temperature and volumetric soil water content, respectively, for all measurements

[‡]Two-way ANAVO was conducted only for mean soil temperature and mean volumetric soil water content, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; and ns, not significant; Min.= minimum, Max.=maximum

Table 4-4 Effects of cover soil type, distance from CWD, sampling time and their interactions on CO_2 emission and CH_4 uptake rates.

Measurement	Year	Cover soils		Distance from CWD		Sampling time		Soil × distance		Soil × time		Distance × time		Soil × distance × 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
CO_2	2012	25.8	0.001	27.8	0.001	111.0	<0.001	5.3	0.054	9.2	<0.001	5.7	0.005	0.4	0.686
	2013	91.4	<0.001	17.4	0.005	158.0	<0.001	4.9	0.065	15.2	<0.001	4.3	0.017	1.0	0.359
CH_4	2012	20.0	0.001	1.5	0.268	0.83	0.437	7.4	0.024	1.7	0.181	1.4	0.252	0.1	0.900
	2013	10.5	0.013	3.0	0.129	0.98	0.383	0.01	0.910	0.01	0.993	0.96	0.389	1.41	0.252

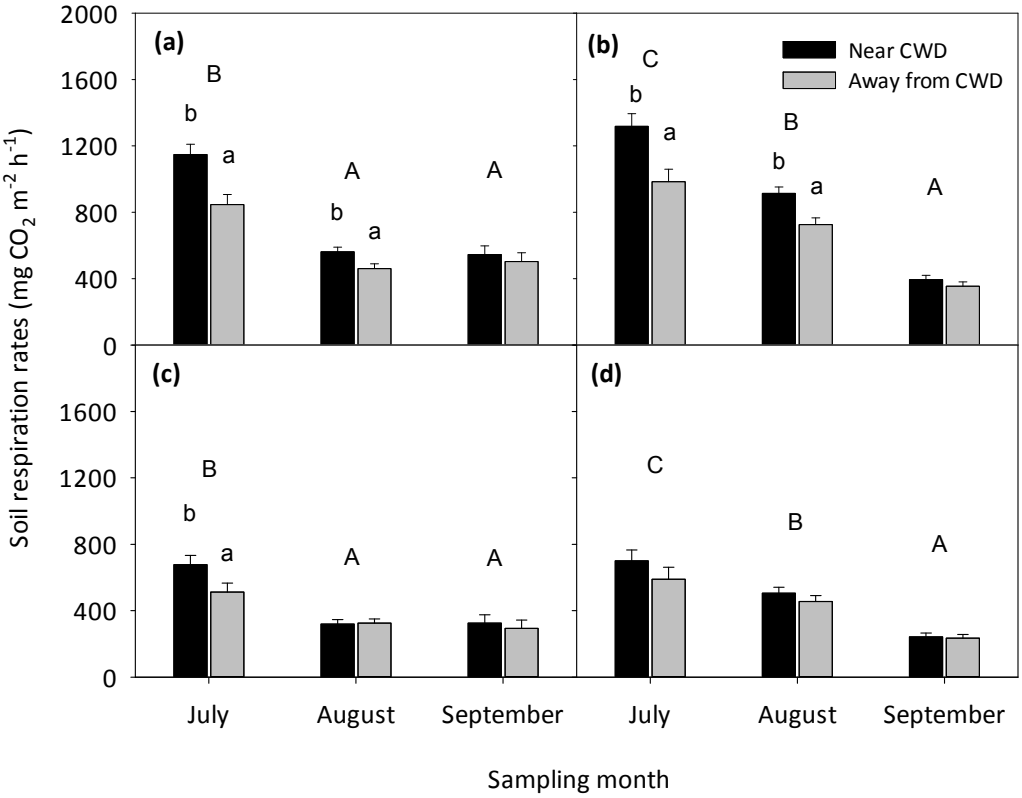

Figure 4-1 Soil respiration rates from cover soils, FMM and PMM, near CWD and away from CWD in a) FMM in 2012, b) FMM in 2013, c) PMM in 2012, and d) PMM in 2013. Error bars are standard error of the mean (n=6). Different lower case letters indicate significant difference between distance from CWD (near vs away) and different upper case letters indicate significant difference between sampling months in each cover soil and sampling year (α=0.05).

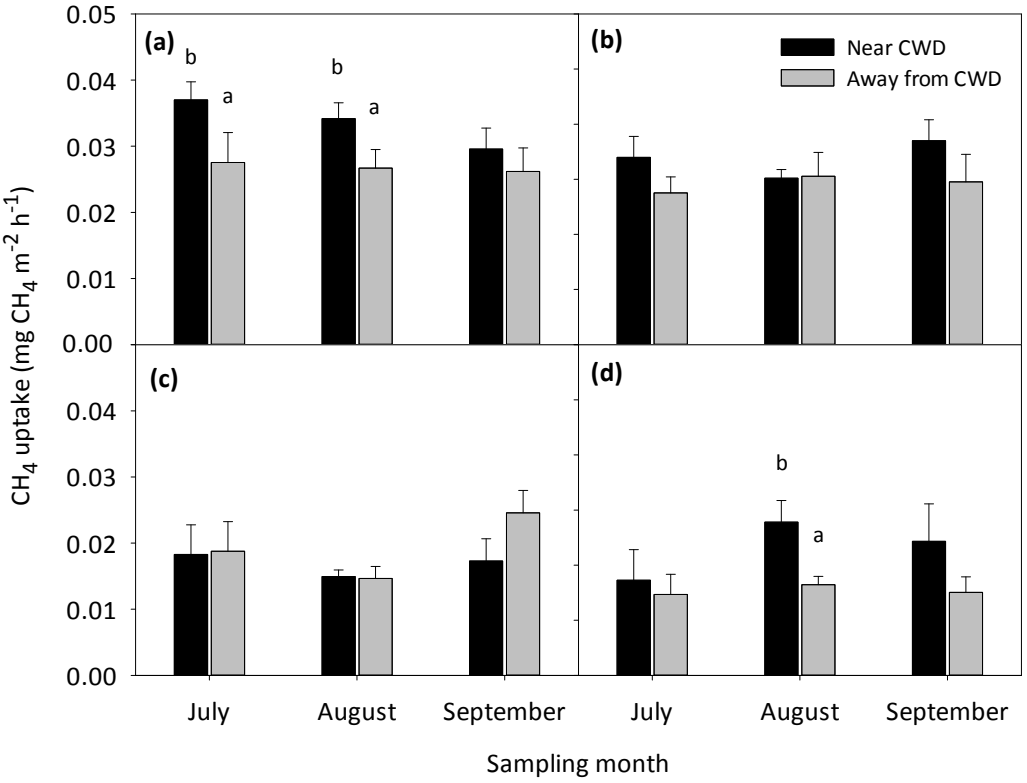

Figure 4-2 CH_4 uptake rates from cover soils, FMM and PMM, near CWD and away from CWD in a) FMM in 2012, b) FMM in 2013, c) PMM in 2012, and d) PMM in 2013. Error bars are standard error of the mean (n=6). Different lower case letters indicate significant difference between distance from CWD (near vs away) (α=0.05).

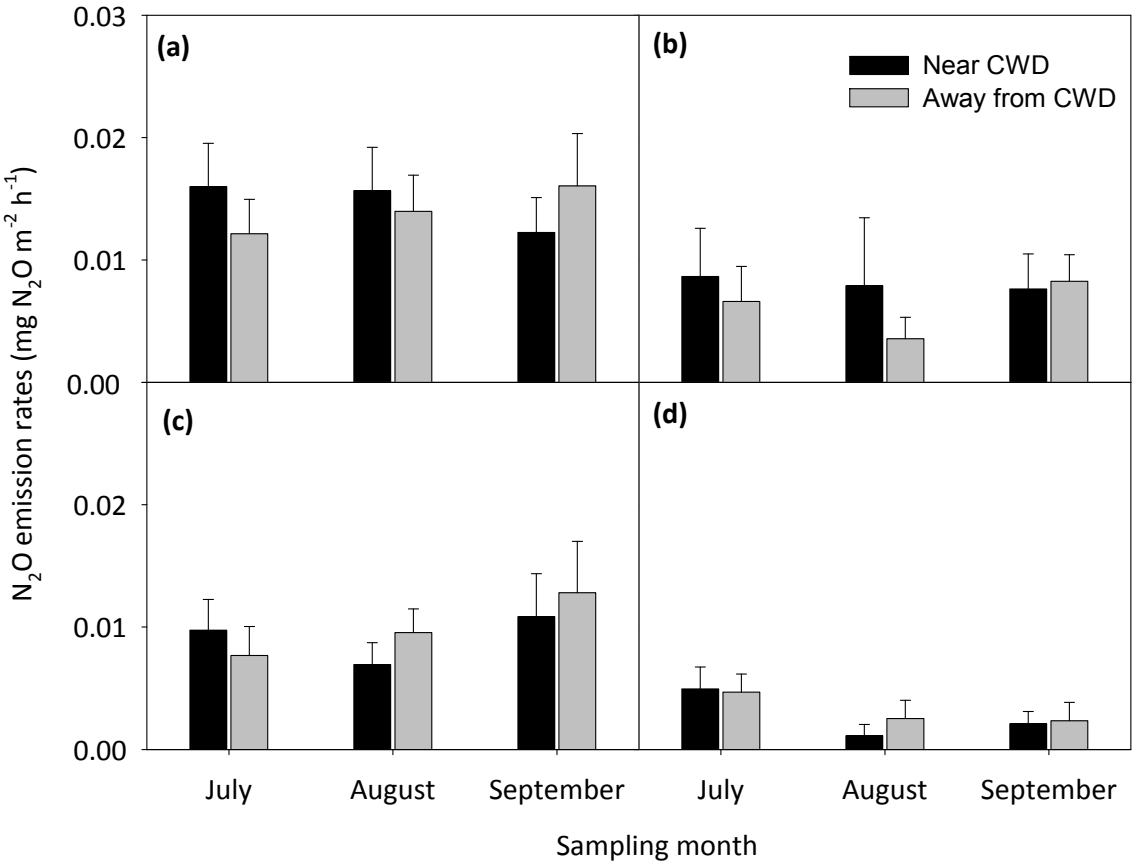

Figure 4-3 N_2O emission rates from cover soils, FMM and PMM, near CWD and away from CWD in a) FMM in 2012, b) FMM in 2013, c) PMM in 2012, and d) PMM in 2013. Error bars are standard error of the mean (n=6).

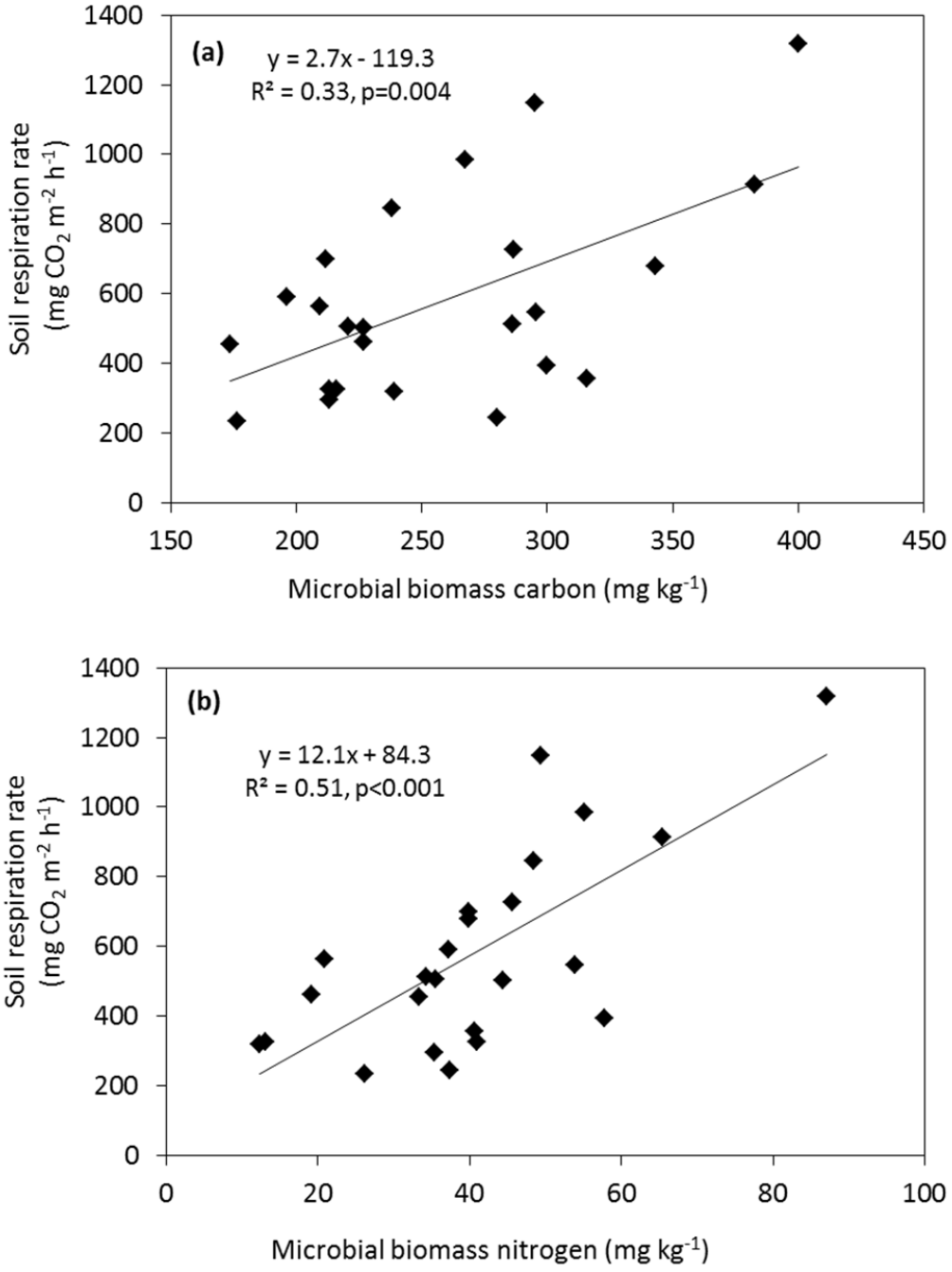

Figure 4-4 Relationships between a) microbial biomass carbon and soil respiration rates and b) microbial biomass nitrogen and soil respiration rates. The mean values of each sampling month and treatment were used.

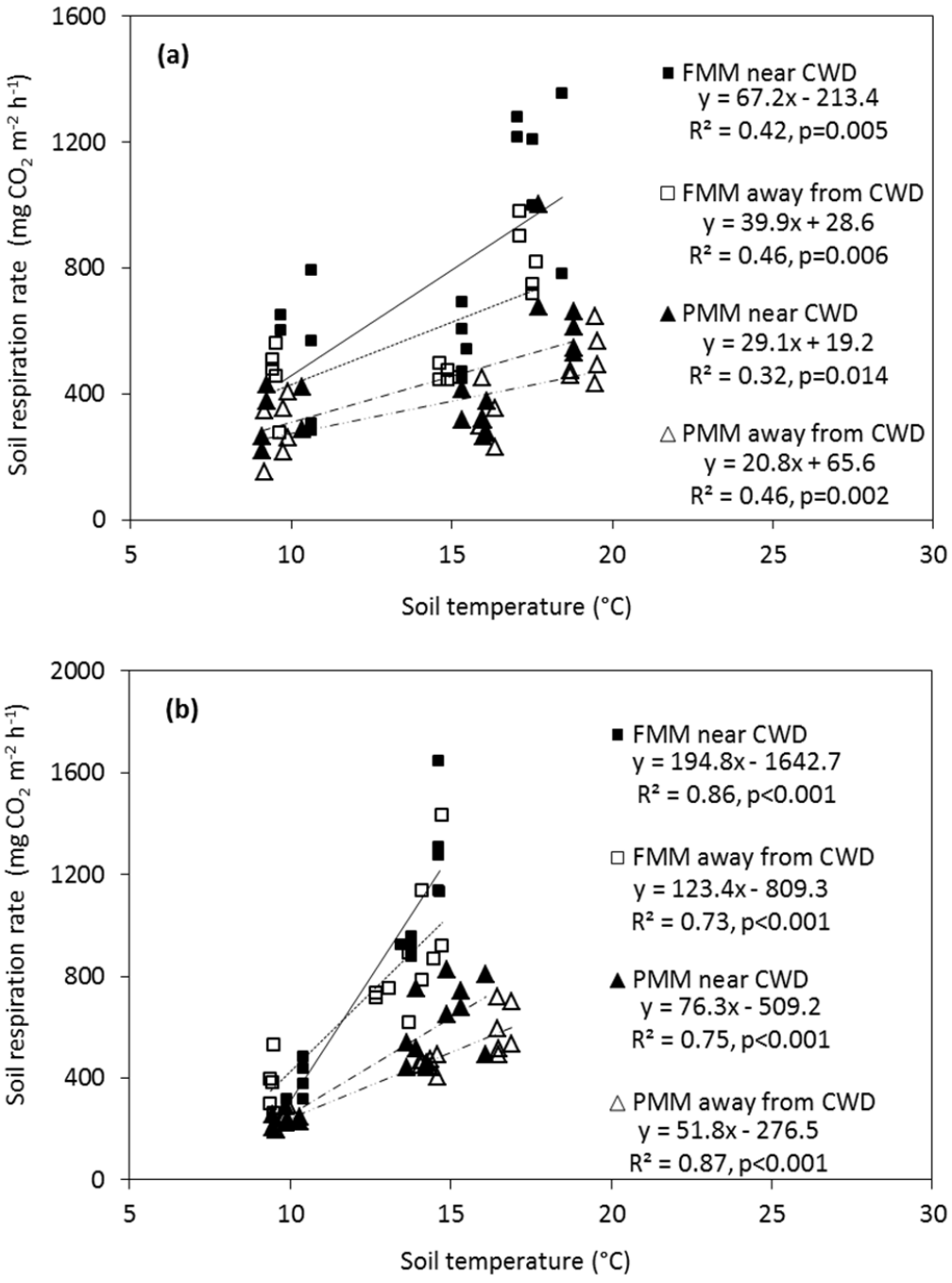

Figure 4-5 Relationships between soil temperature and soil respiration rates in a) 2012 and b) 2013.

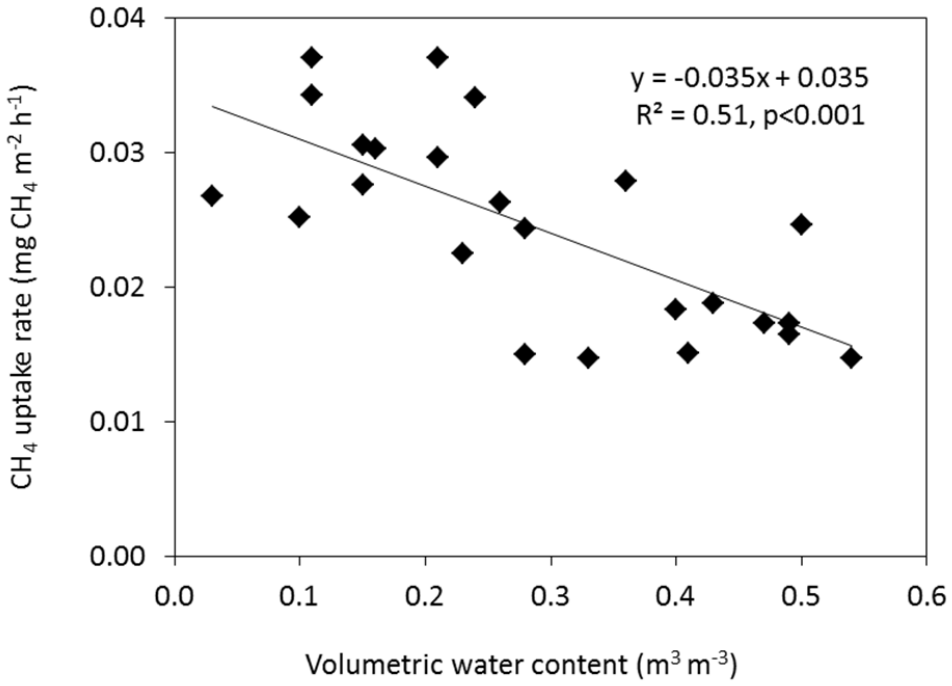

Figure 4-6 Relationships between volumetric soil water content and CH_4 uptake rates. The mean values of each sampling month and treatment were used.

CHAPTER 5. NITROGEN TRANSFORMATION RATES ARE AFFECTED BY COVER SOIL TYPE BUT NOT COARSE WOODY DEBRIS APPLICATION IN RECLAIMED OIL SANDS SOILS

1. Introduction

Open-pit mining in the Athabasca oil sands region in northeastern Alberta, Canada, had disturbed 767 km^2 of mixedwood boreal forests by 2013 (Government of Alberta, 2013). Provincial regulations require the return of such disturbed land to land capability equivalent to pre-disturbance levels (Province of Alberta, 2014) and reclaim the disturbed land to upland forests is a common reclamation practice in the Athabasca oil sands region.

Nitrogen (N) in the soil is the most likely limiting nutrient for plant growth in boreal forest ecosystems and reclaimed lands. Nitrogen transformation and supply rates are closely related to plant growth and forest productivity (Kaye and Hart, 1997; Yan et al., 2012; Duan et al., 2015). Nitrogen transformation rates and availability are affected by soil properties, such as soil temperature (Lang et al., 2010), pH (Cookson et al., 2007; Cheng et al., 2013), water content (Chen et al., 2011; Cheng et al., 2012), carbon (C) to N (C:N) ratio (Hart et al., 1994) and microbial and enzyme activities (Sinsabaugh et al., 1991; Burns et al., 2013). Vegetation cover also affects N availability as influenced by litter dynamics and N uptake by vegetation (Nadelhoffer et al., 1985; Kaye and Hart, 1997). Determining N availability in reclaimed oil sands soils is essential to improve plant growth and accelerate early development of ecosystems established on reclaimed oil sands lands.

Forest floor mineral soil mix (FMM) and peat mineral soil mix (PMM) are common cover soils used for land reclamation to improve soil fertility and plant growth (DePuit, 1984; Sydnor and Redente, 2002). As FMM is salvaged from upland forests and PMM from peat-forming wetlands, FMM and PMM have contrasting properties (Mackenzie and Naeth, 2010; Hahn and Quideau, 2013; Brown and Naeth, 2014; Jamro et al., 2014). For example, FMM has lower C:N ratios (Mackenzie and Naeth, 2010; Jamro et al., 2014), greater microbial biomass (McMillan et al., 2007; Brown, 2010; Jamro et al., 2014; Hahn and

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Quideau, 2013), greater enzyme activities (Jamro et al. 2014) and greater vegetation cover (Mackenzie and Naeth, 2010; Brown and Naeth, 2014; Forsch, 2014). Therefore, N transformation rates and N availability are different between the two cover soils.

Coarse woody debris (CWD), including large branches, logs, standing dead trees, and dead coarse roots, plays important ecological roles in forest ecosystems (Harmon et al., 1986; Stevens, 1997). Large amounts of CWD are produced from clearing mixedwood boreal forests before oil sands mining and applying CWD for oil sands reclamation has recently been tested (Brown and Naeth, 2014). Coarse woody debris can provide more favorable habitats, stabilize soil temperature and increase soil water availability for microorganisms, thereby increasing microbial activity (Kappes et al., 2007; Brown and Naeth, 2014). However, CWD leachate with high C:N ratio may also increase N immobilization (Kwak et al., 2015a). Due to these different effects of CWD on soil properties, effects of CWD on N transformation rates and N availability reported in the literature are inconsistent (Spears et al., 2003; Hafner and Groffman, 2005; Metzger et al., 2008; Lindsay and Cunningham, 2011; Goldin and Hutchinson, 2013). Organic matter content, total N and nitrate (NO_3^-) concentrations were higher near CWD than those in the forest floor without CWD in woodlands in Australia (Lindsay and Cunningham, 2011; Goldin and Hutchinson, 2013). However, soil inorganic N concentrations decreased beneath CWD compared to areas without CWD in a lodgepole pine forest (Busse, 1994) and a mixedwood forest (Hafner and Groffman, 2005) in North America. Gross and net N mineralization and nitrification rates were lower under CWD than in bare mineral soils in post-fire lodgepole pine forests (Metzger et al., 2008), in a mixedwood forest in New York State (Hafner and Groffman, 2005) and in a Douglas-fir forest in Oregon (Spears et al., 2003). However, effects of CWD on N availability in reclaimed oil sands soils have not been studied and such effects need to be evaluated for designing strategies for using CWD for land reclamation.

The objective of this study was to evaluate the effects of CWD on N transformation rates and N availability in reclaimed oil sands soils with either FMM or PMM as a cover soil. A field study was conducted to test the following hypotheses: 1) gross and net N mineralization rates will be greater in FMM than in PMM due to lower C:N ratios and greater soil enzyme and microbial activities in FMM than in PMM; 2) CWD will increase

gross and net N mineralization and supply rates by moderating soil temperature, increasing soil water availability, and increasing microbial activity; and 3) available N concentrations will be greater in FMM than in PMM and near CWD than away from CWD due to the effects of cover soil and CWD on N transformation rates.

2. Materials and Methods

2.1 Study site

The study was conducted on an oil sands company lease located at 56° 58'N, 111° 22'W, which was about 24 km north of Fort McMurray, Alberta, Canada. The site was located in the mixedwood boreal forest and was cleared in 1999 for open-pit oil sands mining. A detailed description of the research site and experimental plots is provided in Brown (2010) and Brown and Naeth (2014).

From 1981 to 2010, mean annual temperature was 1.0 °C and mean annual precipitation was 418.6 mm, with 316.3 mm as rain and 133.8 cm as snow (Environment Canada, 2014). For 2011, 2012 and 2013, the years this study was conducted, mean average temperature from May to October was 12.8, 15.9 and 16.0 °C, respectively, and precipitation in the growing season was 178.8, 244.8 and 120.0 mm, respectively (Environment Canada, 2014).

2.2 Experimental design and plot establishment

The experiment used a factorial design consisting of two types of cover soils (FMM vs PMM) and two sampling distances (near vs away from CWD) with six replications. Study plots, 10 m wide and 30 m long, were established between November 2007 and February 2008. Trembling aspen (*Populus tremuloides* Michx.) CWD with a minimum of 10 cm diameter was salvaged and applied to each plot in February 2008. The CWD was arranged to provide each piece of CWD with maximum contact with the soil surface and cover approximately 10-20% of the land surface in each plot. A mixed fertilizer ($N:P_2O_5:K_2O$) was applied at a rate of 300 kg ha^{-1} (23.5:25.0:8.0) in June 2008 and at 250 kg ha^{-1} (31.5:16.0:5.0) in August 2009, as per standard reclamation practice for the company. Plots were again aerially fertilized in June 2010 and 2011 using granular urea, monoammonium phosphate and muriate of potash at a rate of 250 kg ha^{-1} (Brown, 2010; Forsch, 2014).

In each plot, six 1 × 1 m subplots for soil sampling and soil incubation were established within 5 cm from CWD (CWD located in each subplot) and more than 100 cm away from CWD. The near CWD and away from CWD subplots represent areas that have been affected by CWD or not, respectively. Plots were covered by native forbs, grasses, shrubs and mosses (Brown and Naeth, 2014). Overall vegetation and woody species covers were greater in FMM than in PMM and near CWD than away from CWD and increased over time (Brown and Naeth, 2014; Forsch, 2014).

2.3 Soil sampling and analyses

Soil samples were collected from the 0-10 cm layer using an auger on 23 June and 26 September in 2011; on 24 May, 28 June, 26 July, 29 August, 26 September and 18 October in 2012; and on 26 July, 26 August, 28 September and 18 October in 2013. At each sampling, three soil samples were collected from each treatment, three within 5 cm from CWD and three more than 100 cm away from CWD, from each plot, then bulked to form a composite sample for each treatment and plot. Soil samples were stored on ice packs in a cooler and transported to the laboratory. Fresh soil samples were homogenized, passed through a 2-mm sieve and refrigerated at 4 °C until further analysis. All analyses were completed within 7 days after sampling. A sub-sample of each sample was used for the analysis of ammonium (NH_4^+), NO_3^-, microbial biomass C (MBC) and N (MBN) and dissolved organic C (DOC) and N (DON). The remainder of each sample was air-dried at room temperature and used for pH and electrical conductivity (EC) analyses. A portion of the air-dried sample was ground to fine powder using a ball mill (MM200, REtsch GmbH, Haan, Germany) and analyzed for total C and N and $\delta^{15}N$.

Soil pH was determined with a pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA) and EC with a AP75 portable waterproof conductivity/TDS meter (Thermo Fisher Scientific Inc., Waltham, MA, USA) at a 1:5 ratio of soil weight to deionized water volume. The concentrations of NH_4^+ and NO_3^- were determined via the steam distillation method using magnesium oxide and Devada's alloy sequentially after extraction with 2 mol L^{-1} potassium chloride at a 1:5 ratio of soil weight to potassium chloride solution volume. The liberated ammonia (NH_3) was collected in 0.005 mol L^{-1} sulphuric acid solutions (Keeney and Nelson, 1982). To prevent isotopic cross

contamination among samples, 25 mL reagent grade ethanol was added to distillation tubes and steam distilled for 3 minutes between each sample distillation (Hauck 1982). Ammonium and nitrate concentrations were determined by titration with 0.01 mol L^{-1} sodium hydroxide potentiometrically using an auto titrator (719 S Titrino, Brinkmann Metrohm, USA).

Natural abundance of ^{15}N (expressed as $\delta^{15}N$) of NH_4^+ and NO_3^- serves as an indicator of N transformation processes, since they cause ^{15}N enrichment in the substrate and ^{15}N depletion in the product (Marrioti et al., 1981; Högberg,1997; Choi et al., 2002, 2003). For example, nitrification results in enrichment of ^{15}N in NH_4^+ (substrate) and depletion of ^{15}N in NO_3^- (product). Denitrification or preferential leaching of NO_3^- causes enrichment of ^{15}N in NO_3^-. For samples collected in June, August and October 2012, the distillates containing NH_4^+ were dried at 60 °C after acidifying to pH 3 with 0.05 mol L^{-1} sulphuric acid (Feast and Dennis, 1996) and analyzed for ^{15}N using a stable isotope ratio mass spectrometer (Thermo Delta Plus XP IRMS, Waltham, MA, USA) linked to a CN analyzer (Costech 4010, Valencia, CA, USA). In this study, stable isotope abundances were reported as:

$$\delta^{15}N\ (‰) = [(R_{sample} / R_{standard})\text{-}1] \times 1000$$

where R_{sample} and $R_{standard}$ are the $^{15}N/^{14}N$ ratios of the sample and the standard, respectively. The standard for N is atmospheric N_2 ($R_{standard}$ = 0.3663 atom % ^{15}N).

Soil MBC and MBN were analyzed using the chloroform fumigation-extraction method (Jenkinson et al., 1981). Fresh soil samples were fumigated with ethanol-free chloroform for 24 hours in an evacuated desiccator. Fumigated and unfumigated samples were extracted with 0.5 mol L^{-1} potassium sulphate at a 1:10 ratio of soil weight to potassium sulphate solution volume and filtered using a Whatman No. 42 filter paper. Extractable C and N were analyzed using a TOC-V_{CSN} analyzer (Shimadzu, Kyoto, Japan). To analyze DON and DON concentrations, 5 g of fresh soil samples were extracted with 50 mL of deionized water and filtered using a Whatman No. 42 filter paper. Concentrations of C and N in the filtrate were determined using the TOC-V_{CSN} analyzer. Extractable C was used to represent DOC and the difference between extractable N and available N (NH_4^++NO_3^-) was used to represent DON. Total C and N concentrations and $\delta^{15}N$ were

analyzed using a stable isotope ratio mass spectrometer (Optima-EA; Micromass, Crewe, UK) linked to a CN analyzer (NA series 2, CE instruments, Italy). Nitrogen isotope ratios of FMM and PMM are 17.7 ± 1.1 and 17.6 ± 1.6‰, respectively.

Coarse woody debris samples were collected in July 2012 and oven dried at 60 °C to constant weight. The woody debris that includes bark, sapwood and heartwood was ground and passed through a 0.84 mm sieve. Ground CWD was extracted with deionized water at a 1:10 soil weight-to-deionized water volume ratio, shaken at 250 rpm on a mechanical shaker for 1 hour, and filtered through Whatman No. 42 filter papers. The pH of the extracts were measured using the pH meter described above. A portion of the ground CWD was further ground to fine powder using the ball mill described above. Total C and N in the finely ground samples were analyzed using the automated elemental analyser (NA series 2, CE instruments, Italy).

2.4 In situ N availability measurement

Monthly in situ soil incubation was conducted to determine net N mineralization and net nitrification rates. Field incubation facilitates measurement of the rate of N mineralization reflecting fluctuating field conditions such as soil temperature and water content (Wienhold, 2007). A 4 cm diameter × 13 cm long stainless steel tube was driven into soils in each subplot to cut plant roots and to prevent N losses by plant uptake. Soil cores were covered with an aluminum foil with tiny (approximately 1 mm diameter) holes to minimize addition of N through deposition but to allow air to get into tubes. Non-incubated soil samples were collected at the same time as the incubated soil cores were installed. The incubated soil samples were retrieved after1 month. Inorganic N concentrations in the non-incubated and incubated samples were analyzed as described above. Net N mineralization and net nitrification rates were calculated as the difference of inorganic N ($NH_4^+ + NO_3^-$) or NO_3^- between pre-incubation and incubated soil samples, respectively.

Ion exchange membrane plant root simulator (PRS) probes are widely used for measuring N supply rates in the field (Drohan et al., 2005). Plant root simulator probes absorb soluble forms of ions in soils using an ion exchange mechanism between the ion exchange membrane and the soil solution. Eight pairs of probes were installed in each plot, with 4 pairs installed near CWD and 4 pairs away from CWD at the distances described

above. Each pair of the probes consisted of a cation and an anion probe. The probes were incubated during growing seasons, June to September in 2011, May to October in 2012 and July to October in 2013 and non-growing (winter) seasons, September 2011 to May 2012 and October 2012 to July 2013. At each sampling, probes were collected, washed with deionized water and sent to Western Ag Innovations Inc. Elution was conducted with a 0.5 mol L^{-1} hydrochloric acid solution and concentrations of NH_4^+ and NO_3^- in each elution were analyzed using the colorimetric method on an automated flow injection analysis system (Western Ag Innovations Inc., 2010).

2.5 Soil temperature and water content measurements

Soil temperature and volumetric water content were measured using 12-bit smart temperature sensors (Onset Computer Corporation, Bourne, MA, USA) and volumetric smart soil water sensors (Decagon Devices Inc., Pullman, WA, USA) connected to HOBO micro station data loggers (Onset Computer Corporation, Bourne, MA, USA). Three sensors were installed per treatment at 5 cm depth in FMM and PMM plots near CWD and 100 cm away from CWD. Data were collected hourly during the study period and mean monthly soil temperature and volumetric water content were calculated. Soil temperature and water content data are not reported here but were used for correlation and regression analyses.

2.6 Statistical analyses

Two-way analysis of variance (ANOVA) was conducted to test the significance of the effects of cover soil type and distance from CWD on basic soil properties, available N concentrations and N transformation rates. A repeated measures ANOVA was used to assess the effects of cover soil type and distance from CWD on inorganic N concentration, net N transformation rates, N supply rates and $\delta^{15}N$ of NH_4^+ and NO_3^- over time using the PROC MIXED model. Distance from CWD was used as a split-plot factor and month of each sampling was considered a repeated measures variable for determining seasonal variation. In this analysis, the output statistics passed tests for compound symmetry. Tukey's HSD test was used to determine significance of effects of cover soil type, distance from CWD, month of sampling and their interactions. Before performing the ANOVA, normality of distribution

and homogeneity of variance were checked with Kolmogorov-Smirnov and Levene's tests, respectively. Multiple linear regression analysis was used to determine the relationship between soil parameter and N availability and transformation rates in cover soils. Standardized regression coefficient (β) of each variable was presented to indicate how strongly each predictor variable influences the dependent variable. All analyses were performed using the SAS 9.3 software (SAS Institute Inc., NC, USA) with an α value of 0.05 to indicate statistical significance.

3. Results

3.1 Soil chemical and biological properties

Soil pH and total C and N concentrations were greater ($p<0.05$) in PMM than in FMM but were not affected by distance from CWD (Table 5-1). Soil EC was greater ($p<0.05$) in PMM than in FMM and near CWD than away from CWD ($p<0.01$) in both cover soils.

Soil DOC and DON concentrations were not affected by any of the treatments due to high spatial variability (Figure 5-1). The DOC:DON ratios were greater ($p<0.05$) in PMM than in FMM but were not affected by distance to CWD. The MBC were greater in FMM than in PMM but not different between the two cover soils due to high variability (Figure 5-2). Soil MBN was not affected by cover soil type; however, CWD application increased ($p<0.05$) MBN in cover soils in some samplings in 2012 and 2013.

3.2 Soil inorganic N concentrations and $\delta^{15}N$

Ammonium concentrations were generally stable over the study period without any effects of cover soil or CWD (Figure 5-3; Table 5-2). Nitrate concentrations varied over the study period without any effects of cover soil type or CWD and were lower than 4 mg N kg^{-1} during active growing seasons (June to August) and higher than 10 mg N kg^{-1} in September 2012 and 2013 in both cover soils.

The $\delta^{15}N$ values of NH_4^+ and NO_3^- were greater ($p<0.05$) in PMM than in FMM in each sampling and sharply decreased from June to October in each treatment (Table 5-3). Initial $\delta^{15}N$ values of NH_4^+ and NO_3^- were not different between near CWD and away from CWD positions and decreased more ($p<0.05$) away from CWD than near CWD in August and October in both cover soils.

3.3 N transformation and supply rates

Net N transformation rates in each incubation period were not affected by cover soil type, distance from CWD and their interactions (Figure 5-4; Table 5-4). Net N transformation rates did not show temporal patterns in both cover soils. Although there were no differences in N transformation rates in each incubation period, net nitrification and net N mineralization rates across the incubation periods were significantly greater in FMM than in PMM but were not affected by distance to CWD.

Net N mineralization rates were positively related to soil temperature ($p<0.001$; Figure 5-5a) and MBC ($p=0.045$; Figure 5-5b). Net nitrification rates were negatively related to DOC:DON ratio ($p=0.012$, Figure 5-5d). Based on multiple regression analysis, MBN ($\beta=0.431$, $p=0.008$; Table 5-5) and soil temperature ($\beta=0.353$, $p=0.028$) were the main determinants for net ammonification rates. The DOC:DON ($\beta=-0.476$, $p=0.010$) and soil temperature ($\beta=0.305$, $p=0.085$) were the main determinants for net nitrification rates, and DOC:DON ($\beta=-0.338$, $p=0.044$) and soil temperature ($\beta=0.542$, $p=0.002$) for net N mineralization rates.

Total inorganic N supply rates were significantly greater in FMM than in PMM but were not different between near CWD and away from CWD (Table 5-6). Soil NO_3^- supply rates between June 2011 and May 2012 were significantly greater in FMM than in PMM but were not affected by distance from CWD or its interaction with cover soil type. However, NO_3^- supply rates in 2012 and 2013 and NH_4^+ supply rates in each incubation periods were not different among treatments.

4. Discussion

4.1 Net N mineralization and supply rates were greater in FMM than in PMM

Although net N mineralization rates and N supply rates in each incubation period did not differ between FMM and PMM, mean net nitrification, mean net N mineralization and total inorganic N supply rates across the incubation periods were greater in FMM than in PMM, supporting the first hypothesis. Soil N transformation rates and N availability were linked to contrasting soil properties of FMM and PMM (McMillan et al., 2007; Mackenzie and Naeth, 2010; Brown and Naeth, 2014; Jamro et al., 2014). Similar C:N ratios between the cover

soils may have determined the similar N transformation rates between them within each sampling period. Nitrogen fertilization and N deposition in the study site may have caused similar N transformation rates but the cumulative differences over the entire study duration reflected the inherited soil properties. The higher DOC:DON ratio in PMM than in FMM may cause greater N immobilization rates in PMM (Cookson et al., 2007) and would be linked with the lower N mineralization and nitrification rates in PMM. Dissolved organic matter is considered to be the most readily available energy source for soil microorganisms (McGill et al., 1986; Wang et al., 2003) and DOC:DON ratio can be used as an indicator of substrate quality that affects N transformation rates (Cookson et al., 2007). Net N mineralization and net nitrification rates were negatively related to DOC:DON ratio in cover soils and higher DOC:DON ratio in PMM probably increased NH_4^+ immobilization rates in PMM than in FMM leading to lower net N mineralization rates in PMM (Cookson et al., 2007; Kwak et al., 2015a).

Net N mineralization rates in this study were positively related to microbial biomass and the greater N mineralization rates in FMM than in PMM were linked to the greater microbial biomass in FMM than in PMM. Soil microbial populations consume labile soil organic matter and play important roles in soil organic matter decomposition and N cycling (van Veen and Kuikman, 1990; Ladd et al., 1995). In the same research plots, activities of enzymes such as β-1,4-glucosidase, cellobiohydrolase and peroxidase related to C degradation (Kwak et al., 2015b) and β-1,4-N-acetylglucosaminidase, arylamidase and protease related to N cycling (Jamro et al., 2014) were greater in FMM than in PMM, illustrating that the greater organic matter mineralization and N availability in FMM than in PMM were linked to enzyme activities (Burns et al., 2013; Jamro et al., 2014).

The properties of the contrasting cover soils not only affect N transformation rates but also vegetation establishment and coverage on the reclaimed land surface, while differences in vegetation establishment in turn affect substrate quality and N supply rates. Greater vegetation cover in FMM (Mackenzie and Naeth, 2010; Brown and Naeth, 2014; Forsch, 2014) would mean a greater amount of litter input (Forsch, 2014) and production of root exudates in FMM. The greater fresh organic matter input to the soil may cause a greater positive priming effect in FMM (Kuzyakov et al., 2000). Lower NO_3^--N supply rates in 2012 and 2013 than in 2011 were likely associated with the rapid vegetation development

over that time period in the study site (Brown and Naeth, 2014; Forsch, 2014); the plant roots of the rapidly establishing vegetation vigorously competed with PRS probes for soil mineral N, reducing the amount of NO_3^--N that was adsorbed by the PRS probes and thus the NO_3^--N supply rate.

The $\delta^{15}N$ of NH_4^+ and NO_3^- provides information about N cycling (Marrioti et al., 1981; Högberg, 1997). Decreasing $\delta^{15}N$ of NH_4^+ over time mainly arose from ammonification of organic matter with low $\delta^{15}N$, 17.7 ± 1.1 and 17.6 ± 1.6‰ for FMM and PMM, respectively; the production of NH_4^+ with low $\delta^{15}N$ would dilute the ^{15}N of NH_4^+ in the soil solution (Choi et al., 2002, 2003). Greater $\delta^{15}N$ of NH_4^+ in PMM than in FMM could be attributed to greater ammonium volatilization in PMM due to its higher soil pH and lower ammonification (less dilution) in PMM, rather than effects of nitrification as net nitrification rates were greater in FMM than in PMM. Greater $\delta^{15}N$ of NO_3^- in PMM than in FMM would arise from greater denitrification in PMM due to greater soil water content and pore space filled with water in PMM (0.28-0.54 m^3 m^{-3} and 74.4 ± 1.3%, respectively) than in FMM (0.03-0.26 m^3 m^{-3} and 48.3 ± 1.5%, respectively) (Kwak et al., 2015c).

4.2 Effects of CWD on N transformation and supply rates

Applying CWD to the two reclaimed soils did not affect net N transformation and supply rates measured by the *in situ* soil core and PRS probe incubations. Therefore, the second hypothesis is rejected. Even though net nitrification and NO_3^- supply rates were similar near CWD and away from CWD, the $\delta^{15}N$ of NH_4^+ in August and October were greater near CWD than away from CWD in both cover soils, indicating that gross nitrification rates were greater near CWD than away from CWD causing more enrichment of ^{15}N in NH_4^+ near CWD (Marrioti et al., 1981; Högberg, 1997). Greater gross nitrification rates near CWD would arise from increased microbial population size reflected in microbial biomass (Figure 5-2) near CWD than away from CWD.

The lack of differences in net nitrification and NO_3^- supply rates was caused by increased NO_3^- immobilization due to high C:N ratio of CWD leachate (Spearse et al., 2003; Hafner and Groffman, 2005; Metzger et al., 2008; Kwak et al., 2015a). Applying CWD extract increased N immobilization rates and thus decreased net N mineralization and nitrification rates in a lab incubation experiment that used the same cover soils (Kwak et al.,

2015a). Although the amount of CWD leachate was very small compared to the organic matter content in the cover soils, addition of fresh organic matter would be utilized by microorganisms quickly (McGill et al., 1986; Wang et al., 2003) affecting N transformation patterns; CWD leachate increased N immobilization and decreases net N mineralization (Kwak et al., 2015a). Nitrogen fertilization and N deposition may also have caused similar N transformation and supply rates between near CWD and away from CWD. The study site received fertilizer applications for 4 years after plot establishment (Brown, 2010; Forsch, 2014). Oil sands mining and upgrading related activities emit a large amount of NO_x, approximately 300 ton N day^{-1} in 2000s (Hazewinkel et al., 2008), and NH_4^+ and NO_3^- deposition rates within 30 km from main oil sands area were approximately 1-5 and 1-2 kg ha^{-1} yr^{-1}, respectively, between May 2008 and May 2009 (Proemse et al., 2013). While the N from fertilization application or atmospheric deposition is intercepted by CWD, they are deposit to the soil directly in locations away from CWD. Therefore, the discrepancy between the lack of CWD effects on net N transformation and supply rates and the effect of CWD on $\delta^{15}N$ of NH_4^+ (implying effects on gross N transformation rates) in some of the samplings indicate the need to study gross N transformation rates to gain a better picture of N cycling in different cover soils and under different management regimes in land reclamation.

4.3 Effects of cover soil type and CWD on available N concentrations

Available N concentrations were not different between the two cover soils and between the two positions to CWD, rejecting the third hypothesis. Although net nitrification and net N mineralization rates were greater in FMM than in PMM, available N concentrations were not different between the two cover soils. Greater vegetation cover in FMM than in PMM (Brown and Naeth, 2014; Forsch, 2014) may have caused greater plant N uptake in FMM (Nadelhoffer et al., 1985; Kaye and Hart, 1997). Similar available N concentrations between the cover soils may also have been affected by N fertilization and N deposition as described above.

Applying CWD did not affect net N transformation rates, in turn, resulting in the lack of differences in available N concentrations near CWD and away from CWD. Nitrate concentrations near CWD were lower than away from CWD in some sampling months,

which could arise from increased NO_3^- immobilization due to CWD leachate. Brown and Naeth (2014) also found lower NO_3^- concentrations near CWD than away from CWD in the same research plots in the first two years after plot establishment. Nitrogen fertilization and N deposition in the study site may increase N availability in soils more away from CWD than near CWD as described above. Such interactive effects of plant N uptake, N immobilization, fertilization and N deposition may result in their effects canceling each other out, causing the lack of significant treatment effects on available N concentrations in the cover soils.

5. Conclusions

The two contrasting cover soils used for land reclamation after oil sands mining had different N mineralization and supply rates, with net nitrification, net N mineralization and N supply rates greater in FMM than in PMM. Microbial biomass, DON:DON ratios and soil temperature were strongly related with net N transformation rates in the studied cover soils. However, greater vegetation cover in FMM may have resulted in the lack of differences in available N concentrations between the two cover soils. The greater $\delta^{15}N$ of NH_4^+ near CWD indicated that CWD increased gross nitrification rates and the lack of effect on net N transformation rates or N availability in the studied cover soils was due to increased N immobilization in soils near CWD. Applying FMM as a cover soil for land reclamation post open-pit oil sands mining would be a better choice as a source of soil organic matter and to provide N to promote plant growth and early ecosystem development in oil sands reclamation. Although CWD did not increase N availability in cover soils, applying CWD for land reclamation is recommended to increase spatial variability and microsites for microorganisms and vegetation establishment. The novelty of our research is finding the differential effect of cover soil type and CWD application on net N transformation rates. Research on the effect of cover soil type and CWD application on gross rates of N transformation needs to be conducted in the future to better understand the N cycling processes.

Table 5-1 Chemical properties of forest floor mineral soil mix (FMM), peat mineral soil mix (PMM) and coarse woody debris (CWD).

Treatments		pH	EC ($dS\ m^{-1}$)	Total C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	C:N
Cover soil	Distance from CWD					
FMM	Near	5.90 (0.12)	0.18 (0.01)	39.7 (4.9)	1.4 (0.2)	30.1 (3.7)
	Away	6.05 (0.10)	0.21 (0.02)	40.8 (5.6)	1.4 (0.3)	32.8 (2.9)
PMM	Near	7.06 (0.03)	0.37 (0.02)	68.4 (8.2)	2.4 (0.3)	28.6 (1.6)
	Away	7.18 (0.04)	0.45 (0.03)	72.7 (10.3)	2.4 (0.4)	31.3 (1.3)
Two-way ANOVA						
Cover soil		***	*	*	*	ns
Distance from CWD		ns	*	ns	ns	ns
Soil × distance		ns	ns	ns	ns	ns
CWD		4.56 (0.01)	ND	499.3 (0.3)	3.6 (0.2)	140.2 (0.8)

Values are means with SE (n=6);
* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant;
EC= electrical conductivity, C:N= carbon to nitrogen ratio and ND=not determined.

Table 5-2 Effects of cover soil type, distance from CWD, sampling time and their interactions on soil inorganic nitrogen concentrations

Measurement	Year	Cover soil		Distance from CWD		Time		Soil × distance		Soil × time		Distance × time		Soil × distance × time	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p
NH_4-N	2011	6.2	0.021	0.1	0.788	0.9	0.419	0.6	0.438	0.8	0.465	1.76	0.186	0.01	0.987
	2012	2.7	0.117	2.6	0.126	14.7	<0.001	1.2	0.292	1.7	0.140	2.6	0.030	0.5	0.800
	2013	1.3	0.745	0.7	0.408	162.2	<0.001	0.1	0.745	3.1	0.061	9.6	0.001	0.2	0.827
	Whole[Ŧ]	5.6	0.029	0.7	0.417	21.9	<0.001	0.1	0.833	2.4	0.009	2.1	0.023	0.5	0.912
NO_3-N	2011	7.7	0.012	1.0	0.327	8.1	0.001	1.6	0.225	2.2	0.128	0.5	0.620	1.5	0.248
	2012	0.3	0.585	0.3	0.597	213.7	<0.001	0.8	0.389	0.9	0.467	7.5	<0.001	1.5	0.204
	2013	0.1	0.804	2.9	0.106	53.5	<0.001	0.8	0.385	0.1	0.912	4.4	0.020	0.2	0.815
	Whole	4.0	0.061	0.4	0.558	61.2	<0.001	3.2	0.088	1.9	0.049	3.4	<0.001	0.8	0.671
NH_4-N + NO_3-N	2011	6.6	0.018	0.1	0.791	2.2	0.122	2.0	0.176	0.5	0.634	0.5	0.631	1.8	0.188
	2012	0.6	0.459	0.3	0.597	97.8	<0.001	0.1	0.720	0.9	0.467	7.4	<0.001	0.7	0.594
	2013	0.5	0.488	3.1	0.098	137.1	<0.001	0.7	0.401	0.9	0.418	9.8	<0.001	0.1	0.909
	Whole	4.5	0.048	0.0	0.921	41.6	<0.001	1.6	0.224	2.6	0.014	3.4	<0.001	1.0	0.445

[Ŧ]Whole sampling period (June 2011-October 2013)

Table 5-3 Nitrogen isotope compositions ($\delta^{15}N$) of inorganic soil N in cover soils used for oil sands reclamation.

Sampling month	$\delta^{15}N$ in NH_4-N (‰)				$\delta^{15}N$ in NO_3-N (‰)			
	FMM		PMM		FMM		PMM	
	Near	Away	Near	Away	Near	Away	Near	Away
June 2012	141.1 (4.4)	141.1 (7.6)	164.0 (11.5)	165.6 (7.2)	138.3 (3.2)	142.2 (5.0)	152.3 (1.6)	152.1 (1.6)
August 2012	109.8 (18.3)	61.0 (8.5)	106.4 (7.6)	70.8 (11.0)	59.4 (3.3)	44.3 (3.4)	73.6 (11.0)	53.0 (5.0)
October 2012	38.8 (8.1)	27.3 (2.1)	60.2 (7.5)	34.9 (3.1)	17.9 (2.0)	18.2 (1.9)	29.3 (5.8)	23.0 (6.3)

Repeated measures ANOVA

	NH_4-N		NO_3-N	
	F value	p value	F value	p value
Cover soil	9.57	0.013	13.23	0.005
Distance from CWD	11.53	0.004	5.02	0.051
Sampling time	86.83	<0.001	559.02	<0.001
Soil × distance	0.46	0.507	0.87	0.376
Soil × time	1.39	0.264	0.15	0.865
Distance × time	8.72	0.006	3.95	0.028
Soil × distance × time	0.13	0.726	0.02	0.979

Values are means with SE (n=6)

Table 5-4 Effects of cover soil type, distance from CWD, sampling time and their interactions on soil nitrogen transformation rates.

Measurement	Year	Cover soil		Distance from CWD		Time		Soil × distance		Soil × time		Distance × time		Soil × distance × time	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p
Net ammonification rate	2011	0.3	0.574	1.2	0.292	0.1	0.783	0.1	0.817	1.2	0.281	1.1	0.302	0.2	0.642
	2012	0.1	0.715	1.4	0.260	9.9	<0.001	0.7	0.434	0.6	0.688	1.2	0.343	0.3	0.906
	2013	2.3	0.150	0.1	0.831	0.7	0.402	0.0	0.861	4.1	0.059	0.0	0.902	0.2	0.671
	Whole[Ŧ]	1.3	0.274	0.2	0.685	7.6	<0.001	0.8	0.393	0.9	0.549	1.0	0.432	0.2	0.990
Net nitrification rate	2011	1.8	0.196	0.1	0.839	9.4	0.008	1.2	0.298	1.2	0.290	1.7	0.209	0.0	0.933
	2012	3.4	0.080	3.8	0.065	8.3	<0.001	2.5	0.131	2.1	0.096	1.2	0.331	0.3	0.884
	2013	2.2	0.156	1.4	0.262	23.2	<0.001	1.6	0.218	7.0	0.019	2.4	0.143	0.5	0.506
	Whole	8.0	0.012	0.8	0.393	7.1	<0.001	0.1	0.763	1.5	0.163	1.4	0.193	0.8	0.601
Net N mineralization rate	2011	0.9	0.348	1.0	0.325	5.8	0.028	0.3	0.621	2.6	0.128	0.0	0.975	0.0	0.921
	2012	3.6	0.074	0.3	0.618	5.5	<0.001	2.4	0.139	0.7	0.569	0.6	0.634	0.2	0.959
	2013	3.0	0.105	0.4	0.553	3.9	0.068	0.8	0.401	0.0	0.961	1.0	0.330	0.0	0.984
	Whole	7.7	0.014	0.3	0.605	4.4	<0.001	0.3	0.626	0.8	0.605	0.6	0.768	0.4	0.916

[Ŧ]Whole sampling period (June 2011-October 2013)

Table 5-5 Multiple regression model for net ammonification, net nitrification and net N mineralization rates.

Regression equations	R^2	p value
Net ammonification rate = 0.002 × MBN + 0.01 × Temp – 0.197	0.24	0.006
Net nitrification rate = - 0.004 × DOC:DON +0.01 × Temp + 0.036	0.25	0.012
Net N mineralization rate = -0.003 × DOC:DON + 0.021 × Temp – 0.122	0.34	0.002

MBN = microbial biomass nitrogen, Temp = soil temperature and DOC:DON = dissolved organic carbon to dissolve organic nitrogen ratio.

Table 5-6 Soil N supply rates (µg N per 10 cm^2) measured using Plant Root Simulator (PRS) probes in cover soils used for oil sands reclamation.

Incubation period	NH_4-N (µg N per 10 cm^2)				NO_3-N (µg N per 10 cm^2)				Total inorganic N (µg N per 10 cm^2)			
	FMM		PMM		FMM		PMM		FMM		PMM	
	Near	Away	Near	Away	Near	Away	Near	Away	Near	Away	Near	Away
June 2011 – Sept. 2011	2.1 (1.1)	2.3 (0.5)	1.3 (0.5)	0.6 (0.4)	19.8 (10.2)	13.11 (9.1)	0.6 (0.3)	1.2 (0.6)	21.9 (10.9)	15.4 (7.8)	1.9 (0.6)	1.8 (0.9)
Sept. 2011 – May 2012	ND	ND	ND	ND	15.26 (5.9)	8.0 (4.7)	3.6 (1.3)	3.4 (0.7)	15.3 (5.9)	8.0 (4.7)	3.6 (1.3)	3.4 (0.7)
May – Oct. 2012	1.2 (0.2)	1.0 (0.2)	1.1 (0.3)	1.0 (0.2)	2.4 (1.2)	2.3 (0.3)	1.9 (0.8)	2.3 (0.5)	3.6 (1.3)	3.3 (0.2)	3.0 (0.8)	3.2 (0.4)
Oct. 2012 – July 2013	3.2 (0.8)	2.3 (0.7)	1.8 (0.2)	1.5 (0.3)	3.8 (0.5)	3.5 (0.1)	3.7 (0.3)	4.1 (0.5)	7.0 (1.2)	5.8 (0.6)	5.5 (0.3)	5.6 (0.6)
July – Oct. 2013	3.6 (0.2)	4.2 (0.9)	3.6 (0.1)	4.9 (1.0)	1.5 (0.9)	1.2 (0.4)	1.3 (0.4)	0.8 (0.3)	5.1 (1.0)	5.3 (0.7)	4.9 (0.5)	5.7 (1.1)
Total	8.6 (2.1)	7.9 (1.8)	7.4 (0.7)	8.0 (1.4)	38.5 (12.0)	22.9 (9.1)	9.9 (2.4)	11.7 (0.9)	47.1 (13.6)	30.8 (10.4)	17.3 (2.3)	19.7 (0.9)

Repeated measures ANOVA

	NH_4-N		NO_3-N		Total inorganic N	
	F value	p value	F value	p value	F value	p value
Cover soil	1.03	0.350	3.72	0.094	4.60	0.075
Distance from CWD	0.37	0.561	1.86	0.214	1.79	0.229
Sampling time	19.37	<0.001	9.94	<0.001	3.90	0.013
Soil × distance	0.67	0.474	0.65	0.446	1.48	0.270
Soil × time	0.20	0.900	4.69	0.005	3.98	0.012
Distance × time	0.29	0.835	1.33	0.285	1.03	0.412
Soil × distance × time	0.01	0.998	1.00	0.427	0.72	0.584

Values are means with SE (n=6); ND= not detected.

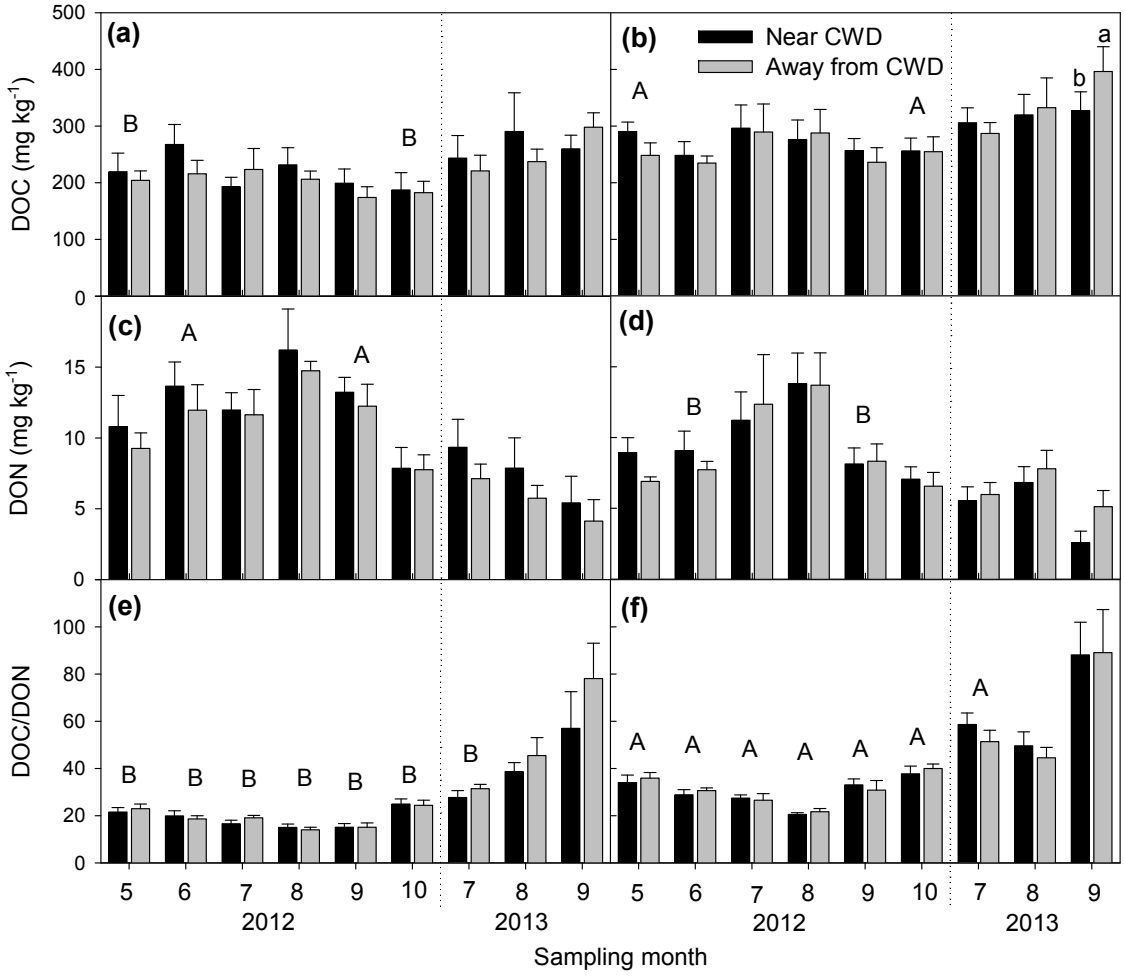

Figure 5-1 Changes during 2012-2013 growing seasons in a) dissolved organic C (DOC) in FMM; b) DOC in PMM; c) dissolved organic N (DON) in FMM; d) DON in PMM; e) DOC to DON ratio (DOC:DON) in FMM; and f) DOC:DON ratio in PMM. Vertical bars are SE (n=6). Different lower case letters indicate significant differences between near CWD and away from CWD within each cover soil type and different upper case letters indicate significant differences between two cover soil types in each incubation period (α=0.05).

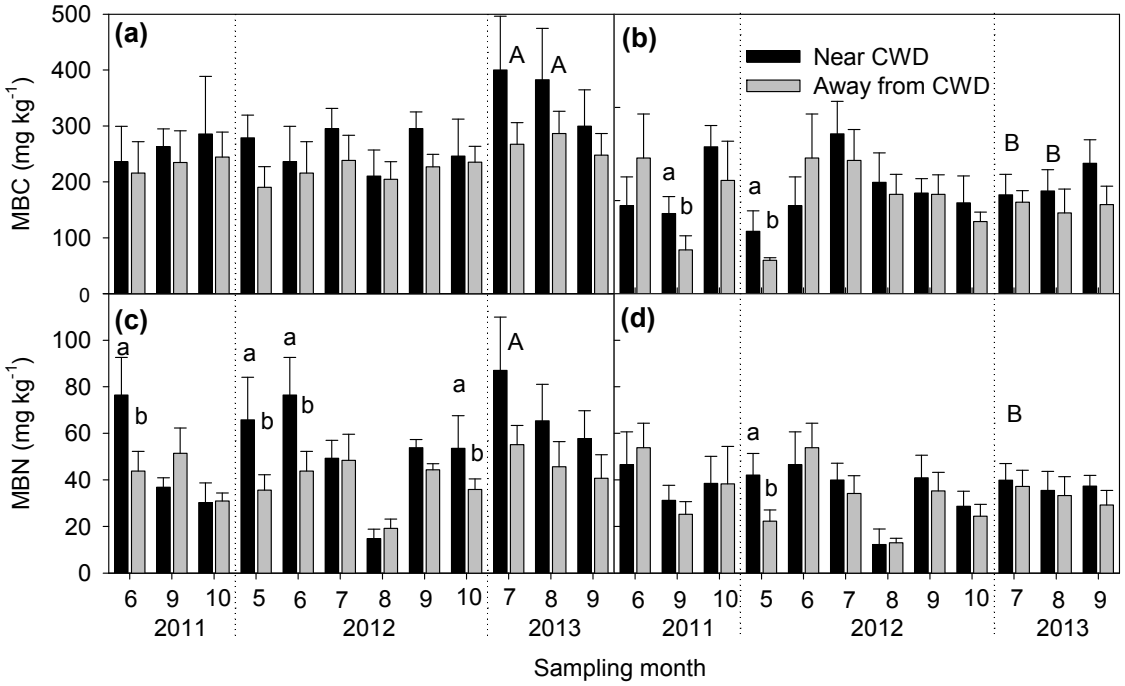

Figure 5-2 Changes during 2011-2013 growing seasons in a) microbial biomass C (MBC) in FMM; b) MBC in PMM; c) microbial biomass N (MBN) in FMM; and d) MBN in PMM. Different lower case letters indicate significant differences between near CWD and away from CWD within each cover soil type and different upper case letters indicate significant differences between two cover soil types in each incubation period (α=0.05).

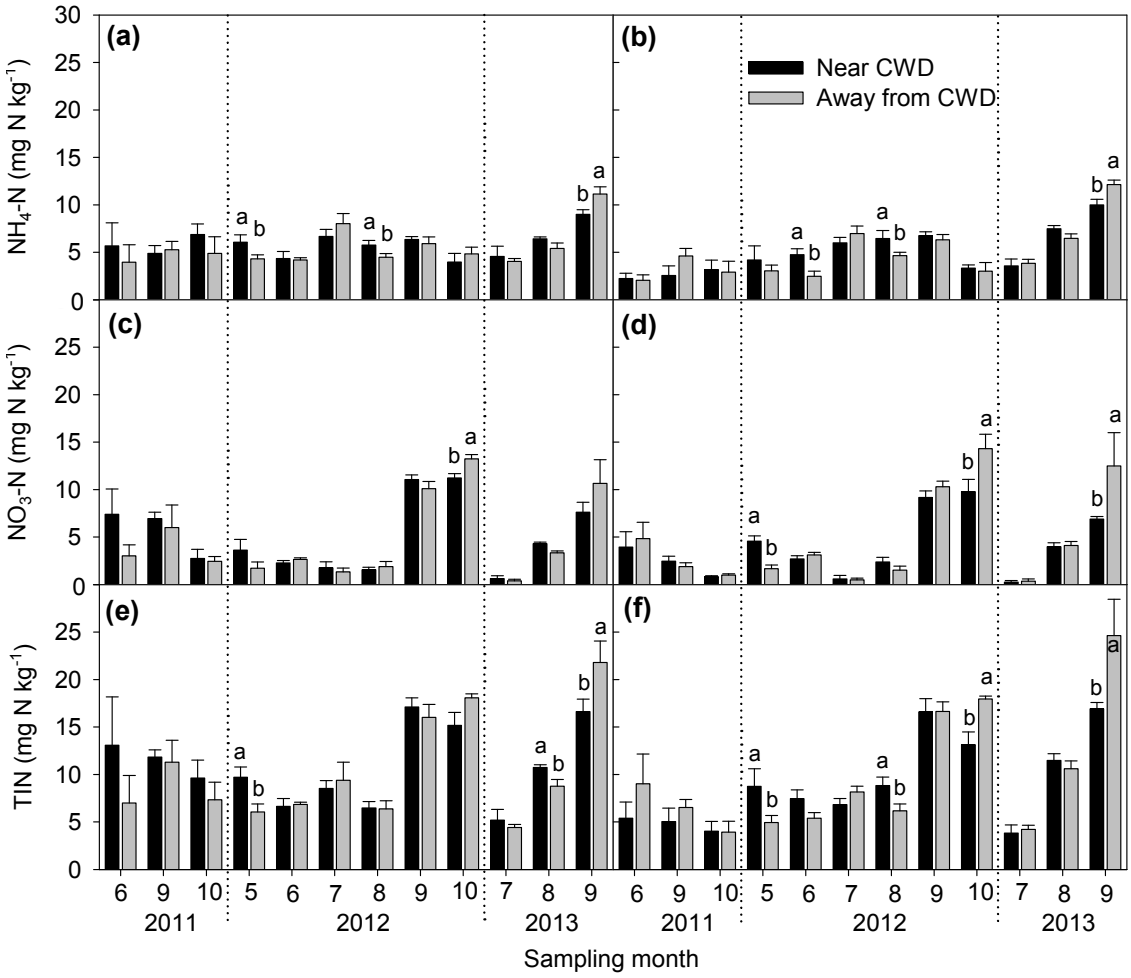

Figure 5-3 Changes during 2012-2013 growing seasons a) NH_4-N in FMM; b) NH_4-N in PMM; c) NO_3-N in FMM; d) NO_3-N in PMM; e) total inorganic N (TIN, NH_4-N+NO_3-N) concentrations in FMM; and f) total inorganic N in PMM. Vertical bars are SE (n=6). Different letters indicate significant differences between near CWD and away from CWD within each cover soil type (α=0.05).

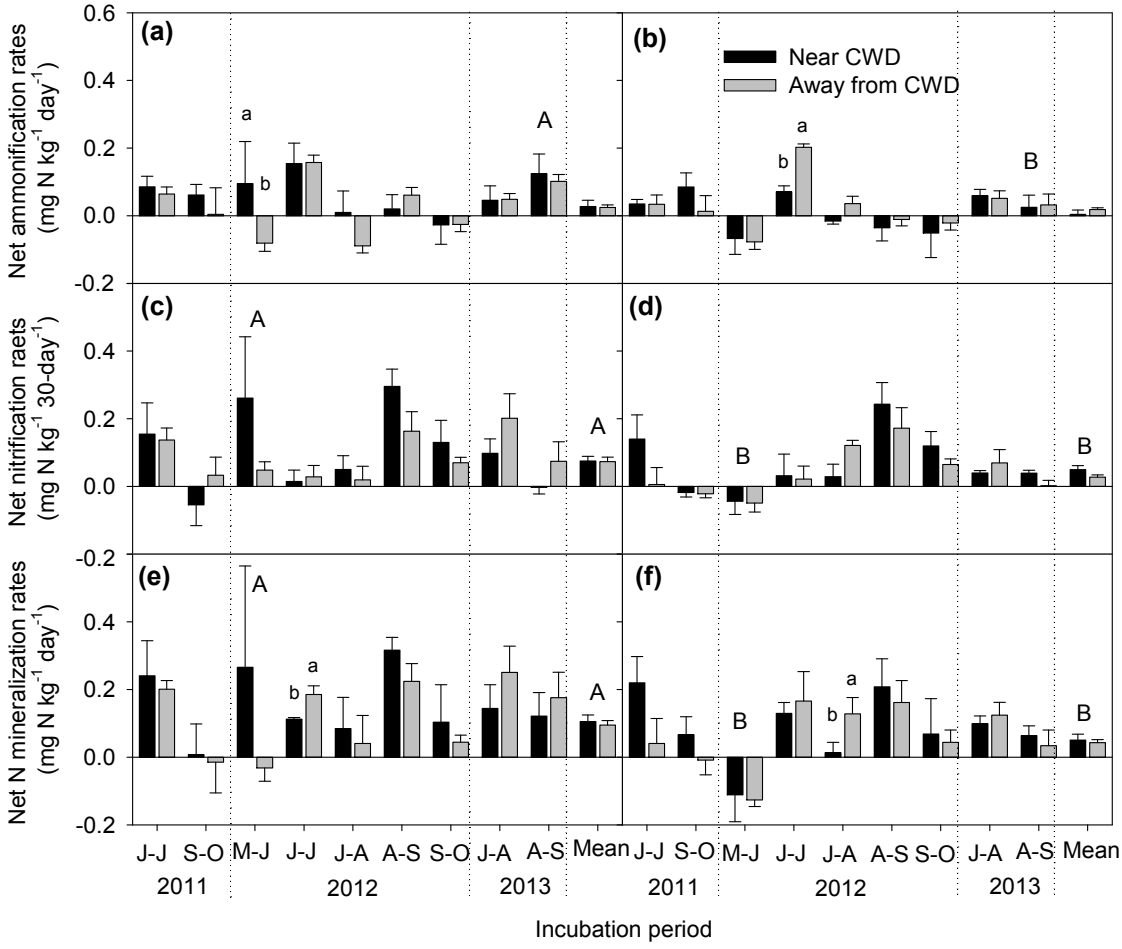

Figure 5-4 Changes during 2012-2013 growing seasons in a) net ammonification rates in FMM; b) net ammonification rates in PMM; c) net nitrification rates in FMM; d) net nitrification rates in PMM; e) net N mineralization rates in FMM; and f) net N mineralization rates in PMM. Vertical bars are SE (n=6). Different lower case letters indicate significant differences between near CWD and away from CWD within each cover soil type and different upper case letters indicate significant differences between two cover soil types in each incubation period (α=0.05).

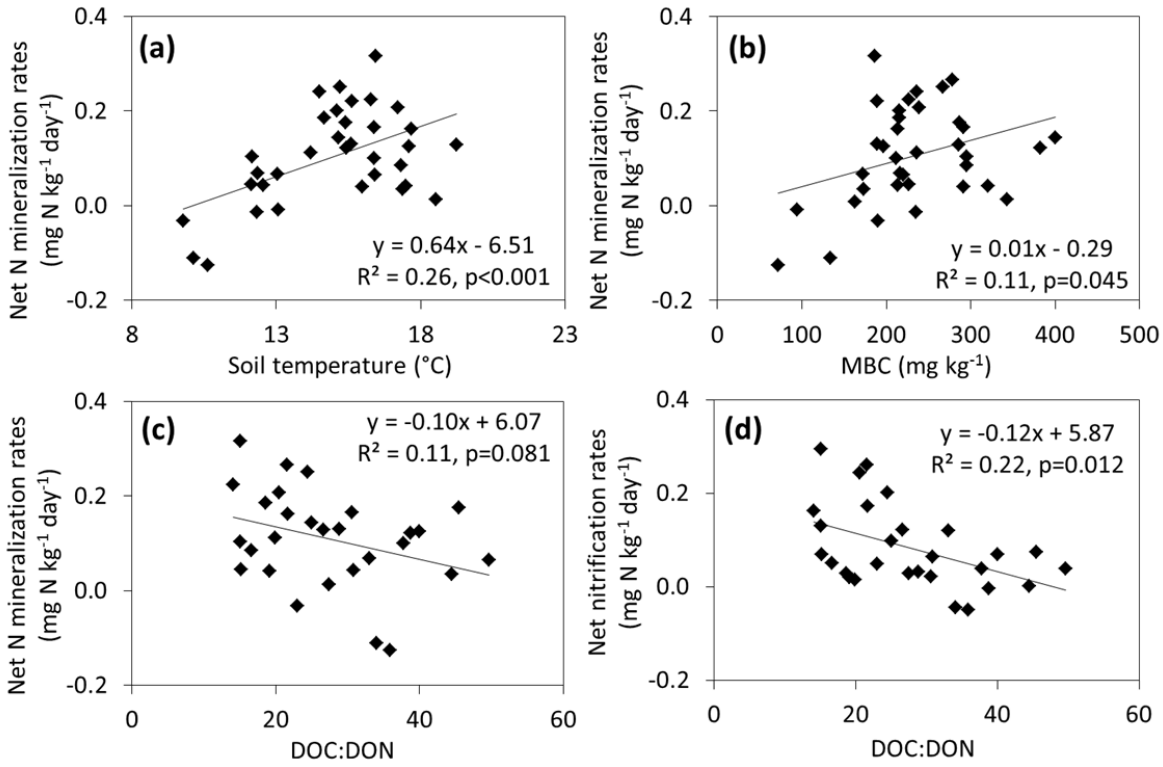

Figure 5-5 Relationship between a) soil temperature and net N mineralization rates; b) microbial biomass C (MBC) and net N mineralization rates; c) DOC:DON and net N mineralization rates; and d) DOC:DON and net nitrification rates. The mean values of each sampling month and treatment were used for the regression analyses.

CHAPTER 6. SYNTHESIS, CONCLUSIONS, AND FUTURE RESEARCH

1. Research Objectives Overview

The overall objective of this research was to assess effects of coarse woody debris (CWD) on microbial community function, enzyme activity, greenhouse gas emissions, nitrogen (N) transformation rates and N availability in cover soils amended with forest floor mineral soil mix (FMM) and peat mineral soil mix (PMM). A laboratory incubation experiment was conducted to assess the chemical effect of CWD extract on gross and net N transformation rates. The CWD extract was used to simulate CWD leachates (with rainwater as a control) that are produced in the field. Microbial community level physiological profile, microbial biomass and extracellular enzyme activities were determined to assess effects of CWD on soil biological properties. Soil respiration and methane uptake rates were measured in cover soils to determine how changed microbial community function and enzyme activity by CWD affect greenhouse gas emission rates. Monthly soil incubation was conducted to measure net N mineralization and nitrification rates and plant root simulator probes were incubated to determine in situ N supply rates.

2. Summary and Synthesis of Research Results

2.1 CWD extract effects on N transformation rates in cover soils

There was no significant difference in gross N mineralization rates between PMM and FMM due to the similar carbon to nitrogen (C:N) ratio and soil pH of the two cover soils. Ammonium (NH_4^+) immobilization rates were greater in PMM than in FMM, associated with greater dissolved organic C and dissolved organic N ratio in PMM. Gross and net nitrification rates were greater in FMM than in PMM. The ratio of gross nitrification to gross NH_4^+ immobilization rates was greater in FMM than in PMM, as the greater NH_4^+ immobilization rates in PMM decreased gross nitrification rates by decreasing NH_4^+ availability.

Addition of CWD extract dramatically increased gross N mineralization rates the first day of incubation in both cover soils mainly due to the priming effect. However, gross N mineralization rates in both cover soils decreased sharply by the CWD extract addition from

the second day of incubation, resulting in lower rates than with rainwater. The CWD extract addition increased NH_4^+ immobilization rates in both cover soils mainly due to the high C:N ratio of the CWD extract. Addition of the CWD extract decreased gross and net nitrification rates in both cover soils mainly because increased NH_4^+ immobilization rates by the CWD extract decreased NH_4^+ availability for nitrification and low pH of the added CWD extract decreased activity of nitrifying bacteria. The nitrification to gross ammonium immobilization ratios decreased by the CWD extract addition, indicating that heterotrophic NH_4^+ immobilization was superior to that of autotrophic nitrification due to the high C:N ratio of the added CWD extract.

2.2 CWD effects on microbial community function and enzyme activity

Soil microbial community level physiological profile was significantly different between FMM and PMM cover soils, associated with different sources of organic matter and plant species composition. Microbial biomass was greater in FMM than in PMM, which was derived from greater microbial biomass from the year of plot establishment and greater litter input in FMM than in PMM (Brown and Naeth, 2014; Forsch, 2014). Average well color development was greater in PMM than in FMM, which arose from greater soil water content and dissolved organic C concentration in PMM. Soil enzyme activities were greater in FMM than in PMM due to greater microbial biomass and vegetation cover in FMM.

Coarse woody debris changed the soil microbial community level physiological profile in FMM. Increased dissolved organic C concentration near CWD changed the soil microbial C utilization pattern in FMM. However, the CWD did not change dissolved organic C concentration and soil microbial community level physiological profile in the PMM cover soil. Coarse woody debris increased microbial biomass and average well color development in both PMM and FMM cover soils. Coarse woody debris increased soil water content and dissolved organic C concentrations, especially in FMM cover soils. Soil enzyme activities were greater near CWD than away from CWD, without any statistically significant difference, probably due to the similar vegetation cover and the soil properties between the near CWD and away from CWD treatments.

2.3 CWD effects on greenhouse gas emission rates in cover soils

Soil respiration rates were greater in FMM than in PMM regardless of the distance from CWD at each sampling time. Greater microbial biomass and vegetation cover in FMM than in PMM led to greater heterotrophic and autotrophic respiration in FMM. The CWD increased soil respiration by 22-33% in FMM but not in PMM. Soil respiration rates were positively related to microbial biomass C and microbial biomass N; greater microbial biomass near CWD led to greater soil respiration near CWD. Soil respiration rates decreased from July to September in 2012 and 2013, and were positively related to soil temperature but not to soil water content.

Methane uptake rates were greater in FMM than in PMM mainly due to the drier condition of FMM than that of PMM. The CWD increased methane uptake rates only in July and August 2012 in FMM due to increased microbial activity by CWD. Methane uptake rates were negatively related to soil water content but not to soil temperature.

2.4 CWD effects on N availability in cover soils in the field study

Net N mineralization and nitrification rates in each incubation period were not significantly different among treatments. However, averaged net N mineralization and nitrification rates were greater in FMM than in PMM, associated with greater microbial biomass and enzyme activity in FMM. Net N mineralization rates were positively related to soil temperature and microbial biomass C. Net N mineralization and nitrification rates were negatively related to the dissolved organic C to dissolved organic N ratio. Total inorganic N supply rates were greater in FMM than in PMM in 2011 but no difference in 2012 and 2013, probably due to increased competition between plant root simulator probes and plant roots in the later years followed by increasing vegetation cover over time. There was no difference in available N concentrations between FMM and PMM mainly due to greater vegetation cover and N uptake in FMM.

There were no significant effects of CWD on net N mineralization, nitrification and N supply rates. Coarse woody debris caused greater ^{15}N discrimination between NH_4^+ and nitrate, indicating that gross nitrification rates were greater near CWD than away from CWD; increased microbial biomass, microbial community function and enzyme activity near CWD enhanced gross nitrification rates. However, increased N immobilization rates near CWD resulted in similar N transformation rates and available N concentrations

between near CWD and away CWD.

3. Conclusions

The cover soils used in this study had contrasting properties, resulting in different biogeochemistry. The soil microbial community level physiological profile was different between FMM and PMM, associated with different organic matter composition and plant species composition between cover soils. The FMM was the preferred cover soil to PMM for upland reclamation after oil sands mining; microbial biomass and enzyme activities were greater in FMM than in PMM resulting in greater soil respiration and N transformation rates in FMM. Methane uptake rates were greater in FMM than in PMM with drier soil condition in FMM. Overall greenhouse gas emission rates were greater in FMM than in PMM; however, plant growth, at the same time, was greater in FMM than in PMM (Brown and Naeth, 2014; Forsch, 2014). Therefore, net balance between greenhouse gas emission from cover soils and net ecosystem productivity need to be assessed to determine overall global warming potential of cover soil application. Applying FMM will increase N availability thereby plant growth relative to PMM; gross and net nitrification rates were greater in FMM while N immobilization rates were greater in PMM. However, there was no difference in available N concentrations due to the fertilization and the greater vegetation cover in FMM than in PMM. These results suggest that applying FMM for land reclamation would accelerate early ecosystem development by providing more microbial biomass, increasing organic matter decomposition and N availability, therefore, increasing plant growth. Results from this study provide support to findings in earlier studies that vegetation cover was greater in FMM than in PMM (Brown and Naeth, 2014; Forsch, 2014).

This study has shown the importance of CWD on soil properties and land reclamation. The CWD provided more favourable habitats for microorganisms with greater soil water content and more stable soil temperature. Coarse woody debris increased microbial community functional diversity and microbial biomass, increasing organic matter decomposition (soil respiration rates) and gross N mineralization rates. Effects of CWD on soil properties were more significant when CWD was applied on FMM, which is coarse texture and drier condition compare to PMM. Applying CWD changed microbial community physiological profile and increased soil respiration in FMM but not in PMM.

Effects of CWD on soil properties would be little if soil has high organic matter content and water content, for example, PMM in this study. However, applying CWD over PMM is still beneficial in increasing organic matter content and vegetation establishment and providing habitats for invertebrates, microorganisms, small mammals and fungal species (Harmon et al., 1986; Stevens, 1997; Brown and Naeth, 2014).

Relatively fresh CWD had little effects on N availability; CWD increased gross nitrification rates (greater $\delta^{15}N$ of NH_4^+ near CWD than away from CWD) but CWD leachate also increased N immobilization rates. Although fresh CWD could increase nutrient immobilization, nutrients contained in CWD biomass will be released at the later years increasing nutrient availability in soils.

Large amounts of CWD are produced during clear-cutting of forest stands prior to open-pit mining and CWD is currently buried, burned and mulched. Microsites and organic matter content can be increased using readily available and potentially wasted CWD materials. Therefore, CWD can promote functional and sustainable land reclamation with relatively little financial implication. Applying CWD for initial stage of land reclamation, when landscape is exposed to changing environment directly, would be most beneficial. Once vegetation cover increased, effects of CWD, especially increases in microsites, become smaller. Further research determining long term effects of CWD on soil biogeochemistry and vegetation development would be needed to develop strategies of CWD application.

4. Future Research Suggestions

4.1 Effects of CWD quality on soil biogeochemistry

This study used fresh aspen CWD of decay class 1 or 2. Nutrient concentrations and decomposer abundance in more decomposed CWD are higher than those in fresh (Harmon et al., 1986). Applying further decomposed CWD may provide more nutrients and microorganisms to the soils, increasing nutrient availability and plant growth. Element compositions vary with tree species, thus composition of CWD leachate would also differ with tree species. Decomposition rate of CWD is dependent on size of CWD as it changes volume to surface area ratio (Harmon et al., 1986). Small CWD will decay faster and

release more nutrients to soils. However, large CWD may provide more microsites and habitats for microorganisms. Evaluating effects of different quality of CWD, such as decay class, tree species and size on soil biogeochemistry would be valuable for developing strategies of CWD application for land reclamation.

4.2 Appropriate CWD cover

This study showed that CWD is beneficial for increasing microbial biomass, microbial community function and organic matter decomposition. However, applying too much CWD for land reclamation could increase nutrient immobilization and decrease work efficiency during transplanting. Therefore, appropriate amounts of CWD for land reclamation must be identified to increase effectiveness of applying CWD.

4.3 Effects of CWD on soil biogeochemistry without acid depositions or fertilizations

The research site received fertilizer for four years after plot establishment and acid deposition rate is relatively high in the area. Fertilizer applications and acid deposition will change microbial community composition and activity, and thus, organic matter decomposition and N transformation rates in soils. Therefore, a study needs to be conducted to determine effects of CWD on soil biogeochemistry in areas without acid depositions or fertilizations.

4.4 Mixing FMM and PMM

In this study, FMM was a better cover soil for land reclamation; however, amount of FMM is limited. The PMM is more readily available than FMM and had higher organic matter content but a less suitable microbial community for upland reclamation (Hahn and Quideau, 2013). To complement of weak point of each cover soil, mixing FMM and PMM could be an alternative approach. Evaluating optimum rates of FMM and PMM can be valuable to make the most suitable cover soil for land reclamation.

4.5 Erosion control

Soil erosion is one of the problems in the reclamation landscape, especially where there are steep dikes, like in the Alberta Oil Sands Region. Placing CWD across the slope changes the flow of water and soil, reducing soil erosion (Harmon et al., 1986; Stevens, 1997).

Further research on the effect of CWD on soil erosion control in the reclaimed landscape would be valuable to decrease erosion loss from cover soils and improve effectiveness of cover soils.

4.6 Global warming potential

This study evaluated effects of cover soil type and CWD on greenhouse gas emission. Land reclamation would also sequester atmospheric carbon dioxide, supporting plant growth. Net balance between greenhouse gas emission from cover soils and net primary productivity in the reclaimed landscape needs to be evaluated to determine effects of land reclamation on global warming potential.

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