University of Alberta

Soil Microbial Communities in Early Ecosystems

by

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Abstract

Microbial communities are responsible for biogeochemical processes in soils such as nutrient cycling and organic matter formation, which are essential to the establishment of vegetation and ecosystem sustainability. Phospholipid fatty acid analysis, microbial respiration and enzymatic activities were used to assess the development of soil microbial communities in two early ecosystems: along a 99 year glacial chronosequence, and in reconstructed soils in the Canadian boreal forest following open-pit mining. In the glacial environment, microbial biomass, respiration and enzymatic activity increased along the chronosequence and became more similar to the reference stand as vegetation developed. Further, in mid-successional stage soils, microbial biomass in plant rhizospheres was double that measured in bulk soil. In the reconstructed soils the use of organic amendments originating from the target ecosystem placed both the vegetation and soil microbial community on a faster trajectory towards ecosystem recovery than did the use of alternative amendments.

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AOSR	Athabasca oil sands region
b	Bulk soil
es	Engelmann spruce
FFM	Forest floor- mineral soil mix
MRPP	Multi-response permutation procedure
NMDS	Non-metric multidimensional scaling
OC	Organic carbon
PLFA	Phospholipid fatty acid analysis
PM	Peat-mineral soil mix
TC	Total carbon
TOC	Total organic carbon
yma	Yellow mountain avens
Pw	Gravimetric water content

1.0 Chapter 1: Introduction

1.1 A Brief history of soil science

Soil science originated in multiple disciplines, and as such, was studied from specific viewpoints; geologists focused on the parent rock, agriculturists on crop production and climatologists on the influence of weather (Wilding et al. 1983). In the 19th century, Justus von Liebig (1803-1873), a German chemist, worked on agricultural chemistry and is most known for his "Law of the Minimum", which states that plant growth is controlled, or proportional to, the nutrient that is most limiting (Brown 1942), a theory still taught in introductory soil science classes today. By 1879, Russian scientist Vasily Vasili'evich Dokuchaev (1846-1903) had theorized that there were multiple factors influencing soils. He identified eight characteristics important in differentiating soils, including the geology of the parent material (source rock), as well as the number, colour, structure, texture, chemical composition, arrangement and thickness of each soil horizon (Wilding et al. 1983). Swiss agriculturist Hans Jenny (1899-1992), later expanded upon the theories developed by Liebig and Dokuchaev. In particular, he further investigated soil nutrients and ion exchange, as well as studied the effect of land management practices, such as tillage, on the quality and quantity of soil organic matter. Jenny also established the specific link between climate influence and various soil properties. Over the course of his long career, Jenny produced several books including Factors of Soil Formation (1941) for which he is best known (Amundsen 2004).

The ideas of von Leibig, Dokuchaev, Jenny, and other pre-eminent soil scientists continue to be the theoretical foundation for others in the field. Consequently, subdisciplines, such as soil physics and fertility have become better known, studied and defined (Wilding et al. 1983). Despite the fact that a widely agreed upon, clear cut definition of soil is still lacking, the modern concept of soil as presented in the Canadian System of Soil Classification (1998) defines soils as: "the naturally occurring, unconsolidated mineral or organic material at least 10 cm thick that occurs at the earth's surface and is capable of supporting plant growth" (Soil Classification Working Group 1998).

Soils support the world terrestrial ecosystems by providing, storing and cycling nutrients and supplying rooting medium. Additionally, soils are fundamental to agriculture, and thus global food supply, as well as many other industries, such as forestry and ecotourism (Brady 2002). Because soils function on a timescale much longer than our own, researching how the soil systems work and develop over time can be difficult. However, understanding soil systems is the first step in developing sustainable environmental practices.

1.2 Studying soil through time

Soil chronosequences, physical gradients along which space is substituted for time, can provide unique opportunities for studying environmental development over long periods of time in the course of a single field season. Chronosequences also allow scientists to investigate how an individual soil forming factor, such as climate, climate change or vegetation, influences soil through time. Additionally,

through the study of chronosequences, competing theories in soil development and formation can be reconciled, the speed and course of soil evolution evaluated and new philosophies and conceptual ideas brought to light (Huggett 1998).

Soil microbial and carbon dynamics in naturally occurring newly developing areas, such as glaciers, fire sites, volcanic outcrops or oasified dry areas, are becoming of greater importance with increased human disturbance creating "newly developing" areas. However, microbial succession can be difficult to study owing to the limited number of, and limited access to, barren environments (Svoboda and Henry 1987). By attempting to reclaim open-pit mines, chemically contaminated soils and infrastructure demolition sites, humans have now inadvertently created areas on which to study soil microbial community development and succession. Further, reclamation is done with the objective of "kick starting" or accelerating ecosystem development beyond what would naturally occur. By studying the natural development of ecosystems or the succession of specific environmental components in undisturbed landscapes, the realization of the aforementioned reclamation goal can be assessed. Additionally, the relative importance of additional factors such as vegetation or specific vegetative species in the glacier environment may inform areas of future reclamation research.

The word succession comes from the Latin word *succedere* which means both "to come after" and "to succeed" (Svoboda and Henry 1987). Succession has been defined as an: "ecosystem development which involves changes in species

structure, is reasonably directional and therefore predictable, is community controlled (even though the physical environment determines the pattern, the rate of change, and often sets the limits as to how far development can go), and culminates in a stabilized ecosystem" (Odum 1971). In this research, it is assumed that succession does result in Odum's (1971) suggestion of a stabilized or climax ecosystem, wherein the rate of change in this community is negligible compared to that in earlier successional stages. This climax ecosystem is therefore used as a control or a reference stand to which younger stage ecosystems aspire. Microbial succession is controlled by complex interactions among soil properties and carbon resources (Tscherko et al. 2004). Therefore the role of soil microbial communities and the factors that influence these communities during succession must be examined before ecosystem succession can be fully understood.

1.3 The role of soil microbial communities

Soil microbial communities are responsible for biogeochemical processes in soils such as nutrient cycling and organic matter formation, which are essential to the establishment of vegetation and ecosystem sustainability (Jones 1998; Tscherko et al. 2003). Further, microorganisms help stabilize soil against wind and water erosion, and aid in the accumulation and release of nutrients (Belnap et al. 1999; Jones 1998; Kastovska et al. 2005; Tscherko et al. 2003). Thus, invasion of "new" environments by plants, is dependent upon microbial community development and succession (Kastovska et al. 2005). Owing to this, the composition and functional ability of the soil microbial community can be used as indicators of

biogeochemical processes and soil development (Moore-Kucera and Dick 2008). By studying soil microbial communities through time, scientists may gain a better understanding of ecosystem succession and soil formation.

Soil microbial communities and vegetation are linked as plant communities determine litter composition (Saetre and Baath 2000) and produce root exudates (Miethling et al. 2000), which are the primary source of energy and nutrients for soil microbial communities (Broughton and Gross 2000; Schutter and Dick 2001). Soil organic matter is created through the chemical breakdown of organic material by soil microorganisms (Sinsabaugh 2010) as well as by physical processes such as pedoturbation (Brady 2002). Generally, soil organic matter can be thought of as a series of different organic C pools: the labile pool, which contains readily decomposable C, and several stabilized pools in which the material is at different degrees of decomposition (Norris et al. 2009). Stabilization of organic matter can occur through one or more of the following mechanisms: physical seclusion, wherein the microbial community is unable to access the material because of its isolation inside soil aggregates; chemical stabilization, in which the material is adsorbed onto fine soil particles; biochemical stabilization, where the material becomes recalcitrant due to the structure of the substance; and spatial-temporal stabilization, wherein the material is inaccessible by the soil microbial community due to differences in space at a given time (e.g. the microbes lack physical or temporal access to the resource) (Norris et al. 2009). Organic matter colors the soil in dark brown or black, aids in soil structure formation and is the soil ecosystem's dynamic nutrient supply (Brady 2002). By learning more about

carbon and soil microbial dynamics, knowledge pertaining to the complex interactions that drive ecosystem processes can be gained.

In the boreal mixed-wood forest, soil microbial communities have been found to be highly influenced by aboveground vegetation (Bach et al. 2008). For example, aspen (*Populas tremuloides* Michx.) dominated stands host soil microbial communities that have greater total biomass and are structurally different from the communities found under spruce (*Picea glauca* (Moench) Voss) dominated stands (Flanagan and Vancleve 1983; Hannam et al. 2007; Lindo and Visser 2003). Abiotic conditions such as soil pH (Priha et al. 2001), temperature and moisture may play an equally as important role as vegetation in driving soil microbial community composition (Fierer et al. 2003; Zak et al. 1999; Zogg et al. 1997). To illustrate, patterns in soil microbial community composition were so affected by seasonal precipitation that the influence of vegetation was masked during times of high precipitation, but perceivable during low rainfall seasons (Swallow et al. 2009). Similarly, microbial biomass has been shown to increase in the spring and decrease in the autumn when environmental conditions are drier in temperate grasslands (Bardgett et al. 1999).

1.4 Athabasca oilsands mining and reclamation

The Athabasca oilsands region (AOSR), located near Fort McMurray in Alberta, Canada (57°00’ N, 111°28’ W) has become one of the most talked about mining sites around the globe. Oil mining in the area began in 1906 and has since expanded rapidly to include approximately 91 oilsands projects which account for

over 50% of the oil and gas produced in Canada (Government of Alberta 2010). Two of largest oil sands mining companies in the AOSR, Syncrude Canada Ltd and Suncor Energy Inc., use trucks and shovels to remove the top 40 m of geological material and then carry the bitumen-rich sands to the appropriate extraction facilities (Lanoue 2003). Prior to the onset of mining, surface materials (0-3 m) are removed in lifts and stored separately from the deeper geological material, called overburden, until they can be used to reconstruct soils (Lanoue 2003). During reclamation, soil-like profiles are created by placing organic amendments atop surface mineral materials, including overburden or tailings sand (a byproduct of the bitumen refining process). Currently, the two main types of organic amendments used in upland reclamation in the AOSR consist of either peat or forest floor material obtained by salvaging peat (up to meters deep) or forest floor (several centimeters deep) from nearby peatlands or upland forests. Organic reclamation treatments are then obtained by mixing these materials with the mineral soil below (Fung and Mackyk 2000), resulting in either a peat-mineral soil mix (PM), or a forest floor-mineral soil mix (FFM) that range in organic: mineral soil between approximately 40% and 70% (Lanoue 2003).

Forest floor layers overlying mineral soils in natural areas are rich sources of native plant seeds and propagules. Evidence from recent research indicates that salvaged FFM applied to reclaimed areas exhibits a native revegetation response superior to typical PM reclamation practices (Mackenzie and Naeth 2006). Further work suggests that FFM may stimulate microbial activity as indicated by increased respiration rates, microbial biomass, and net and gross nitrification rates

(McMillan 2007). In terms of the soil microbial community, the benefits and drawbacks of using each type of reclamation material requires further research.

Reclaimed sites on a trajectory towards sustainable ecosystem function should become more similar to natural sites as native vegetation and associated organic matter re-develop over time. Establishment of biogeochemical recycling between soils, microbes, and plants is one of the most critical factors required to establish long-term sustainability in reclaimed landscapes. Thus, researching the influence of reclamation techniques on the soil microbial community over time may help in the development of effective and sustainable reclamation strategies.

1.5 The research potential of glacial retreat environments

Despite ecologically and geographically diverse ecosystems, many of the same biogeochemical processes such as nutrient cycling and organic matter formation are performed by similar microorganisms in all soil environments. However, individual environments have different resource constraints and rates at which these processes occur making them complex despite their ubiquity (Brady 2002). Given that both glacier retreat environments and reclamation sites undergo primary succession, it may be possible to compare microbial development patterns between these two ecosystem types. For example, understanding soil microbial community development in glacial retreat environments may help to define reasonable reclamation timelines, the necessity of certain resources as well as the ecological consequences of individual environmental components.

Polar and glacial regions were once thought to be isolated and barren environments, but research has now shown that neither of these assumptions is true (Marshall 1996). A closer look at glacier habitats reveals an ecosystem like many others, rich with life and impacted by global environmental phenomena (Battin et al. 2001; Marshall 1996). In addition to being home to many microorganisms, glaciers also retreat and subsequently reveal "new" environments (Hämmerli et al. 2007). Melting glaciers expose approximately $1x10^{17}$ to $1x10^{21}$ viable microbes annually (Castello and Rodgers 2005). These previously entombed, and later liberated microbes, along with microbes living in sediments that become exposed, are thought to act as "starters" or 'seeds' for microbial colonization of glacial retreat environments (Grzesiak et al. 2009). Additionally, soils recently exposed by glacial retreat, have been found to be highly suitable for colonization by allochthonous bacteria in the first 4-5 years following exposure (Rehakova et al. 2010; Schmidt et al. 2008). Microbial succession is fundamental to ecosystem development as primary colonizers improve soil conditions, thereby making the habitat more favourable for higher-lever organisms (Kastovska et al. 2005).

Research shows that nutrients exist at high enough levels for metabolic activity to take place *in situ* under the ice and thus influence glacial melt water chemistry (Foght et al. 2004). Microbial communities are thought to be capable of producing CO_2, organic acids, methane, and reduced or oxidized forms of sulfur, nitrogen and iron beneath glaciers. These metabolic byproducts directly affect the *in situ* subglacial environment and also the melt water leaving the subglacial

environment (Foght et al. 2004). These melt waters tend be acidic (Foght et al. 2004) and, as soil water acidity increases from subglacial and barren sites to vegetated sites (Kastovska et al. 2005), the melt waters may aid in lowering the pH of proglacial soils. Additionally, in recently exposed soils, cyanobacteria and algae stabilize soil against wind and water erosion and aid in soil formation. Further, soil bacteria transform algal litter into organic matter that can be used as a source of nutrients for other organisms (Kastovska et al. 2005), as well as mobilize nutrients (e.g.: Brunner et al. 2011; Mapelli et al. 2011) and play an important role in nitrogen fixation (e.g.: Brankatschk et al. 2011).

While microorganisms help create conditions conducive to vegetation development, once established, plants provide new resources that can be exploited by the microbial community through the production of litter and root exudates (Miethling et al. 2000). Interactions between plants and microbes have an important impact on soil microbial successional patterns (Kaye and Hart 1997; Ohtonen et al. 1999). Correspondingly, in glacier retreat environments, microbial functional diversity has been shown to increase with plant development and organic matter accumulation (Tscherko et al. 2003). Similarly, enzymatic activity and total microbial biomass of microbial communities sampled within the plant rhizospheres have been found to be significantly higher than in samples taken from the bulk soil (Miniaci et al. 2007; Tscherko et al. 2004). However, soil microbial community-plant interactions have thus far been reported to be species specific (Bardgett and Walker 2004).

Opportunities to study sites undergoing primary development and vegetative establishment have not previously been widely available (Svoboda and Henry 1987). However, as global warming increases the rate at which glaciers retreat, more and more land is exposed and open for primary microbial community succession (Chen et al. 2006a; Chen et al. 2006b).

1.6 Research questions and objectives

1. One objective of this study was to determine the long-term influence of two different organic amendments on microbial community development in reconstructed soils following open-pit mining in the Athabasca oilsands region of northeastern Alberta, Canada. I hypothesized that the combined effect of upland native organic matter and associated vegetation would promote soil microbial communities more similar to those found in natural forest stands as the soil and reclaimed sites develop through time compared to those sites reconstructed using lowland organic matter.

2. The second objective was to assess the relative importance of abiotic conditions on the soil microbial community on reclaimed and natural forested sites, by manipulating soil moisture in a laboratory incubation experiment. I hypothesized that by increasing moisture, and thus lifting soil moisture limitation, the soil microbial communities on the reclaimed sites (found to have significantly less soil moisture that the natural sites (McMillian et al., 2007) would become more similar to those found on the natural reference stands.

3. Finally I aimed to assess soil microbial community succession and determine the influence of two plant species, yellow mountain avens (*Dryas drummondii* Rich.), and Engelmann spruce (*Picea engelmannii* Parry) on soil microbial community succession along a Canadian glacier chronosequence. I hypothesize that soil microbial community composition and activity will increase with increasing plant cover and successional stage. Secondly, we predict that soil microbial activity will be higher in samples taken within the plant rhizospheres when compared to those taken from the bulk soil.

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2.0 Chapter 2: Long-term Effects of Organic Amendments on Plant and Soil Microbial Community Development in the Canadian Boreal Forest

Prepared for Plant and Soil

2.1 Introduction

In natural areas, forest floor layers overlying mineral soils contribute to ecosystem recovery after disturbance as they are rich sources of native plant seeds and propagules (Pare et al. 1993; Whittle et al. 1997), and provide energy and nutrients to the soil microbial community (Fyles and McGill 1987; Vancleve et al. 1993). Biogeochemical cycling between soils, microbes, and plants is one of the most critical factors required to insure long-term sustainability in forest ecosystems. While the soil microbial community is responsible for processes such as nutrient cycling (Wagner 1998) and organic matter accumulation (Bradshaw 1984; Frouz et al. 2007), which are central to the support of plant growth (Wagner 1998), soil microbial communities have also been found to be highly influenced by vegetation (Bach et al. 2008). For example, in the boreal mixed-wood forest of western Canada, aspen (*Populas tremuloides* Michx.) dominated stands host soil microbial communities that have greater total biomass and are structurally different from the communities found under spruce (*Picea glauca* (Moench) Voss) dominated stands (Flanagan and Vancleve 1983; Hannam et al. 2007; Lindo and Visser 2003).

Plant communities determine litter composition (Saetre and Baath 2000), a primary source of energy and nutrients for the soil microbial community (Schutter and Dick 2001; Broughton and Gross 2000). Several authors have reported faster litter decomposition rates when decomposition takes place beneath the plant species from which the litter comes from (e.g.; Ayres et al. 2009a; Ayres et al. 2009b; Bocock et al. 1960; Gholz et al. 2000). This phenomenon, dubbed the "home-field advantage" (Gholz et al. 2000) has been linked to the adaptation of the soil microbial community to the substrates encountered most frequently, allowing the community accelerated access to the most abundant sources of nutrients in its specific environment (Ayres et al. 2009a; Ayres et al. 2009b; Bocock et al. 1960).

Abiotic conditions such as soil pH (Priha et al. 2001), temperature and moisture may play an equally as important role as vegetation in driving soil microbial community composition (Fierer et al. 2003; Zak et al. 1999; Zogg et al. 1997). To illustrate, patterns in soil microbial community composition were so affected by seasonal precipitation that the influence of vegetation was masked during times of high precipitation, but perceivable during low rainfall seasons (Swallow et al. 2009). Similarly, microbial biomass was shown to increase in the spring and decrease in the autumn when environmental conditions are drier in temperate grasslands (Bardgett et al. 1999). Climatic differences and specifically soil moisture regimes have been proposed as important drivers of soil microbial community composition in cases of significant temporal seasonal differences (Bardgett et al. 1999; Bell et al. 2009; Prevost-Boure et al. 2011; Swallow et al.

2009). While many studies have investigated seasonal effects on the soil microbial community, to our knowledge no study characterizing composition of the soil microbial community has spanned more than three years (e.g.; Bell et al. 2008; Bell et al. 2009), and captured the entirety of site climatic conditions.

Soil microbial communities, are thus controlled by an intricate and dynamic combination of biotic and abiotic conditions. A better understanding of the drivers of soil microbial community composition will further current knowledge in ecosystem processes and lead to improved sustainable management of natural resources. Reclaimed landscapes provide unique opportunities to manipulate environmental conditions and study specific drivers of soil microbial communities in isolation of other confounding factors. The overall objective of this study was to determine the long-term influence of two types of organic amendments on soil microbial community development following open-pit mining in the Athabasca oilsands region of northwestern Alberta, Canada. Currently two sources of amendments are used in the region: peat, which is salvaged from the abundant lowland pre-mining areas, and forest floor, which is obtained from upland boreal forest stands similar to the reclamation target ecosystems. The application of forest floor materials to reclaimed areas has been shown to provide a source of propagules, and results in a greater native revegetation response compared to sites reclaimed with peat (Mackenzie and Naeth 2006). However, whether the greater presence of native plants promotes soil microbial communities more similar to those on natural forest sites is yet unknown. We hypothesized that the combined effect of upland forest floor organic matter and associated native vegetation

would promote soil microbial communities more similar to those found in natural forest stands as compared to soils reconstructed using lowland organic matter. We characterized the soil microbial community from field samples collected over seven consecutive years. Additionally, we assessed the relative importance of abiotic conditions on the soil microbial communities of both reclaimed and natural forested sites, by manipulating soil moisture in a laboratory incubation experiment. Here, we hypothesized that by increasing moisture, and thus lifting soil moisture limitation, the soil microbial communities on the reclaimed sites, typically found to have significantly less soil moisture that the natural sites (McMillan 2007), would become more similar to those found on the natural reference stands.

2.2 Materials and methods

2.2.1 Study area

The research sites were located approximately 40 km north of Fort McMurray, Alberta (56.72°N 111.37°W) in the Central Mixedwood Region of the Canadian boreal forest (Natural Regions Committee 2006), within the Athabasca Oil Sands Region (AOSR). Glaciolacustrine sediments and morainal till make up 45% of the soil parent materials in the area, while an additional 15% of the landscape is covered by colluvial and glaciofluvial deposits. Medium to fine textured Gray Luvisols (Haplocrysalfs according to the USDA classification) are the most prevalent mineral soils, although Dystric Brunisols (Dystrocryepts) and Gleysols (Cryaquepts) are found on approximately 10% and 5% of the region, respectively.

The remaining 40% is occupied by organic soils, which occur within bogs and treed fens (Natural Regions Committee 2006; Soil Classification Working Group 1998). Trembling aspen (*Populus tremuloides* Michx.), white spruce (*Picea glauca* (Moench) Voss), and jack pine (*Pinus banksiana* Lamb.) dominate the forest landscape on the gently undulating plains. The region receives an average of 456 mm of precipitation annually (Table 2-1), and has an average of 69 frost-free days (Environment Canada 2011). Summers are short and cool with an average temperature of 16.3^{0}C while the long winters that can extend from September to May experience an average temperature of -21.5^{0}C (Turchenek and Lindsay 1982).

Resource extraction in the Athabasca Oil Sands Region (AOSR) disturbs ecosystems at the landscape scale. Two of largest oil sands mining companies in the AOSR, Syncrude Canada Ltd and Suncor Energy Inc., use trucks and shovels to remove 40 m of geological material and then carry the bitumen-rich sands to the appropriate extraction facilities. Prior to the onset of mining, surface materials (0-3 m) are removed in lifts and stored separately from the deeper geological material, called overburden, until they can be used to reconstruct soils (Lanoue 2003). During reclamation, soil-like profiles are created by placing organic amendments atop surface mineral materials, including overburden or tailings sand (a byproduct of the bitumen refining process). Currently, the two main types of organic amendments used in upland reclamation in the AOSR consist of either peat or forest floor material obtained by salvaging peat (up to meters deep) or forest floor (several centimeters deep) from either adjacent peatlands or upland

forests. Peat may be used or directly, or organic reclamation treatments may be obtained by mixing either peat or forest floor materials with the mineral soil below (Fung and Mackyk 2000), resulting in either a peat-mineral soil mix (PM), or a forest floor-mineral soil mix (FFM). There treatments typically range in organic: mineral material ratios between approximately 40% and 70% (Lanoue 2003).

Three sites, each containing plots reclaimed with FFM and PM were used in this study. Sites 1 and 3, constructed in 1998 and 2004, respectively were both located on the Syncrude Canada Ltd. mine site, while Site 2, established in 2000, was positioned on the Suncor Energy Inc. reclamation area. The PM and FFM reclamation treatments for all sites were harvested from peatlands and aspen-dominated upland forests located in the Athabasca River valley and placed at a depth of 5-20 cm on the reclaimed plots. White spruce and aspen were planted on Sites 1 and 2, while no planting was done on Site 3. Both reclamation treatments on Site 2 were fertilized with N-P-K in 2000, 2001 and 2003. As described by McMillan (2007), vegetation on the reclaimed sites was dominated by common yarrow (*Achillea millefolium* L.), fireweed (*Epilobium augustifolium* L.), and graceful cinquefoil (Potentilla gracilis Dougl Ex Hook var. *gracilis*), peavine sp. (*Lathyrus* sp.), sweet clover sp. (*Melilotus* sp.) and wild strawberry (*Fragaria virginiana* Mill.).

Luvisolic soils (Haplocrysalfs) supported the three mature forest (all > 70 years of age) reference stands included in this study. The reference sites included two

aspen-dominated sites, one located 4 km south of the mine site and a second located on the Syncrude Canada Ltd. mine site, as well as a white spruce- aspen-dominated stand, also located on the Syncrude Canada Ltd. mine site.

2.2.2 Field assessment and soil sampling

The natural aspen-dominated stands supported understory vegetation typical of this type of ecosystem, that include low bush cranberry (*Viburnum edule* Michx.), rose (*Rosa* sp.), green alder (*Alnus crispa*, (Ait.) Pursh), Canada buffaloberry (*Shepherdia canadensis*, (L.), hairy wild rye (*Elymus innovatus*, (Beal) Pilg), bunchberry (*Cornus canadensis*, L.), wild sarsaparilla (*Aralia nudicaulis* L., Araliaceae), dewberry (*Rubus pubescens*, Raf.) and fireweed (*Epilobium augustifolium* L.). The white spruce-aspen-dominated stand had an understory vegetation similar to that of the aspen-dominated sites with the addition of stair-step moss (*Hylocomium splendens* (Hedw.)), Scheber's moss (*Pleurozium schreberi* (Michx.)) and knight's plume moss (*Ptilium crista-castrensis* (Hedw)).

The mine lease owners conducted several vegetation assessments at the reclaimed sites between 1999 and 2008. Vegetation assessments at Site 1 were conducted from 1999 to 2003 and concluded that the FFM treatments had significantly higher percent cover of native and woody species and significantly less percent cover of weedy species than the PM treatments (Brown et al. 2003; Mapfumo 2003). That study also concluded that while all plots were becoming more similar to the natural forest control, in terms of species composition and abundance, plots reclaimed with FFM made accelerated progress (Brown et al. 2003; Mapfumo

2003). At Site 2, plots reclaimed with FFM were found to have significantly more living vegetative cover and litter production than the plots reclaimed with PM in 2005 (AMEC Earth and Environmental 2003; AMEC Earth and Environmental 2006). On Site 3, an assessment completed in 2004 found plots reclaimed with FFM to have significantly higher plant richness, diversity and total density than plots reclaimed with PM (Mackenzie and Naeth 2006).

In August 2010, five 5 m^2 plots were selected for further vegetation characterization at 5 m intervals along a 25 m transect on both reclamation treatments at all reclaimed sites, as well as at the two natural sites located on the Syncrude Canada Ltd. mine site. Within each plot, percent cover of graminoids, forbs, shrubs, trees, bare ground, litter and moss were recorded (AMEC Earth and Environmental 2003; AMEC Earth and Environmental 2006). Each class was assessed on a 0-100% basis. Average number of trees present in the reclaimed plots (including Site 3, where volunteer trees were present) was also recorded.

Soil samples (0-7.5 cm) were taken annually from 2004 to 2010 for microbial characterization. Yearly sampling occurred in August and began on Sites 1 and 2 in 2004; Site 3 was added in 2005 and sampling continued on all three sites until 2010. In terms of the natural sites, the off mine aspen-dominated stand was sampled in 2004; the second aspen-dominated stand located on the Syncrude Canada Ltd. mine site was sampled in 2009 and 2010, and the white spruce-aspen stand was sampled in 2010. In 2005, samples were collected in both May and August, and in 2010 samples were taken in May, August and September. On both

treatments at all three sites, as well as at the natural sites, five replicate soil samples were collected using a metal cylindrical soil corer 7.5 cm in diameter at 5-meter intervals along a 25 m transect. Identifiable plant residues were discarded, and the remaining samples were put into individual sterile Whirlpack™ bags. All equipment was washed with ethanol between treatments to mitigate cross contamination. The samples were placed in coolers and kept cold using ice packs until their arrival at the laboratory, where they were immediately stored at -82°C until they were freeze dried in preparation of the phospholipid fatty acid extraction. Samples collected in September 2010 were also used to measure soil pH. In September 2009, additional samples were collected for a moisture manipulation experiment from all reclaimed sites and the on-site aspen-dominated natural stand. Initially, subsamples from each plot were gathered to determine gravimetric moisture content. Bulk samples of about 20 L of material were then collected to a depth of approximately 10 cm from various, randomly selected, locations on the site. Samples were subsequently air dried and sieved to 4 mm prior to the moisture manipulation experiment.

2.2.3 Laboratory analyses

Gravimetric water content was calculated on a dry-weight basis by oven drying soils at 65°C for three days (ISO 11465 1993). To measure pH, 0.01 *M* calcium chloride was added to oven-dried soil (not sieved) using a 2:1 solution:soil dilution ratio as described in (Kalra and Maynard 1991). Texture was determined on oven-dried, sieved (< 2 mm) soils, using the hydrometer method as outlined in

(Sheldric and Wang 1993). Total carbon and nitrogen contents in ball ground (Retsch, MM200) samples were measured using a Costech 4010 Elemental Analyzer System (Costech Analytical Technologies Inc., Valencia, CA, USA) fitted with a thermal conductivity detector.

Four moisture levels were selected for this experiment, 60% (a water content similar to that measured in the natural stands), 45%, 30% and 15% (a water content similar to that measured in the reconstructed soils) (Table 2-1) all of which were calculated on a dry-weight gravimetric basis. Three replicates from the natural site and each treatment from all three reclaimed sites were prepared for the four moisture levels selected using deionized water (for a total of 84 samples). The samples were incubated in sterile Whirlpack™ bags for six months in a dark room kept at 22°C. Bags were opened weekly to avoid samples becoming anaerobic. They were weighed monthly and the moisture level was readjusted to its original moisture content by adding deionized water as needed.

Phospholipid fatty acid (PLFA) analysis was used to characterize the soil microbial community composition for three separate data sets (total of 420 samples): 1. a seven year chronosequence in which samples collected in August from 2004 to 2010 were included; 2. a comparison of seasonal versus annual differences; and 3. the moisture manipulation experiment. Samples were taken from the deep freeze, and freeze dried, prior to PLFA analysis to remove moisture and maintain the integrity of the PLFAs. Polar lipids were extracted from the soil samples (1.5 g) through a modified Bligh and Dyer extraction (Frostegard and

Baath 1996; Frostegard 1996). Extracts were then isolated using pre-packed silicic acid columns (Agilent Technologies, Wilmington, DE). Lastly, a mild alkaline methanolysis was used to form fatty acid methyl esters (PLFAs), which were then separated and quantified with an Agilent 6890 Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE) fitted with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column. Peaks were identified using the MIDI peak identification software (MIDI, Inc., Newark, DE) and a bacterial fatty acid standard (Hannam et al. 2006; Swallow et al. 2009). PLFA data were expressed on a nmol PLFA g^{-1} basis after being relativized by the internal standard.

2.2.4 Statistical analyses

All PLFAs (≤ 20 carbons in length) identified by the MIDI software were included in the analysis. Total microbial biomass was calculated by summing all PLFAs on a nmol PLFA g^{-1} basis. A Hellinger transformation was used to relativize the data (Legendre and Gallagher 2001). Non-metric multi-dimensional scaling (NMDS) ordinations were used to explore the PLFA data in the long term community composition study, the moisture manipulation experiment and the comparison of seasonal versus annual differences using PC-Ord software version 5 (MjM Software Design, Gleneden Beach, OR). The Multi-Response Permutation Procedure (MRPP) was used to test for significant differences among microbial grouping patterns linked to treatment, site, year or moisture level identified in the NMDS ordination. Results from an MRPP analysis include the following three variables: the T value, which represents the difference(s) between

groups, a more negative value signifying a greater difference; the A value, which represents the variation within groups, where 1 signifies that all points within the group are identical and 0 indicates a variation equal to that of random numbers; and the p value, which represents the significance of these values (Zimmerman et al. 1985). The Sorensen (Bray-Curtis) dissimilarity index was used for all ordinations and MRPPs. An alpha of 0.05 was used for all statistical tests.

The R package (version 2.12.1, R Development Core Team) was used to test assumptions and conduct Wilcoxon rank sum tests comparing the total microbial biomass between treatments and among years, sites and moisture levels. A Bonferroni adjustment was used for multiple comparisons to maintain the family-wise Type I error rate at 0.05. Data collected from the vegetation assessment were used to calculate arithmetic means and standard deviations; however, no further statistical tests were conducted on the vegetation data.

2.3 Results

2.3.1 Plant and soil characteristics

The natural sites possessed, on average, over double the vegetative cover (226%) and number of trees per plot (2.7) of any given reclaimed site (Fig. 2-1). Quantitative differences between the two reclamation treatments (forest floor versus peat amendments) were visually apparent at each site in terms of vegetative cover and plant functional group distribution. Higher percent cover of trees, shrubs and forbs was found on plots reclaimed with the forest floor material (FFM) when compared to those reclaimed with the peat (PM). Additionally,

higher numbers of trees per plot and total vegetative cover were observed on plots reclaimed with FFM (1.5 and 138%, respectively) compared to those reclaimed with PM (0.2 and 87%, respectively). Sites reclaimed with FFM were thus more similar to the natural sites than those reclaimed with PM in terms of total plant cover and number of trees. Additionally, the percent cover of both forbs and shrubs found on the natural sites was more similar to plots reclaimed with FFM than to plots reclaimed with PM. Our results confirm the results from the previous vegetation studies completed on these sites (AMEC Earth and Environmental 2006; Brown et al. 2003; Mackenzie and Naeth 2006; Mapfumo 2003).

Within a given site, soil texture was similar between reclamation treatments (Table 2-2). While all soils had a comparable loamy texture, Site 2 contained the sand percentage, and Site 3 site contained the most clay. Soils from the natural sites and the FFH reclaimed plots had comparable pH values (Table 2-2). The pH of soils from plots reclaimed with PM was higher by an average of 0.7 pH units when compared to the plots reclaimed with FFM and the natural control site. As previously reported by McMillan et al. (2007), soil gravimetric water content was lower on all reclaimed plots than on the natural reference stands (Table 2-2). The natural stands contained five to six times the soil gravimetric water content of any of the reclaimed sites. On the reclaimed sites, soil water content varied among sites rather than between reclamation treatments. The natural reference stands also contained approximately double the organic carbon of any of the reclaimed sites. Additionally, while the carbon to nitrogen ratios did not show any consistent differences between organic amendment types on the three reclaimed sites, the

soil organic carbon content of the PM plots contained approximately double that of the FFM plots. That was most likely due to differences in thicknesses of the original peat and forest floor horizons, which resulted in different organic:mineral mixtures when the organic materials were salvaged together with the underlying mineral horizons.

2.3.2 Long term microbial community composition monitoring

Wilcoxon rank sum tests failed to detect any significant differences in total microbial biomass between plots reclaimed with FFM and plots reclaimed with PM on any site, or for any given year. Consequently, total soil microbial biomass data from both organic amendment treatments on each site were compiled to assess the relationship between biomass and time since initiation of reclamation. Soil microbial biomass was found to increase with time since reclamation on all reclaimed sites and ranged from 75 (2004) to 354 (2010) nmol PLFA g^{-1} (Fig.2-3). However, within the reclaimed plots, microbial biomass remained at least 50% below the values that were measured in 2004, 2009 and 2010 at the natural sites, i.e. 576 (2004), 987 (2009), and 623 nmol PLFA g^{-1} (2010). An NMDS ordination of the soil microbial community composition as measured annually on all reclaimed sites from 2004 to 2010 produced a three dimensional solution, which explained 40.1%, 21.1% and 29.9% of the variation on the axes respectively (Fig. 2-2). A stress of 13.9 was reached after 115 iterations. Year was found to be a stronger grouping factor (T=-60.42) than organic amendment type (T= -13.69) or site (T= -23.37). A secondary matrix including climatic data (Table 2-1) did not

reveal any significant correlations that could help explain the annual differences in soil microbial communities. The effect of season versus year of sampling was also investigated (data not shown). Specifically, an NMDS ordination of the soil microbial communities sampled at the reclaimed sites in August as well as May 2005, and May and September of 2010 generated a two dimension solution which explained 57.2% and 32.0% of the variation on the axes respectively, with a final stress of 16.9 after 234 iterations. The MRPP analysis identified year (T: -45.98; A: 0.01) to be a stronger grouping factor than season (T: -19.07; A: 0.02).

The NMDS analysis of the complete 2004-2010 soil microbial community chronosequence (Fig. 2-2) further showed that all years were significantly different from one another ($p < 0.05$). Years chronologically closer were not found to be more similar to one another in all cases. For instance, 2004 was most similar to 2009 (T: -14.90; A: 0.07), and 2007 was most similar to 2004 (T: -24.29; A: 0.11), although 2005 was most similar to 2004 (T: -15.32; A: 0.08); and 2010 was most similar to 2009 (T: -5.63; A: 0.03). Consequently, time since reclamation was not the sole primary driver of soil microbial composition at these reclaimed sites. Additionally, no significant relationship was identified between the pattern of years found in the NMDS analysis and climatic variables such as total annual precipitation, total August rainfall or average August temperature (Table 2-1).

Several additional MRPP analyses were used to specifically assess the influence of site and reclamation treatment on soil microbial community composition within each individual year (Table 2-3). Significant differences among reclamation sites

and between organic amendment treatments were found every year (with the exception of treatment in 2005). From 2004 to 2007, site was found to be a stronger grouping factor than reclamation treatment. However, in later years (2008 to 2010), reclamation treatment became a stronger grouping factor and surpassed that of site in 2008 and 2010 (e.g.; in 2008, site T = -7.11 vs. reclamation treatment: T: -8.46). This suggests that while site was initially the most important driver, the influence of organic amendment type on soil microbial composition increased with time since reclamation.

The soil microbial community composition of the reconstructed soils remained highly statistically different ($p \leq 0.001$) from that of the reference forest stands in all three years in which the natural soil microbial community was assessed; i.e. in 2004, 2009 and 2010 (Table 2-4). Indicator species analysis (performed in PC-Ord) did not reveal any identifiable PLFA that could help explain the differences between communities. However, the separation between both reclamation treatments and the natural reference sites decreased with time, as can be seen from the increasingly less negative T values in the MRPP analyses. These decreased from -11.42 for FFM and -11.54 for PM in 2004 to -6.28 for FFM and -10.33 for PM in 2010. Furthermore, the soil microbial community associated with the FFM treatments appeared to be converging towards the natural soil microbial community more quickly, as a greater increase in the T values was found in plots associated with FFM compared to plots associated with PM. To explain, in 2004, the difference between the T values for PM vs. Natural (-11.54) and FFM vs. Natural (-11.42) was 0.12, while in 2010, the difference between them was 4.05.

2.3.3 Moisture manipulation experiment

The NMDS ordination of the soil microbial communities at all four moisture levels produced a three-dimensional solution, explaining 14.6%, 52.5% and 26.2% of the variation on the three axes respectively, with a final stress of 10.02 after 111 iterations (Fig. 2-4). Variation linked to differences in moisture levels was much higher for samples collected from the reconstructed soils than for those from the natural forest soil. Additionally, as moisture level increased, the soil microbial communities on the reclaimed sites became less similar to the natural soil microbial communities, as can be seen from increasing T values in individual MRPP tests (at 15% moisture, T= -8.07; 30% moisture, T= -11.31; 45% moisture, T= -11.09; and 60% moisture, T= -12.22).

Moisture did not appear to be the principal driving factor for the differences found between the reclaimed and natural soil microbial communities. Instead, site (including the three reclaimed sites and the natural forest stand) was found to be the strongest grouping factor, as it had a more negative T and a larger A value (T: -15.66; A: 0.12), than moisture level had (T: -6.76; A: 0.05). All sites and treatments (including the natural LFH) were found to be significantly different from one another. The reconstructed soil microbial communities were more different from those found in the natural forest (FFM vs. Natural LFH: T: -12.20; A: 0.11; PM vs. Natural LFH: T: -13.29; A: 0.11) than they were from each other (FFM vs. PM: T: -3.45; A: 0.18). However, both the FFM and PM soil microbial communities became less similar to the natural sites with increasing moisture.

2.4 Discussion

2.4.1 The influence of native organic matter and vegetation on soil microbial community

Total microbial biomass (total PLFAs) on the natural sites (576-987 nmol PLFA g^{-1}) was comparable to the ranges reported in undisturbed aspen and mixedwood forest soils of western Canada, such as 816 – 1626 nmol PLFA g^{-1} measured in central Alberta (Hannam et al. 2007) and 282-407 nmol PLFA g^{-1} reported from the Athabasca Oil Sands Region (Dimitriu et al. 2010). Previous research assessing the full range of reclamation prescriptions in the Athabasca Oil Sands Region found total microbial biomass at the reclaimed sites to vary between 38-267 nmol PLFA g^{-1} (Dimitriu et al. 2010). In this study, total microbial biomass from the reconstructed soils (Fig. 2-3) ranged from 75 (2004) to 354 (2010) nmol PLFA g^{-1} at Site 1, 38 (2004) to 201 (2010) nmol PLFA g^{-1} at Site 2 and 35 (2005) to 161 nmol PLFA g^{-1} at Site 3.

Total microbial biomass on all reclaimed sites increased approximately 480% throughout our seven-year monitoring period (Fig. 2-3), yet it failed to reach levels comparable to the natural reference stand. The exact timeline required for full microbial recovery following soil reconstruction is difficult to predict and depends both on environmental conditions and the reclaimed target ecosystem. For instance, following surface coal mining in the semiarid region of Wyoming, 13 sites were reclaimed to a target ecosystem dominated by shrubs and understory grasses; microbial biomass carbon recovered to predisturbance levels after 11 to

26 years at 12 of the 13 reclaimed landscapes studied (Anderson et al. 2008). However, several other studies in same region (Mummey 2002a; Mummey 2002b) as well as studies conducted in *Eucalyptus marginata* (Donn ex Sm.) forest ecosystems from western Australia (Banning et al. 2011), a German forest and agricultural landscape (Insam and Domsch 1988), and in the Athabasca Oil Sands Region (Dimitriu et al. 2010) have reported that soil microbial community composition and biomass of reclaimed soils remained different from those of the reference stands even after 20 years since reclamation. Reclaimed sites in this study appear to be on trajectory towards ecosystem recovery in terms of soil microbial biomass, however 6 (Site 3), 10 (Site 2) and 12 (Site 1) years after reclamation the reconstructed soils only support, on average, 20% of the total microbial biomass found in the natural reference soils. This clearly establishes that the time needed to reach ecosystem recovery at these sites will extend well beyond a decade.

At Site 1, measured plant cover was 10% and 30% on the FFM and PM reclaimed sites, respectively (Fig.2-1). The 1999 records from the same site indicated plant cover to be 2% and 1% for the same treatments (Mapfumo 2003). Additionally, a 15% increase in plant cover was observed on the two reclaimed treatments found at Site 2 from 2002 to 2005 (AMEC Earth and Environmental 2003; AMEC Earth and Environmental 2006). A positive correlation between soil microbial biomass and plant cover typically gets established as barren reclaimed sites become revegetated (DeGrood 2005; Ponder and Tadros 2002). Similarly, in our study, the annual increase in microbial biomass (Fig. 2-3) followed increases in plant

cover. Total microbial biomass changes have also been suggested to be related to changes in community composition (Bardgett et al. 1999; Lovell et al. 1995). Correspondingly, we found that as total microbial biomass increased, the soil microbial community composition of the reclaimed sites became more similar to that of the natural reference stands (Table 2-4), supporting this hypothesis.

Overall, we found the plots reclaimed with FFM to support vegetation more similar to the natural forest stands than the plots reclaimed with PM (Fig.2-1). Differences in vegetation between the two reclamation treatments were linked to both total plant cover and species composition. Our results agreed with previous vegetation surveys completed on these sites which noted significant differences in vegetation between the two treatments (AMEC Earth and Environmental 2003; AMEC Earth and Environmental 2006; Brown et al. 2003; Mackenzie and Naeth 2006; Mackenzie and Naeth 2010; Mapfumo 2003). We found that the soil microbial community composition of reclaimed sites remained statistically different from the natural stands throughout the study. However, the soil microbial community composition on plots reclaimed with FFM became more similar to those found on the natural reference stands than the plots reclaimed with PM (Table 2-4), as did the vegetation (Fig. 2-1).

While total soil microbial biomass was similar for the two reclamation treatments when calculated on a per-gram of soil basis, results change when total biomass is calculated on a per-gram of soil carbon basis. Indeed, since the PM treatments contained approximately twice as much soil carbon as the FFM plots (Table 2-2),

microbial biomass within the PM plots (g of soil carbon^{-1}) would be much lower than within the FFM plots. This indicates that the composition of the organic amendment, more so than its total amount, was affecting the soil microbial community within the reclaimed sites. A detailed characterization of the carbon in reconstructed soil in the Athabasca oil sands region can be found in Turcotte et al., 2009. Soil pH was found to be lower, and comparable to the natural sites, on the plots reclaimed with FFM compared to the PM plots (Table 2-2). Since pH is closely related to soil microbial community composition (Baath et al. 1995; Dimitriu and Grayston 2009), the greater similarity between FFM reclaimed plots and natural sites could also be related to pH. Finally, we observed a divergence between the soil microbial community compositions found on sites reclaimed with FFM and those reclaimed with PM through time (Table 2-4). Additionally, we identified an increase, with reclamation age, in the importance of the type of reclamation treatment (FFM *versus* PM) as a grouping factor in the MRPPs (Table 2-3). Taken together, these results provide compelling evidence that differences between the FFM and PM treatments not only may be due to initial differences in the composition of the amendments, but also would be linked to differences in the plant communities growing on the two types of reconstructed soils. In turn, these would provide diverging types of litter inputs and carbon resources for the soil microbial communities.

2.4.2 Soil microbial community response to moisture

We hypothesized that because gravimetric soil moisture in the reconstructed soils (8-17%) was significantly lower than that of the undisturbed soils (60%), by increasing moisture, and hence lifting this potential limitation, the microbial communities on the reclaimed sites would become more similar to those found on the references stands. Our results, however, showed a completely opposite trend, since we found that increasing soil moisture in the laboratory experiment increased the difference between reclaimed and natural soil microbial community composition (Fig. 2-4). Previous work has found increased moisture to increase microbial respiration and microbial biomass nitrogen in soils reclaimed with both FFM and PM (McMillan 2007). The authors found that soils reclaimed with FFM had higher respiration rates and microbial biomass N than those reclaimed with PM; though, neither the rate of respiration or microbial biomass N in either reconstructed soil ever reached the levels observed in the natural reference soil (kept at its original in situ moisture content) (McMillan 2007). Here we conclude that differences in the soil microbial composition between the reclaimed sites and the natural forest stands were not solely due to a moisture limitation, but rather to more complex and indirect soil-plant interactions. This conclusion is further supported by the lack of correlation found between sampling date (year), the grouping factor found to have the greatest influence on soil microbial community composition of reclaimed sites (Fig. 2-2), and either annual or August precipitation.

As well, our results suggest that boreal forest soil microbial communities may be more robust to environmental change than previously hypothesized, as when the soil microbial communities of both the reconstructed and natural soils were assessed together, the natural soil (from the aspen-dominated natural forest stand) appeared more resistant to changes in moisture content than the reconstructed soils (Fig. 2-4). Many studies have concluded that natural soil microbial communities are sensitive to fluctuation in environmental conditions (Bardgett et al. 1999; Swallow et al. 2009; Wilkinson et al. 2002); however, this conclusion has been based mainly on observational differences in the soil microbial communities linked to different sampling dates. Soil microbial community resistance to changes in soil moisture is thought to be determined by the inherent characteristics of the microorganisms present (Vangestal et al. 1993). Jangid et al. (2011) identified land use history as having a stronger influence on soil microbial communities than vegetation or soil properties. Thus, reclamation or site history may be the reason the reconstructed soils, both FFM and PM (after 6-12 years since reclamation) responded similarly to one another but differently from the natural site. Furthermore, the variation in response to moisture observed between the reconstructed and natural soils is a clear indicator that the soil microbial communities in the reclaimed plots differ from or are not yet functioning as those in the natural stands.

2.4.3 Importance of native organic matter and vegetation for reconstructing soils

The use of FFM as a soil organic amendment has been shown to be advantageous in terms of native upland forest vegetation establishment and survival when reclaiming sites in northeastern Alberta (AMEC Earth and Environmental 2006; Brown et al. 2003; Mackenzie and Naeth 2006; Mapfumo 2003). Here we found that the use of FFM also promoted the development of soil microbial communities more similar to those found on natural forest stands than did the use of organic materials salvaged from surrounding peatlands. Dubbed 'the home field advantage', previous work has shown that soil microbes adapt to the substrates they most frequently encounter (Gholz et al. 2000). Thus, the accelerated development of the "natural", or target, soil microbial communities we observed on the plots reclaimed with FFM, may be linked to the similarities in plant communities found between these plots and the natural forest reference stand.

Tables and Figures

Table 2-1 Total annual and August precipitation, and mean August temperature in Fort McMurray, AB, Canada, from 2004 to 2010, and 30 year normal (Environment Canada 2010).

	Year						
	2004	2005	2006	2007	2008	2010	1971-2000
Total annual precipitation (mm)	327	376	362	267	422	328	456
Annual August precipitation (mm)	17	65	37	70	155	76	73
Mean August temperature (^{0}C)	12.7	14.1	15.5	12.9	16.4	15.4	15.3

Table 2-2 Selected topsoil properties (0-7.5 cm or as indicated) at the natural and reclaimed sites (FFM: plots reclaimed with forest floor-mineral soil mix; PM: plots reclaimed with peat-mineral soil mix) in the Athabasca Oil Sands Region (OC: organic carbon; Þw: gravimetric water content).

Site	Treatment	Texture [a]	OC ($g\ Kg^{-1}$)	C/N	Þw [d] (%)	pH [a]
Site 1	FFM	loam	50 [b]	21.3 [b]	14	5.3
	PM	loam	100 [b]	21.5 [b]	17	5.9
Site 2	FFM	loamy sand	40 [b]	48.0 [b]	8	5.0
	PM	sandy loam	80 [b]	29.1 [b]	8	6.0
Site 3	FFM	clay loam	40 [c]	22.9 [c]	10	5.4
	PM	clay loam	80 [c]	31.9 [c]	13	6.2
Natural Forest Stand	-	-	165[b]	18.3[b]	60	4.7

[a] sampled September 2010;

[b] McMillian 2005 sampled to a depth of 7 cm;

[c] Mackenzie 2006 sampled to a depth of 10 cm;

[d] sampled September 2009

Table 2-3 Multi-response permutation procedure (MRPP) results for soil microbial community composition (PLFA) in the Athabasca Oil Sands Region. Treatment compares FFM (plots reclaimed with forest floor-mineral soil mix) to PM (plots reclaimed with peat-mineral soil mix). Site compares three reclaimed sites (Site 1, Site 2 and Site 3) to one another.

	Treatment			Site		
Year	T	A	*p*	T	A	*p*
2004	-4.19	0.04	0.002	-20.53	0.22	<0.001
2005	-1.66	0.022	0.069	-5.03	0.10	0.001
2006	-3.50	0.039	0.007	-7.62	0.12	<0.001
2007	-1.94	0.020	0.044	-2.23	0.02	0.020
2008	-8.46	0.087	<0.001	-7.11	0.11	<0.001
2009	-7.08	0.096	<0.001	-7.58	0.12	<0.001
2010	-9.68	0.095	<0.001	-7.77	0.11	<0.001

Table 2-4 Multi-response permutation procedure (MRPP) results for soil microbial community composition (PLFA) in the Athabasca Oil Sands Region comparing samples collected from plots reclaimed with FFM (forest floor-mineral soil mix) and PM (peat-mineral soil mix) to those collected from natural forest sites (Natural).

Year	Comparison	T	A	*p*
2004	FFM vs. Natural	-11.42	0.18	<0.001
	PM vs. Natural	-11.54	0.16	<0.001
2009	FFM vs. Natural	-4.10	0.07	0.001
	PM vs. Natural	-6.70	0.10	0.001
2010	FFM vs. Natural	-6.28	0.08	0.001
	PM vs. Natural	-10.33	0.17	0.001

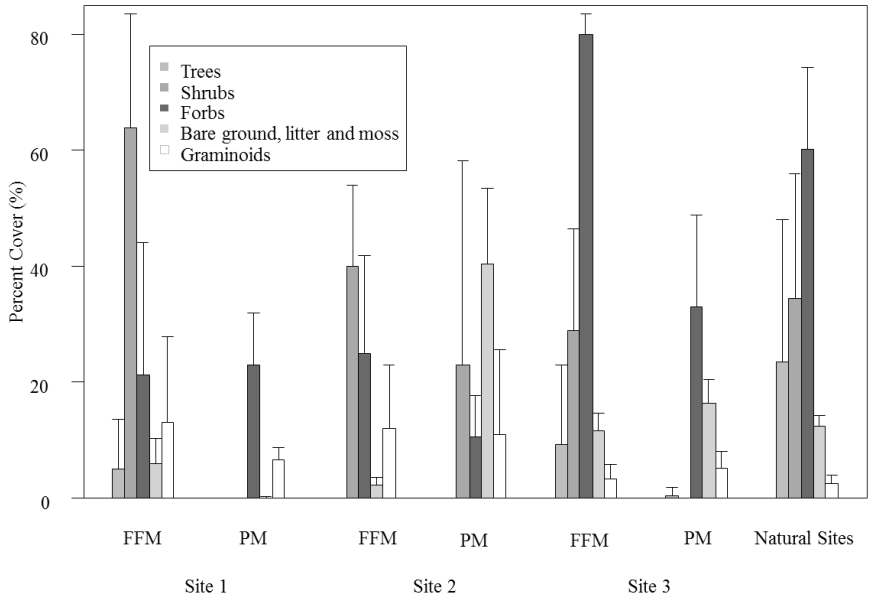

Figure 2-1 Percent cover of bare ground trees, shrubs, forbs and graminoids growing on natural forest sites and plots reclaimed with FFM (forest floor-mineral soil mix) and PM (peat-mineral soil mix) in the Athabasca Oil Sands Region. Error bars correspond to one standard deviation from the mean.

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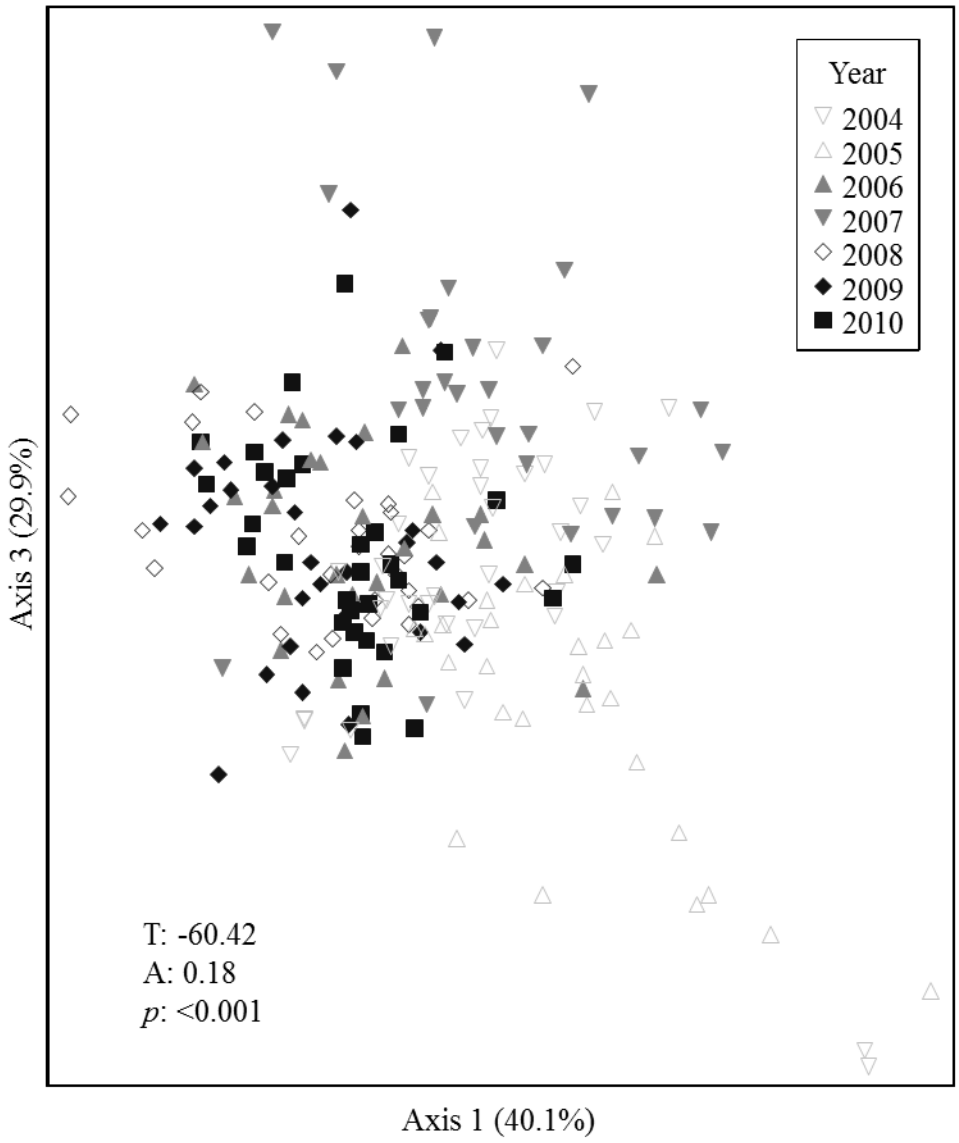

Figure 2-2 NMDS ordination of topsoil (0-7.5 cm) PLFA profiles sampled annually in August (2004 to 2010) on three reclaimed sites in the Athabasca Oil Sands Region.

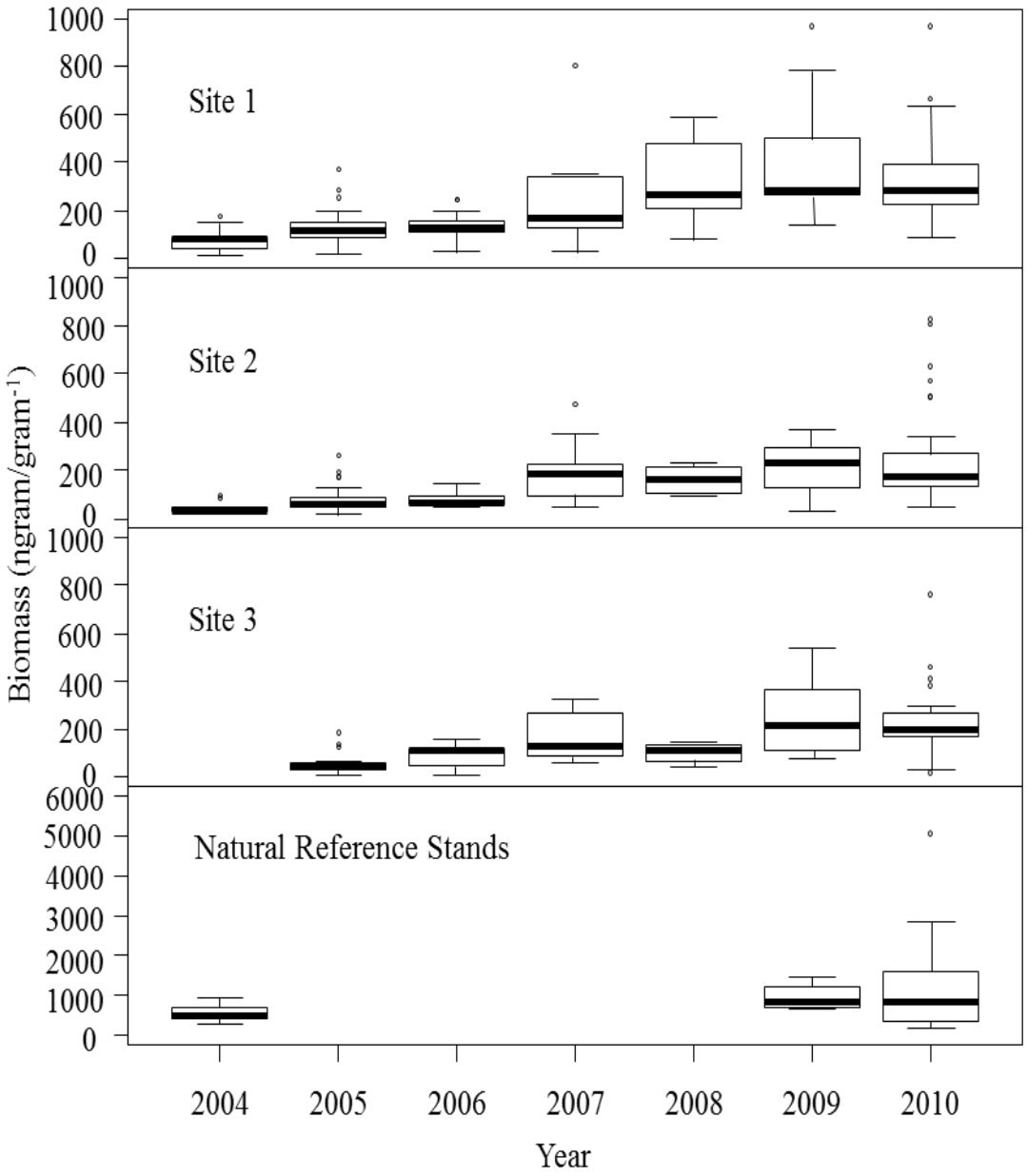

Figure 2-3 Total topsoil (0-7.5 cm) microbial biomass sampled annually in August (2004 to 2010) on three reclaimed sites and three natural sites (displayed together) in the Athabasca Oil Sands Region. The median value is displayed as the line through the box. The first quartile (Q1, 25%) and the third quartile (Q3, 75%) make up the box. The whiskers of the boxplot indicate that the range of the observations is within the whiskers at either end of the box with the exception of outliers (beyond 1.5*(Q3-Q1), which are represented as single points.

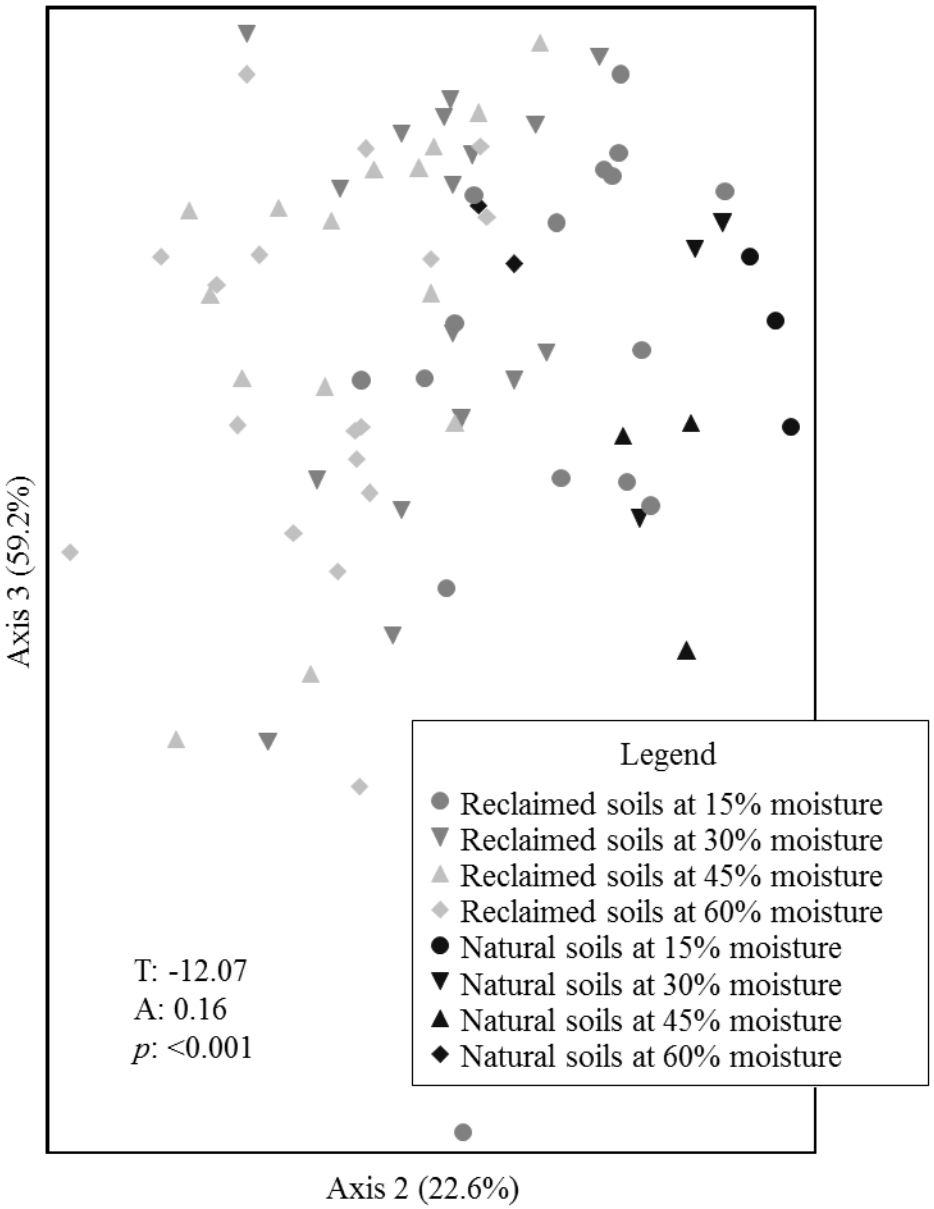

Figure 2-4 NMDS ordination of topsoil (0-7.5 cm) PLFA profiles from natural and reclaimed sites in the Athabasca Oil Sands Region, which were incubated for six months at four gravimetric moisture contents (15%, 30%, 45% and 60%).

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3.0 Chapter 3: Shifts in Soil Microbial Community Biomass and Resource Utilization along a Canadian Glacier Chronosequence

Prepared for Soil Biology and Biogeochemistry

3.1 Introduction

Glacier retreat environments provide unique opportunities in which to study primary ecological succession (Tscherko et al. 2003). Melting glaciers expose approximately $1x10^{17}$ to $1x10^{21}$ viable microbes annually (Castello and Rodgers 2005) which, along with microbes living in sediment beneath glaciers, are thought to act as "starters" or "seeds" for microbial colonization of glacial retreat environments (Grzesiak et al. 2009). Additionally, soils recently exposed by glacial retreat have been found to be highly suitable for colonization by bacteria originating from distant locations (allochthonous), in the first 4-5 years following exposure (Rehakova et al. 2010; Schmidt et al. 2008). Microorganisms serve to change soil conditions, thereby making the habitat more favorable for higher-lever organisms (Kastovska et al. 2005). To illustrate, microbes are responsible for biogeochemical processes in soils such as nutrient cycling and soil organic matter formation, which are essential to the establishment of vegetation in early successional stages, and ecosystem sustainability in later successional stages (Belnap et al. 1999; Jones 1998; Kastovska et al. 2005; Tscherko et al. 2003). Hence, colonization of these "new" environments by plants is dependent upon prior microbial community colonization (Kastovska et al. 2005).

While microorganisms help create conditions conducive to vegetation development, once established, plants provide new resources that can be exploited by the microbial community through the production of litter and root exudates (Miethling et al. 2000). Synergistic relationships between plants and microbes have an important impact on patterns of soil microbial development and succession (Kaye and Hart 1997; Ohtonen et al. 1999). Correspondingly, in glacier retreat environments, microbial functional diversity has been shown to increase with plant development and organic matter accumulation (Tscherko et al. 2003). Similarly, microbial biomass and enzymatic activity in rhizosphere soil samples are typically higher than in samples taken from the bulk soil (Miniaci et al. 2007; Tscherko et al. 2004), although not all pioneer plants have a measurable influence on the soil microbial community (Bardgett and Walker 2004). Soil microbial community succession is also influenced by abiotic factors such as soil pH, soil texture, and water content (Bekku et al. 2004b; Kastovska et al. 2005; Kastovska et al. 2007). Thus, complex interactions among soil abiotic properties and carbon resources control microbial succession (Tscherko et al. 2004).

Despite previous research into primary succession in terms of vegetation development (e.g.; Mizuno 1998; Stocklin and Baumler 1996), soil formation (Egli et al. 2001; Sondheim and Standish 1983; Tisdale et al. 1966) and microbial functional diversity (Klingensmith and Vancleve 1993; Schipper et al. 2001; Tscherko et al. 2003), the processes by which newly exposed ecosystems undergo succession are still relatively poorly understood (Grzesiak et al. 2009). Furthermore, the majority of information available about microbial succession in

glacier retreat environments is based upon empirical evidence and inference from individual study sites. Thus, as soil microbial community successional patterns have been shown to differ among glacier forefields (Sigler et al. 2002; Tscherko et al. 2003), it is essential that the information pertaining to microbial succession comes from numerous globally distributed environments, in order to capture the entirety of this intricate phenomenon. While studies of microbial succession along glacier forefields have been conducted in Austria (e.g.; Tscherko et al. 2003, 2004 and 2005), Norway (e.g.; Bekku et al. 2004a and 2004b; Kastovska et al. 2005 and 2007), Finland (e.g.; Merila et al. 2010), Switzerland (e.g.; Conen et al. 2007; Edwards et al. 2006; Miniaci et al. 2007; Sigler et al. 2002) and Peru (e.g.; Nemergut et al. 2007), few have been undertaken in North America (e.g.; Bardgett and Walker 2004; Ohtonen et al. 1999), and even fewer in Canada (e.g.; Day 2010; Yoshitake et al. 2006).

The overall objective of this study was to assess soil microbial community succession and determine the influence of two plant species, yellow mountain avens (*Dryas drummondii* Rich.), and Engelmann spruce (*Picea engelmannii* Parry) on soil microbial community composition and activity along a Canadian glacier chronosequence with sedimentary parent material. Yellow mountain avens is a pioneer plant common at the Robson glacier that is present in both early and late successional plant communities and fixes atmospheric nitrogen within its root nodules (Lawrence et al. 1967). Engelmann spruce is the climax species in the mature forest stands present in the area (BC Parks 2001), as well as the first tree species to appear along the chronosequence. We quantified, for the first time, the

potential peroxidase and phenol oxidase enzymatic activities of soil microbial communities along this glacial chronosequence. We hypothesized that soil microbial community composition and activity would increase with increasing plant cover and successional stage. Secondly, we predicted that soil microbial activity would be higher in samples taken from plant rhizospheres when compared to the bulk soil. Specifically, we aimed to: (i) characterize ground cover and soil characteristics; (ii) measure soil microbial community composition and activity; and (iii) compare samples taken from bulk soil and the rhizospheres of yellow mountain avens and Engelmann spruce along the chronosequence.

3.2 Materials and methods

3.2.1 Study area

The study area was located in Mount Robson provincial park along the Robson glacier (53.1˚N, 119.1˚ W), in British Columbia, Canada. Standing 3954 m tall, Mount Robson reaches the highest point in the Canadian Rocky Mountains and supports a large ice- and snowfield. The region receives 881 mm of precipitation annually, 281 mm of which falls during the summer. Mean annual temperature is 1°C but ranges from -11°C to 11°C although average temperatures above 10°C typically occur in only one month of the year (BC Parks 2001). Vegetation in the park ranges from Alpine tundra at higher elevations to sub-boreal spruce; however the majority of the park is dominated by mature coniferous stands (> 140 years of age), mainly Englemann Spruce (*Picea engelmannii* Parry) (BC Parks 2001). Pioneer communities in the region are dominated by sweetvetch

(*Hedysarum sp.*) and mountain avens (*Dryas sp.*) followed by willow (*Salix sp.*), bearberry (*Arctostaphylos alpina* Reh. and Wil.) and other shrubs (Tisdale et al. 1966). The Robson glacier has deposited a terminal moraine as well as 10 recessional moraines over the past 200 years (Blundon and Dale 1990; Cooper 1916; Heusser 1956). The parent geological material consists of sedimentary material including quartzites, limestones, and dolomites of the Paleozoic age (Tisdale et al. 1966). Soils in the area tend to be well-drained, contain from 40 to 70% coarse fragments (> 2 mm) and have a relatively high $CaCO_3$ content (Sondheim and Standish 1983). Although the most recently exposed soils in the area are regosolic (Cryorthents according to the USDA classification), Orthic Eutric brunisols (Typic Cryepts) have formed on the oldest moraines (Blundon and Dale 1990; Sondheim and Standish 1983; Tisdale et al. 1966).

The retreat of the Robson glacier has been well documented by the park and the Alpine Club of Canada for the past 100 years (Blundon and Dale 1990; Sondheim and Standish 1983; Tisdale et al. 1966). For our study, sampling occurred along a transect running from the toe of the glacier, north-west towards Berg Lake (Fig. 3-1). As in previous studies (Blundon and Dale 1990; Conen et al. 2007; Ohtonen et al. 1999; Tscherko et al. 2003), the glacial retreat area was divided in successional "stages" or areas that differed in time since deglaciation and had visually distinct vegetation (Fig. 3-1). A nearby mature forest reference stand was also included in the study, for which the precise time since deglaciation is unknown (>> 100 years). A total of 5 stages was identified along the 1 km long, 99-yr chronosequence and these were numbered sequentially with increasing time

since deglaciation. We can estimate that the five stages are approximately 20 years apart. Stage 1 was located at the toe of the glacier and lacked any vegetation. Stage 2, although still primarily barren of vegetation, hosted a few plant species, including the sparsely growing yellow mountain avens (*Dryas drummondii* Rich). Stage 3, while still containing prominent areas of bare soil, had greater vegetation diversity. Full vegetation cover was still not present at Stage 4, but few bare spots were observed. Again, a greater diversity of plant species appeared at this stage, and included, for the first time, Engelmann spruce. Additionally, the accumulation of an organic layer was noted and recorded to be 1 cm in depth beneath the trees. Stage 5 was deglaciated 99 years before sampling (as recorded by the Alpine Club of Canada's historical marker); full vegetation cover was observed and a greater number of trees was present. Organic layer accumulation was also recorded to be 1 cm in depth beneath the trees. The reference stand was dominated by false azalea (*Menziesia ferruginea* Sm.), white-flowered rhododendron (*Rhododendron albiflorum* Hook.), black huckleberry (*Vaccinium membranaceum* Douglas ex Torr.), five leave bramble (*Rubus pedatus* Sm.), bunchberry (*Cornus canadensis* L.), oak fern (*Gymnocarpium dryopteris* L. New. Oak), lodgepole pine (*Pinus contorta* Douglas), subalpine fir (*Abies lasiocarpa* Hooker) and Engelmann spruce (BC Parks 2001).

3.2.2 Field sampling

Soil samples were taken to a depth of approximately 7.5 cm after removal of live vegetation if needed be. In Stage 1, five replicate samples were taken from the

bare ground since no vegetation was present. In Stages 2 to 5, six samples were taken, three under yellow mountain avens and three on bare soil (Stages 2 and 3) or beneath Engelmann spruce (Stages 4 and 5). A total of three samples were taken in the reference stand, also under Engelmann spruce. As the litter layer was > 7.5 cm deep, the samples collected were organic and contained little to no mineral material.

At each soil sampling point, a 10 m^2 plot was established and a ground cover survey was conducted. Using examples of percent cover as a visual aid (Government of British Columbia 2011), the percent cover of barren soil (< 2 cm), coarse mineral material (> 2 cm), yellow mountain avens, graminoids and forbs, shrubs, organic ground cover (e.g. litter, fungi, bryophytes) and Engelmann spruce were estimated in each plot on a 0-100% basis.

As many soil samples as possible were sieved to 2 mm in the field, in order to minimize the amount of coarse fragments to be transported down the mountain. Samples from Stages 2, 5 and the reference stand were sieved in the laboratory due to time constraints in the field. The weight of coarse fragments (> 2 mm) was recorded using a portable handheld scale, and on average was 70% wt. Samples were collected on either August 31st or September 1st, 2010, and, with the exception of samples to be used for phospholipid fatty acid (PLFA) analysis (which were stored at -80^{0}C until time of analysis), samples were kept in the refrigerator (4^{0}C) until the analyses could be performed.

3.2.3 Laboratory analyses

Prior to determining soil physical and chemical properties, samples were oven-dried at 65°C for three days (ISO 11465 1993). To measure pH, 0.01 *M* calcium chloride was added to soil using a 2:1 solution: soil ratio for Stages 1-5 and a 6:1 ratio for the reference stand, as described in Kalra and Maynard (1991). Total carbon (TC), total nitrogen (N) and total organic carbon (TOC), which was pre-treated with HCl to remove carbonates, were measured in ball ground (Retsch, MM200 grinder) subsamples using a Costech 4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) fitted with a thermal conductivity detector. The $\delta^{13}C$ values were obtained using the same Costech ECS 4010 Elemental Analyzer coupled to a Finnigan Delta Plus Avantage Isotope Ratio Mass Spectrometer (ThermoFinnigan, Bremen, Germany). Internal standards, which were calibrated against the international reference standard (Pee Dee Belemnite), were used to calculate and express results using the standard δ notation. For the determination of soil texture, in each stage, samples collected under the same conditions (e.g., under Engelmann spruce, yellow mountain avens, or from bulk soil) were composited on a weight basis; texture was then determined using the hydrometer method as outlined in Sheldric and Wang (1993).

Prior to extraction, soil samples underwent freeze-drying to eliminate moisture while retaining the integrity of the PLFAs. As adapted from Hannam et al. (2006), and based on their TOC content, approximately 20-30 g of samples from Stages

1-3, 5-10 g for soils from Stages 4 and 5, and 0.3 g from the reference stand were used in the analysis. Using a modified Bligh and Dyer extraction, the polar lipids were extracted from the soil, isolated using pre-packed silicic acid columns (Agilent Technologies, Wilmington, DE) and finally subjected to a mild alkaline methylation which served to form fatty acid methyl esters (Frostegard and Baath 1996). An Agilent 6890 Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE) fitted with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column, with hydrogen as the carrier gas was employed to separate and quantify the PLFAs. The MIDI peak identification software (MIDI, Inc., Newark, DE) was used to identify and name peaks (Hannam et al. 2006; Swallow et al. 2009).

Substrate induced respiration (SIR) analysis was carried out on all soil samples within a week of returning to the laboratory, using the MicroResp™ multi-SIR system that employs 96 deep wells to effectively measure the CO_2 evolved from the respiration of whole soil microbial communities (Campbell et al. 2003; Chapman et al. 2007). Specifically, we measured the ability of the soil microbial community to respire three carbon substrates: protocatechuic acid, a dihydroxybenzoic acid; glucose, a substrate commonly used in soil analysis (e.g.; Anderson and Domsch (1978), cited approximately 1500 times); and N-acetyl glucosamine, a molecule containing nitrogen and commonly found in bacterial cell walls (Brooks and Baddiley 1969). In addition, we used water to determine basal respiration. The analysis was restricted to the three carbon monomers due to the limited amount of sample collected. In brief, 0.40 g of fresh soil was added to

each deep well. The three substrates were then prepared so that 30 mg of substrate per gram of soil water could be delivered in 25 µl of water, and they were pipetted into the deep well plates. Agar, mixed with cresol red, potassium chloride and sodium bicarbonate was used to make indicator plates. A spectrophotometer microplate reader was employed to take a blank reading of absorbance (570 nm) of the indicator plate before it was sealed to the deep well plate. Samples were then incubated for 6 hours at 25°C before a second reading of the indicator plate was taken. Data for protocatechuic acid, glucose, and N-Acetyl glucosamine were normalized to the respiration of water in each sample and all values were expressed as µg CO_2- C/g/hr.

Soil enzymatic activity was measured on fresh soil samples within 10 days of collection. The methods used to measure enzymatic activity were adapted from Sinsabaugh et al. (2003). Both phenol oxidase (POX; E.C. 1.10.3.2) and peroxidase (PER; E.C. 1.11.1.7) were measured using 3,4-dihydroxy-L-phenylalanine as a colorimetric substrate, while β-Glucosidase (BGLUCO; E.C. 3.2.1.21) activity was measured using 4-methylumbelliferyl-β-D-glucopyranosidase as a fluorimetric substrate. Approximately 1 g of sample was buffered with 125 ml of 50 mM, pH 5.0 sodium acetate, and shaken for thirty minutes. A 200 µl volume from the resulting soil solution and 50 µl of 200 µM substrate solution (either 3,4-dihydroxy-L-phenylalanine or methylumbelliferyl-β-D-glucopyranosidase) were pipetted into 96 well plates; 16 replicates were used for each sample. Additionally, 8 blank wells, containing 50 µl of acetate buffer

and 200 μl of sample suspension, and 16 negative control wells, 8 containing 50 μl substrate solution and 200 μl of acetate buffer, and 8 containing 250 μl of buffer, were included. For the fluorescence spectroscopy, 8 quench standard wells, containing 200 μl soil solution and 50 μl 4-methylumbelliferone, were included for each sample, as were a total of 8 reference standard wells, each containing 200 μl buffer and 50 μl 4-methylumbelliferone.

The enzyme assay plates were left to incubate in the dark for 18 hours (peroxidase), 5 hours (phenol oxidase) or 3 hours (β-Glucosidase). To measure phenol oxidase activity, 10 μl of 0.3% hydrogen peroxide was injected into each well prior to the 5-hour incubation period, while to measure β-Glucosidase activity, 20 μl of 0.5 M NaOH was added to each well following the 3-hour incubation period. A spectrophotometer microplate reader was used to read and quantify absorbance at 460 nm, and fluorescence at 360 nm excitation and 460 nm emission. To calculate the final peroxidase activity, phenol oxidase activity was subtracted from the peroxidase activity measured. After correction for standards and quenching (β-Glucosidase), data were expressed in μmol/hr/g of soil.

3.2.4 Statistical analyses

All PLFAs (≤ 20 carbons in length) identified by the MIDI software were included in the analysis. Soil microbial biomass was calculated using total PLFAs on a nmol PLFA g^{-1} basis. The metabolic quotient, qCO_2 was calculated as the ratio of basal respiration to total microbial biomass (i.e; total PLFAs). A Hellinger

transformation was used to further relativize the data (Legendre and Gallagher 2001). Non-metric multi-dimensional scaling (NMDS) ordinations were used to explore the PLFA data using PC-Ord software version 5 (MjM Software Design, Gleneden Beach, OR). Multi-Response Permutation Procedure (MRPP) tests were then used to assess the significance of microbial grouping patterns identified in the NMDS (Zimmerman et al. 1985). The Sorensen (Bray-Curtis) dissimilarity index was used for all NMDS ordinations and MRPPs. An alpha of 0.05 was used for all statistical tests.

Data collected from the vegetation assessment, enzyme and SIR analyses, were analyzed by calculating arithmetic means and standard deviations using Microsoft Excel (version 2010). The R package (version 2.12.1, R Development Core Team) was used to test the assumption of normality (Shapiro-Wilk test) and homogeneity of variances (Bartlett test). The same R package was used to perform Spearman's rank correlations (ρ) between respiration rate and enzyme activity data as well as between respiration rate, enzyme activity and soil chemical properties. No further statistical analyses to compare values among the different successional stages were conducted as this was an individual case study. Instead, trends in total microbial biomass, respiration rates, enzymatic activity and metabolic quotient along the chronosequence and in the reference stand were explored, again using the same R package.

3.3 Results

3.3.1 Plant and soil characteristics

Plant cover increased with time since deglaciation (Fig. 3-2). While no vegetation was present at Stage 1, by Stage 5 full plant cover (i.e.; 0% barren soil) was observed. An increased number of ground cover classes (e.g.; graminoids and forbs, shrubs and Engelmann spruce) was found with increasing time since deglaciation. Engelmann spruce trees first appeared in Stage 4 (approximately 1 m in height), as did several other species of graminoids, forbs and shrubs (e.g.; *Salix* sp and *Dryas* sp). Stage 5 had the greatest plant cover as well as the highest species diversity. Also at Stage 5, spruce trees were present in greater number and had a greater average height (approximately 2 m in height) compared to Stage 4. Yellow mountain avens, one of the first plants to appear after deglaciation, was found throughout the chronosequence (Stages 2-5 and including the reference stand), although it decreased in number after Stage 3.

Soil texture remained sandy (sand> 70% wt) and relatively similar in all sampled soils along the chronosequence (Table 3-1). Carbonates were found in all samples taken from Stages 1-5, as can be seen from the relatively high inorganic carbon concentrations (TC –TOC) ranging from 7.0 to 8.5%. Linked to the presence of carbonates, the pH values stayed around neutral (6.9-7.5) throughout the chronosequence. The decreasing trend in $\delta^{13}C$ values along the chronosequence indicated a dilution of carbonates ($\delta^{13}C$ ~ 0 ‰) by plant carbon (e.g.; $\delta^{13}C$ of -27.7 ‰ in the reference stand forest floor), which was paralleled by the measured

increase in soil organic carbon (TOC) with time since deglaciation. No differences between samples taken beneath yellow mountain avens and the bulk soil, or between the rhizospheres of yellow mountain avens and Englemann spruce, were observed for any of the chemical and physical variables measured (Table 3-1). For all chemical chemical properties measured, the greatest difference observed was between the reference stand and the chronosquence (Stages 1-5). In the reference stand, the organic layer was thicker than the sampling depth (7.5 cm). Hence, TOC in the reference stand (44.0%) was over ten times that measured in Stage 5 (4.7%). Further, while the C/N ratios ranged from 8-15 along the chronosequence, C/N increased to 35 in the reference stand organic layer. Additionally, the reference stand samples contained no carbonates and subsequently exhibited a much lower pH (4.5) than along the chronosequence.

3.2.2 Microbial biomass, metabolic quotient, and community composition

Total soil microbial biomass increased with time since deglaciation (Fig. 3-3), but at Stage 5, it still only was ~9% (138 nmol PLFA g^{-1}) of that of the natural reference stand (1057 nmol PLFA g^{-1}). No consistent trend between biomass and the specific sampling location (i.e., beneath yellow mountain avens or Engelmann spruce) was observed. However, in Stage 3, biomass in the rhizosphere of yellow mountain avens (14 nmol PLFA g^{-1}) was approximately double that of the bare soil (6 nmol PLFA g^{-1}), and in Stage 4, biomass in the rhizosphere of Engelmann

spruce (121 nmol PLFA g^{-1}) was almost 5 times that of samples taken from the rhizosphere of yellow mountain avens (25 nmol PLFA g^{-1}). The biomass values measured in the rhizospheres of the two plant species in Stage 5 were relatively similar (rhizosphere of yellow mountain avens: 154 nmol PLFA $gram^{-1}$; rhizosphere of Engelmann spruce: 122 nmol PLFA $gram^{-1}$).

Similarly to total microbial biomass, basal respiration increased with time since deglaciation (Fig. 3-3). The metabolic quotient (qCO_2), i.e.; the rate of basal respiration relative to the total microbial biomass (total PLFAs), showed high amounts of variation in Stages 1 and 2, but then decreased sharply in Stage 3 (Fig. 3-3). Stabilization of qCO_2 appeared to be reached in Stage 3 when samples were taken in the rhizosphere of yellow mountain avens (qCO_2 in bulk soil at Stage 3= 0.12; qCO_2 in the rhizosphere of yellow mountain avens at Stage 3= 0.01). To summarize, the metabolic quotient ranged from 0.1 to 1.4 in Stages 1, 2 and 3-b (bulk soil), but varied little and remained below 0.04 in all subsequent samples.

An NMDS ordination of the soil microbial community composition as characterized from the PLFAs on all samples collected produced a two dimensional solution, which explained 54.8% and 41.1% of the variation on the two axes respectively (Fig. 3-4). The final stress was 8.1 after 75 iterations. Despite differences in soil microbial biomass, compositional differences were not detected among the different stages sampled along the glacial chronosequence, nor did soils sampled in the reference stand exhibit a different composition. Several MRPP tests, using either stage, or specific sampling location (bare

ground, or plant species beneath which the sample was taken) as grouping factors failed to find any significant difference among groups ($p > 0.05$). Therefore, regardless of time since deglaciation and species beneath which the samples were taken, the soil microbial community composition, as defined by PLFA, remained statistically indistinguishable throughout the chronosequence and in the reference stand.

3.3.3 Microbial community respiration and enzymatic activities

Overall, enzymatic activity increased with time since deglaciation. In general, the activity rate of all three enzymes was higher in the reference stand than at any point along the chronosequence. Variation in enzyme activity rates among samples (replicates within a given stage) was relatively high throughout the chronosequence, although it was greatest in Stage 5 and the reference stand. Phenol oxidase and peroxidase activities remained relativity similar throughout the chronosequence with the exception of phenol oxidase from samples taken beneath Engelmann spruce in Stage 5, which tended to be higher and more similar to the reference stand. Respiration levels following the addition of all three carbon substrates, protocatechuic acid, glucose, and N-acetyl glucosamine, increased with time since deglaciation (Fig. 3-5). Protocatechuic acid, a phenolic compound, was respired at the highest rate throughout the chronosequence while glucose was respired at the highest rate in the reference stand only.

Respiration rates of all three carbon substrates, protocatechuic acid, glucose, and N-acetyl glucosamine were highly correlated with percent soil nitrogen (Table 3-

2). No significant correlation between any of the soil chemical properties and the activity rates of phenol oxidase or peroxidase was found. The correlation between glucose respiration and β-glucosidase activity was both high ($\rho = 0.78$) and significant ($p = < 0.001$), as were the correlations of both β-glucosidase activity and glucose respiration to total organic carbon (TOC), soil N, and microbial biomass (total PLFAs). Hence, the two aforementioned soil measures (glucose respiration and β-glucosidase activity) are highly related. Compared to protocatechuic acid and N-acetyl glucosamine, glucose was respired at notably low rates during Stages 1-3, since glucose respiration was below 0.8 μmol/hr/g, while protocatechuic acid and N-acetyl glucosamine ranged from 1.2-4.3 μmol/hr/g (Fig. 3-5). Likewise, β-glucosidase activity was below detection limits (<0.001 μg CO_2- C/g/hr) in Stage 1 and Stage 2-b (bulk soil), and just at the detection limit in Stage 2-yma (rhizosphere of yellow mountain avens), and in Stage 3-b. Therefore, we conclude that early stage soils are not readily able to utilize glucose.

3.4 Discussion

3.4.1 Soil microbial community composition

No differences in soil microbial community structure were identified along the glacial chronosequence (Fig. 3-4). Previous studies using both PLFA analysis (Merila et al. 2002; Ohtonen et al. 1999; Tscherko et al. 2005) and molecular techniques (Edwards et al. 2006; Nemergut et al. 2007) report soil microbial community composition changes along glacier chronosequences. Specifically,

using PLFA analysis, Tscherko et al. (2005) reported that while early successional stage communities had similar compositions, in the later stages, microbial composition differed with time since deglaciation. Similarly, Ohtonen et al. (1999) found that the proportion of fungal PLFAs increased with successional stage. Further, both of these studies showed that the soil microbial communities found in plant rhizospheres were distinct from those found in bulk soil (Ohtonen et al. 1999; Tscherko et al. 2005). In addition, Merila et al. (2002) reported strong relationships between PLFA, soil pH and C/N ratios. Along the chronosequence studied here, we did not observe a clear trend in pH or C/N values (Table 3-1), which may explain the similarity in PLFA results (Fig. 3-4). However, despite differences in pH and C/N between the reference stand and the chronosequence (Table 3-1) no difference in soil microbial community composition along the chronosequence or in the reference stand was detected (Fig. 3-4). Conversely, Lamb et al. (2011) suggested that resistance to change may be an inherent characteristic of soil microbial communities. The authors propose that while the metabolism of soil microbial communities may change in response to environmental changes, structural changes are much slower to occur.

3.4.2 Microbial biomass and plant rhizosphere-microbe interactions

Overall, total microbial biomass (total PLFAs) increased from Stage 1 (1 nmol PLFA g^{-1}) to Stage 5 (138 nmol PLFA g^{-1}), corresponding to over a 100 fold increase, yet failed to reach levels comparable to the reference stand (1507 nmol PLFA $gram^{-1}$; Fig. 3-3). Increases in microbial biomass along glacial

chronosequences have been previously shown to be related to increases in plant cover (Ohtonen et al. 1999; Tscherko et al. 2005), and soil nitrogen and pH (Tscherko et al. 2004). Similarly, we found increases in microbial biomass to be concurrent with increased time since deglaciation, plant cover (Fig. 3-2) and soil N (Table 3-1). In glacier environments, the increase in microbial biomass has been found to extend beyond 80-100 years along chronosequences (Ohtonen et al. 1999; Sigler et al. 2002; Tscherko et al. 2003), and even beyond 300 years in a few case studies (Bekku et al. 2004b; Yoshitake et al. 2006).

In Stage 3, biomass in the soil taken from the rhizosphere of yellow mountain avens (14 nmol PLFA g^{-1}) was approximately double that of the bare soil (6 nmol PLFA g^{1}) while the biomass measured in the rhizosphere of yellow mountain avens and the bulk soil in Stage 2 were comparable (bulk soil: 0.6 nmol PLFA $g^{-1;}$ rhizosphere: 1 nmol PLFA g^{-1}). This suggests that yellow mountain avens-microbe relationships change with successional stage and take approximately 40 years to develop. Bardgett and Walker (2004) reported that in soils deglaciated 12-15 years prior, total PLFAs significantly increased and soil microbial community composition differed under three pioneer species, while four additional plant species, including yellow mountain avens, had no effect on the soil microbial community. Further, *Poa alpina* L. has also been found to only influence soil microbial biomass in mid and late successional stages (Tscherko et al. 2004). This change in rhizosphere effect has been attributed to the harsh soil conditions in early successional stages, which supersede the plant influence. In later successional stages, as soil conditions are more favorable, the microbial

community becomes receptive to increased plant and carbon resources (Tscherko et al. 2004). Additionally, previous work has shown that yellow mountain avens fixes N only in mid to later successional stages, not necessarily due to a lack of N fixing *Frankia* but rather because the harsh soil conditions render N fixation excessively energy intensive (Kohls et al. 1994).

Additionally, we found that biomass beneath Engelmann spruce (121 nmol PLFA g^{-1}) was almost 5 times that of samples taken under yellow mountain avens (25 nmol PLFA g^{-1}) in Stage 4, while no difference between the two sampling locations was observed in Stage 5 (Fig. 3-3). Edwards et al. (2006) suggested that as full plant cover is reached and nutrient availability increases, rhizodeposition becomes relatively less important as resources in the surrounding area are more abundant. Thus, in later successional stages, the influence of individual plant species is reduced (Edwards et al. 2006). This may therefore explain why the large difference in biomass between the rhizospheres of yellow mountain avens and Engelmann spruce was only observed in the intermediary Stage 4. Yet, despite several studies into the effects of non-woody species on the soil microbial community in glacial environments (Edwards et al. 2006; Miniaci et al. 2007; Tscherko et al. 2004), little research into the effect of individual tree species in these environments has been done (Bardgett et al. 1999, which examined only the first 12-15 years of succession).

3.4.3 Shifts in soil microbial community resource utilization and efficiency

Our results indicate that early successional stage soil microbial communities are not readily able to utilize glucose. Glucose was respired at a rate less than or equal to the more chemically complex substrates in all stages along the chronosequence, while it was respired at the highest rate only in the reference stand (Fig. 3-5). Additionally, β-glucosidase activity was detected in very low amounts along the Robson chronosequence until Stage 4 (Table 3-3). The activity of β-glucosidase activity has been linked to increases in soil organic matter concentration (Sinsabaugh et al. 2008), which coincides well with our findings in that β-glucosidase activity was highly correlated with both total organic C and N (Table 3-2). Further, β-glucosidase activity and glucose respiration were highly correlated to each other (Table 3-2). Glucose is commonly used to measure soil microbial activity and biomass (Anderson and Domsch 1978 cited approximately 1500 times) as it is thought to be readily and universally used by soil microbial communities. However, here we conclude that that the ability of the soil microbial community to utilize glucose is low in early successional stages and increases with time since deglaciation. Similarly, low sugar assimilation has recently been reported in the early successional stage soils of Antarctica (Grzesiak et al. 2009) and in some boreal forest soils with young vegetative communities (Gronli et al. 2005). Thus we suggest that alternative or multiple substrates be used in place of glucose when soil microbial analyses are being performed on early successional stage soils.

We measured a clear decrease in qCO_2 from Stages 1 and 2 to Stage 3 and subsequently a stabilization of this indicator that was maintained even into the reference stand (Fig. 3-3), indicating a reduction in the maintenance energy required, or an increase in energy utilization efficiency (Insam and Haselwandter 1989). Previous studies have had difficulties in finding consistent trends in qCO_2 along transects (Merila et al. 2002), which was speculated to be due to the confounding influence of stresses, such as drought or grazing, on this indicator (Ohtonen et al. 1999; Wardle and Ghani 1995). However, here we observed a clear trend with time since deglaciation. This suggests that the soil microbial community became more efficient in their use of energy as time since deglaciation increased (Fig. 3-3), which may be indicative of more favorable conditions for microbial growth (resource availability, pH, moisture etc.) (Anderson 1994; Insam and Haselwandter 1989) and thus decreased environmental stress (Wardle and Ghani 1995).

3.4.4 Soil, plant and microbial successional patterns

The overall trends we measured in increasing total organic carbon, soil nitrogen (Table 3-1) and plant cover (Fig. 3-2) with successional stage have been observed in many studies (Kastovska et al. 2005; Tscherko et al. 2005; Zinger et al. 2011). Further, similar to previously reported results (Kastovska et al. 2005), we observed an increase in microbial respiration of carbon substrates with time since deglaciation (Fig.3-5). The respiration rates of protocatechuic acid, glucose, and N-acetyl glucosamine as well as the enzymatic activity of β-glucosidase were

highly correlated with percent soil nitrogen (Table 3-2), indicating that the soil microbial community was responding to successional changes in the soil environment.

Additionally, we found increasing phenol oxidase and peroxidase activities with successional stage (Fig. 3-5). While β-glucosidase is involved in breaking the bonds between disaccharides, together, phenol oxidase and peroxidase are used by soil microbes to degrade more recalcitrant carbon resources such as lignin, as well as for the attainment of nutrients, ontogeny and defense (Sinsabaugh 2010). These enzymes are most commonly produced by fungi (e.g.; Szklarz et al.1989) and are thus an indicator of fungal activity. Moreover, the activity of these enzymes has been shown to vary with plant composition (Fioretto et al. 2000; Sinsabaugh 2010). Correspondingly, we found increasing levels of activity (Fig. 3-5) with increasing plant cover (Fig. 3-2) and thus with increased resource availability. Phenol oxidase and peroxidase are known to have higher spatial-temporal variation than other enzymes (Sinsabaugh 2010), which may explain the lack of significant correlations between these enzymes and chemical soil properties, the respiration of carbon substrates or enzymatic activity of each other or β-glucosidase (Table 3-2). Overall, in Stage 5, phenol oxidase and peroxidase activities were respectively 52% and 28% of that of the reference stand (Fig. 3-5), suggesting that successional changes will require > 100 years for the Robson glacier setting.

3.5 Conclusions

In general, microbial biomass, enzymatic activity, microbial respiration, plant cover and percent organic carbon and nitrogen increased with successional stage. A high correlation between protocatechuic acid, glucose, and N-acetyl glucosamine as well as the enzymatic activity of β-glucosidase and soil N indicated that the soil microbial community was responding to changes in the soil environment. Further, a reduction in the maintenance energy required was observed from the decrease and subsequent stabilization of metabolic quotient (qCO_2) along the chronosequence. Plant-microbe interactions took 40 years since deglaciation to get established as yellow mountain avens had a measurable effect on microbial biomass only in the mid-successional stage. Low rates of glucose respiration and β-glucosidase activity in the early successional stages, as well as a high correlation between these two measures, indicate that early successional stage soil microbial communities are not readily able to utilize glucose. Hence, alternative or multiple substrates should be used in place of glucose when soil microbial analyses are being performed on early successional stage soils.

Tables and Figures

Table 3-1
Selected chemical and physical topsoil (0-7.5 cm) properties along a glacial chronosequence at the Robson Glacier, Mount Robson Provincial Park, British Columbia, Canada (TOC: total organic carbon; TC: total carbon). Stage 1 is located at the toe of the glacier while Stage 5 was deglaciated 99 years prior to sampling. The reference stand is a mature forest stand dominated by Engelmann spruce. Brackets indicate one standard deviation from the mean. Stage 1 (n=5), Stages 2-5 (n=6, 3 for yma and 3 for b or es), Reference Stand (n=3).

Stage	TC (%)	$\delta^{13}C$ (‰)	TOC (%)	N (%)	C/N*	pH	Texture
1	7.1 (0.2)	0.1 (0.2)	0.11 (0.03)	0.01 (0.01)	12 (2)	7.4 (0.3)	sand
2-b	7.1 (0.1)	0.1 (0.1)	0.07 (0.01)	0.01 (0.01)	8 (1)	7.5 (0.3)	loamy sand
2-yma	7.1 (0.1)	0.4 (0.7)	0.10 (0.01)	0.01 (0.01)	8 (1)	7.3 (0.2)	sand
3-b	8.0 (0.3)	0.5 (0.2)	0.15 (0.03)	0.01 (0.01)	15 (6)	7.1 (0.3)	sand
3-yma	8.3 (0.3)	-0.2 (0.2)	0.26 (0.10)	0.02 (0.01)	14 (2)	6.9 (0.6)	sand
4-yma	8.8 (0.2)	-1.2 (1.1)	0.58 (0.36)	0.05 (0.02)	12 (2)	7.4 (0.3)	sand
4-es	9.1 (0.6)	-3.6 (3.7)	1.37 (1.49)	0.12 (0.14)	12 (2)	7.4 (0.1)	sandy loam
5-yma	10.9 (0.6)	-8.3 (1.8)	2.42 (0.78)	0.21 (0.07)	12 (2)	7.1 (0.1)	loamy sand
5-es	11.8 (3.3)	-9.4 (6.0)	4.73 (4.23)	0.28 (0.22)	15 (4)	6.9 (0.6)	loamy sand
Reference Stand	44.4 (2.3)	-27.7 (1.0)	44.01 (1.78)	1.27 (0.14)	35 (5)	4.5 (0.9)	(organic)

b: sampled from bulk soil;
yma: sampled from the rhizosphere of yellow mountain avens;
es: sampled from the rhizosphere of Engelmann spruce;
* C/N calculated using TOC

Table 3-2

Spearman's rank correlations (ρ) for enzyme activity rate, respiration rate, microbial biomass and chemical properties of topsoil (0-7.5 cm) measured along a glacial chronosequence at the Robson Glacier, Mount Robson Provincial Park, British Columbia, Canada. (n=32). Correlations that are both strong (ρ > 0.70) and significant (α=0.05, with bonferroni correction 0.05/45=0.001) are bolded and denoted by *.

Correlation factor	Peroxidase	Phenol oxidase	β-glucosidase	Glucose	N-acetyl-glucosamine	Protocatechuic acid
Peroxidase	-	-	-	-	-	-
Phenol oxidase	0.11	-	-	-	-	-
β-glucosidase	0.51	0.42	-	-	-	-
Glucose	0.41	0.24	**0.77 ***	-	-	-
N-acetyl-glucosamine	0.16	0.19	0.61	0.62	-	-
Protocatechuic acid	0.45	0.33	0.65	0.59	0.59	-
TOC	0.53	0.28	**0.88 ***	**0.80 ***	0.66	0.67
N	0.48	0.33	**0.84 ***	**0.80 ***	**0.72 ***	**0.73 ***
C/N	0.67	0.20	0.55	0.55	0.25	0.45
pH	-0.27	-0.10	-0.45	-0.49	-0.23	-0.46
Microbial biomass	0.43	0.31	**0.88 ***	**0.78 ***	0.66	0.66

TOC: Total organic carbon;
TC: Total carbon

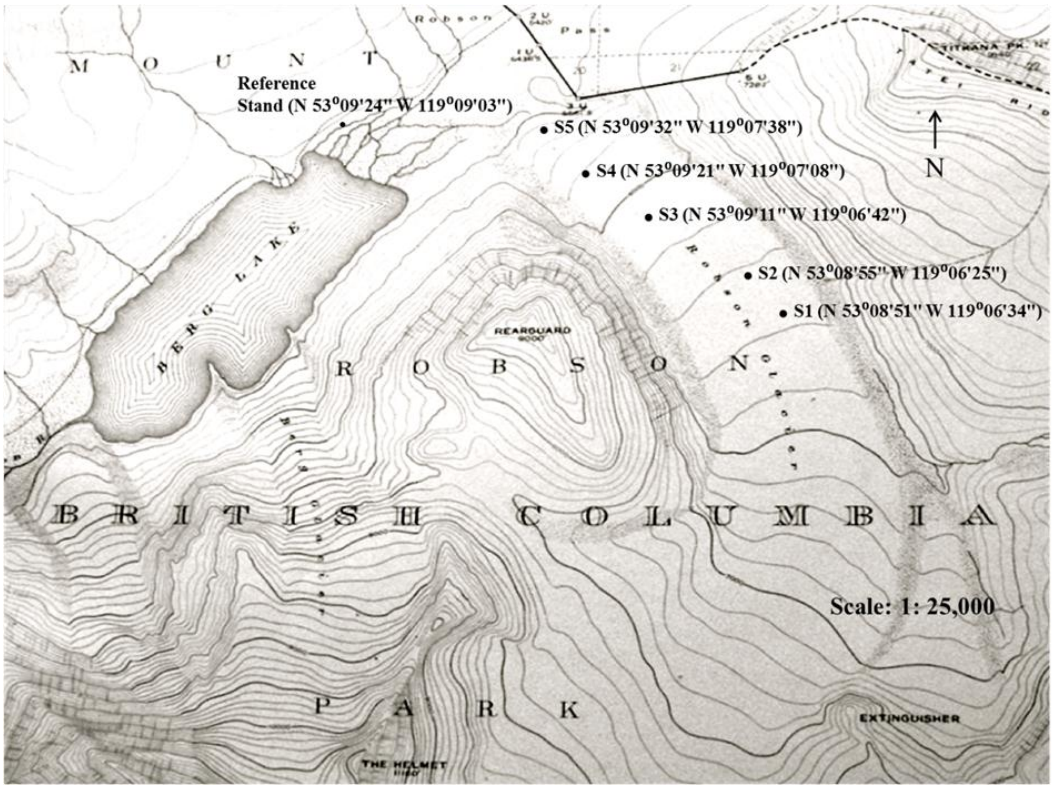

Figure 3-1 Schematic representation of the location of the five stages (S1-S5) and the reference stand sampled. (modified from UNBC GIS and remote sensing laboratory 2011).

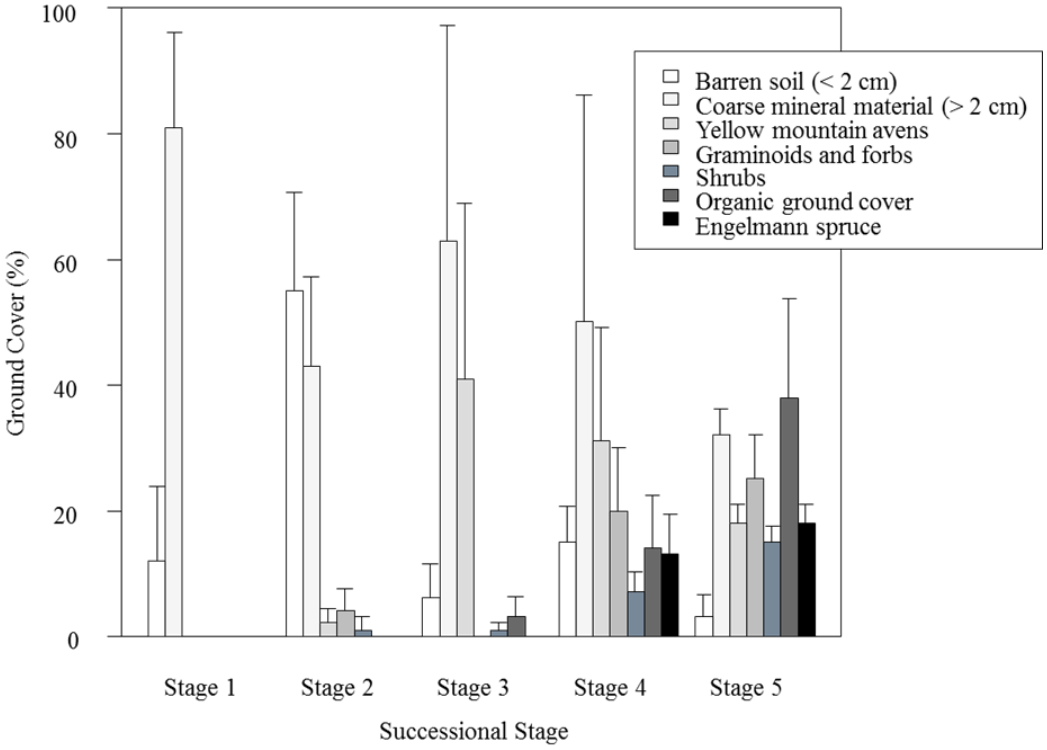

Figure 3-2 Percent cover of barren soil (> 2 mm); coarse mineral material (> 2 mm); organic ground cover; yellow mountain avens; graminoids and forbs; shrubs; and Engelmann spruce growing along a glacial chronosequence at Robson Glacier, Mount Robson Provincial Park, British Columbia, Canada. Stages are numbered with increasing time since deglaciation. Stage 1 was located at the toe of the glacier while Stage 5 was deglaciated 99 years prior to sampling. The reference stand is a mature forest stand dominated by Engelmann spruce. Error bars correspond to one standard deviation from the mean. Stage 1 (n=5), Stages 2-5 (n=6), Reference Stand (n=3).

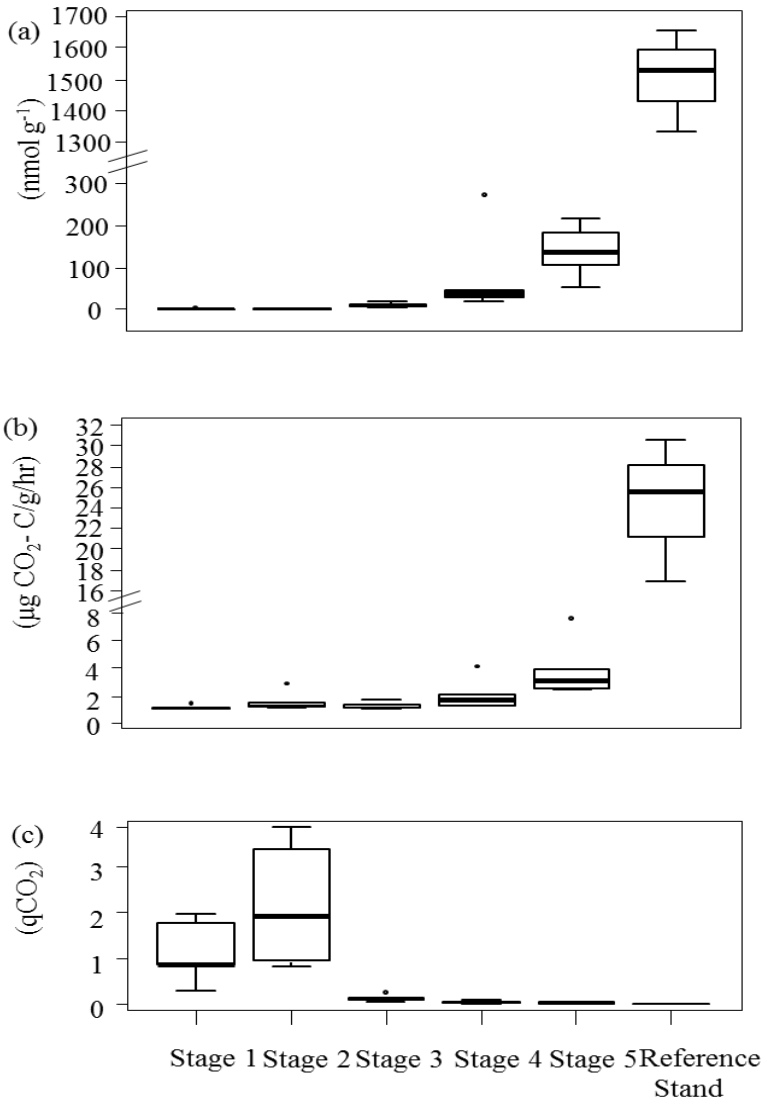

Figure 3-3 Total topsoil (0-7.5 cm) (a) total microbial biomass (plfa) (nmol gram^{-1}), (b) basal respiration (µg CO2- C/g/hr) and (c) metabolic quotient (qCO2) sampled along a glacial chronosequence at Robson Glacier, Mount Robson Provincial Park, British Columbia, Canada. Stages are numbered with increasing time since deglaciation. Stage 1 was located at the toe of the glacier while Stage 5 was deglaciated 99 years prior to sampling. The reference stand is a mature forest stand dominated by Engelmann spruce. The median value is displayed as the line through the box. The first quartile (Q1, 25%) and the third quartile (Q3, 75%) make up the box. The whiskers of the boxplot indicate that the range of the observations is within the whiskers at either end of the box with the exception of outliers (beyond 1.5*(Q3-Q1), which are represented as single points. Stage 1 (n=5), Stages 2-5 (n=6), Reference Stand (n=3).

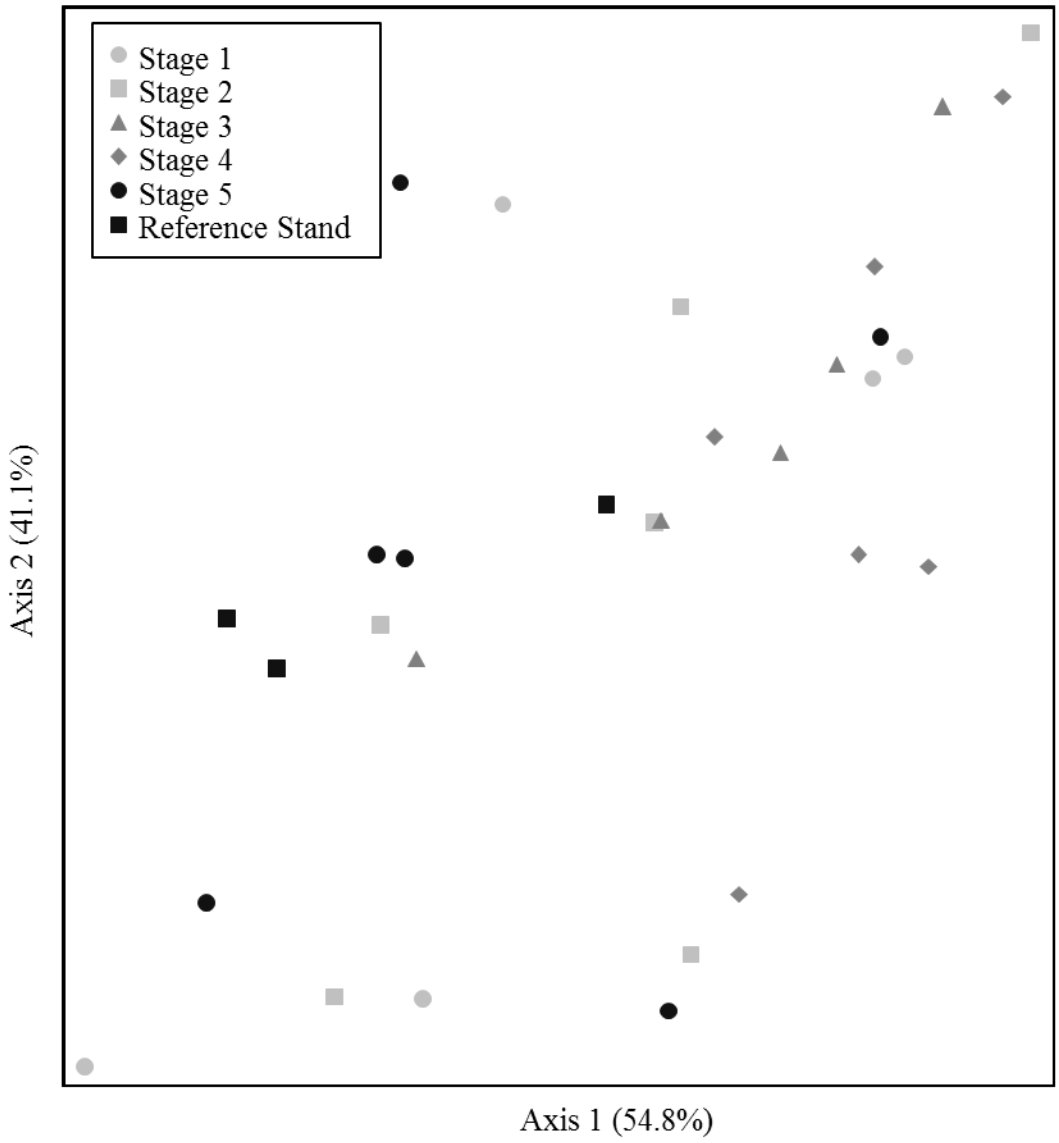

Figure 3-4 NMDS ordination of topsoil (0-7.5 cm) PLFA profiles sampled along a glacial chronosequence at Robson Glacier, Mount Robson Provincial Park, British Columbia, Canada . Stages are numbered with increasing time since deglaciation. Stage 1 was located at the toe of the glacier while Stage 5 was deglaciated 99 years prior to sampling. The reference stand is a mature forest stand dominated by Engelmann spruce.

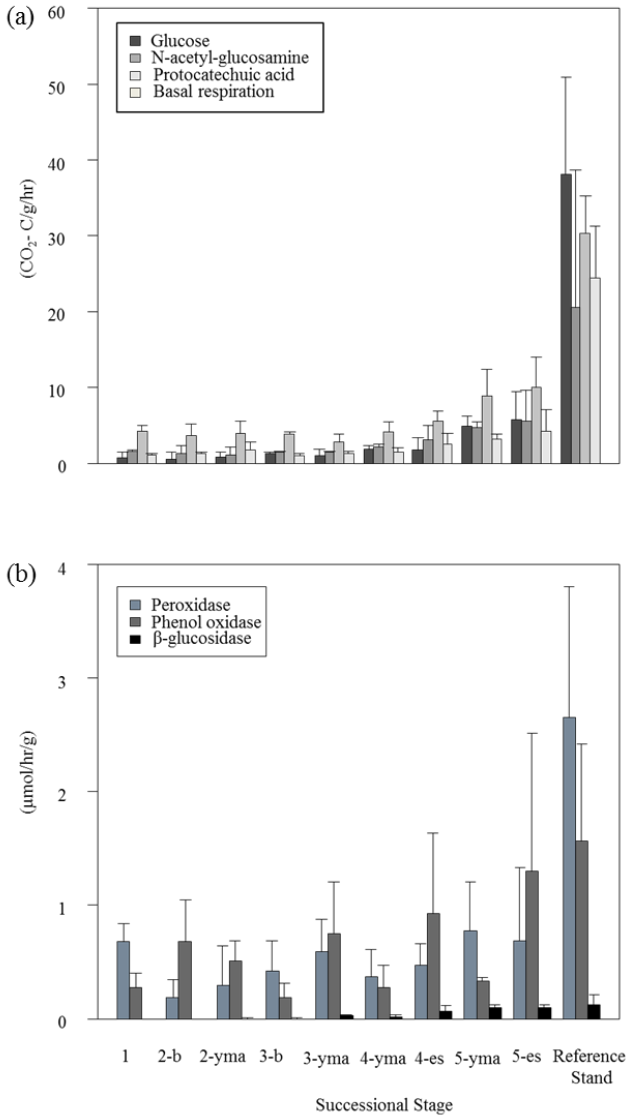

Figure 3-5 (a) Rate of substrate respiration of glucose, N-acetyl glucosamine, protocatechuic acid, and water using the MicroResp™ method (Campbell et el, 2003) and (b) rate of enzymatic activity of peroxidase, phenol oxidase and β-glucosidase by the soil microbial community found in topsoil (0-7.5 cm) sampled along a glacial chronosequence at Robson Glacier, Mount Robson Provincial Park, British Columbia, Canada. Stages are numbered with increasing time since deglaciation. Stage 1 was located at the toe of the glacier while Stage 5 was deglaciated 99 years prior to sampling. The reference stand is a mature forest stand dominated by Engelmann spruce. Brackets indicate standard deviation from the mean. Stage 1 (n=5), Stages 2-5 (n=6, 3 for yma and 3 for b or es), Reference Stand: (n=3).

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4.0 Chapter 4: Summary and Conclusions

4.1 Summary

The specific objectives of this research were:

1. To determine the long-term influence of two different organic amendments on microbial community development in reconstructed soils following open-pit mining in the Athabasca Oil Sands Region of north-eastern Alberta, Canada given that the application of forest floor materials to reclaimed areas has been shown to provide a source of propagules, and results in a greater native revegetation response compared to sites reclaimed with peat.

2. To assess the relative importance of abiotic conditions on the soil microbial community on reclaimed and natural forested sites, by manipulating soil moisture in a laboratory incubation experiment.

3. To assess soil microbial community succession and determine the influence of two plant species, yellow mountain avens (*Dryas drummondii* Rich.), and Engelmann spruce (*Picea engelmannii* Parry) on soil microbial community succession along a Canadian glacier chronosequence at Mount Robson Provincial Park.

4.1.1 Long-term influence of organic matter amendments on microbial community development and associated vegetation

Phospholipid fatty acid (PLFA) analysis was used to characterize soil microbial community composition in the top 7.5 cm of soils reconstructed using peat-mineral soil mix (PM) and forest floor mineral soil mix (FFM) as well as several natural reference stands over a 7 year monitoring period. Soil microbial biomass (total PLFAs) was found to increase with time since reclamation on all reclaimed sites. However, within the reclaimed plots, biomass remained at or below 50% of the values that were measured in 2004, 2009 and 2010 at the natural sites. Analysis of the complete 2004-2010 soil microbial community chronosequence showed that all years were significantly different from one another, yet years chronologically closer were not found to be more similar to one another in all cases. Consequently, time since reclamation was not the sole primary driver of soil microbial composition at these reclaimed sites. Soil microbial community composition of the reconstructed soils remained highly statistically different from the reference forest stands in all three years when the natural soil microbial community was assessed; i.e. in 2004, 2009 and 2010; however, differences in soil microbial community composition between both reclamation treatments and the natural reference sites decreased with time. Moreover, the soil microbial community associated with the FFM treatments appeared to be converging towards the natural soil microbial community more quickly than the plots associated with PM.

An assessment of the percent cover of graminoids, forbs, shrubs, trees, bare ground, litter and moss on both reclamation treatments at all reclaimed sites as well as at the two natural sites located on the Syncrude Canada Ltd. mine site was conducted. The natural sites possessed, on average, almost double the vegetative cover and number of trees per plot of any given reclaimed site. Quantitative differences between the two reclamation treatments (forest floor versus peat amendments) were apparent at each site in terms of vegetative cover and plant functional group distribution. Higher percent cover of trees, shrubs and forbs was found on plots reclaimed with the forest floor material (FFM) when compared to those reclaimed with the peat (PM). Sites reclaimed with FFM were thus more similar to the natural sites than those reclaimed with PM in terms of total plant cover and number of trees.

4.1.2 Importance of water content on the soil microbial community

Samples from sites reconstructed with FFM and PM as well as a natural reference site were incubated at 60%, 45%, 30% and 15% moisture (dry-weight gravimetric basis) for six months in order to assess the influence of moisture on soil microbial community composition. The initial hypotheses argued that because gravimetric soil moisture in the reconstructed soils (8-17%) was significantly lower than that of the undisturbed soils (60%), by increasing moisture, and hence lifting this potential limitation, the microbial communities on the reclaimed sites would become more similar to those found on the references stands. The results, however, showed a completely opposite trend, in that increasing soil moisture in

the laboratory experiment increased the difference between reclaimed and natural soil microbial community composition. Variation linked to differences in moisture levels was much higher for samples collected from the reconstructed soils than for those from the natural forest soil, although both the FFM and PM reconstructed soil microbial communities became less similar to the natural sites with increasing moisture, and hence exhibited the same response to moisture increases. Thus, moisture did not appear to be the principal driving factor for the differences found between the reclaimed and natural soil microbial communities.

4.1.3 Soil microbial community succession and the influence of yellow mountain avens and Engelmann spruce

Phospholipid fatty acid (PLFA), substrate-induced respiration (SIR) and enzyme activity rate analyses were used to assess soil microbial community succession along a 99-year glacial chronosequence. A nearby mature forest was included as a reference stand. Samples were taken from barren soil and from within the rhizosphere of yellow mountain avens and Engelmann spruce where possible. No difference in soil microbial community composition along the chronosequence or in the reference stand was detected. The data show that plant-microbe interactions change with successional stage. Plant-microbe interactions took 40 years since deglaciation to get established, as microbial biomass was not measurably influenced by yellow mountain avens in early successional stages, yet in the mid-successional stage, it doubled in the plant rhizosphere as compared to the bulk soil. Low β-glucosidase activity rates and glucose respiration rates in the early

successional stage soils indicated that the early successional stage soil microbial communities were not readily able to utilize glucose. Overall, increased microbial biomass, enzyme activity and substrate respiration along the chronosequence, as well as significant correlations between the microbial respiration of N-acetyl-glucosamine, protocatechuic acid, glucose and percent soil N indicated that the soil microbial community was responding to changes in the soil environment. In conclusion, despite large increases in microbial biomass (almost 10000%), β-glucosidase activity (8000%), phenol oxidase activity (200%), and respiration (SIR) (300%) between Stage 1 and Stage 5, microbial biomass was only 9% of that in the reference stand, respiration levels approximately 20% of that of reference stand and average enzymatic activity about 50% of that in the reference stand. Thus, soil microbial community succession is a process that continues beyond 100 years at the Robson glacier.

4.2 Project limitations and suggestions for future research

The research projects summarized above addressed soil microbial community development along successional chronosequences in reconstructed soils in the Athabasca Oil Sands Region and in the glacial retreat environment at Mount Robson Provincial Park. There are many avenues for continued research, including the isolation of individual abiotic factors such as temperature, aspect and slope, the determination of the direct effect of additional individual plant species on the soil microbial community and the use of different analytical

techniques to characterize the microbial communities. Some of these are briefly discussed below.

4.2.1 Isolation of abiotic factors

The three reclaimed sites used in the long-term monitoring of soil microbial community composition in the Athabasca Oil Sands Region were reclaimed several years apart (from 1998 to 2004) on sites with different aspects and slopes. Additionally, the depth to which the organic matter was placed differed among sites, as did the length of time during which the materials were stockpiled. This meant that a plethora of potential confounding factors existed and may have decreased the likelihood of finding differences in soil microbial community compositions among years and treatments. To address this, several sites or replicated plots should be created within the same year, using organic amendments placed at the same depth. If of interest, the influence slope and aspect could be investigated through replicating these factors in several plots.

As mentioned in previous chapters, increased soil moisture has been found to increase microbial respiration and microbial biomass nitrogen in soils reclaimed with both FFM and PM (McMillan 2007). The authors found that soils reclaimed with FFM had higher respiration rates and microbial biomass N than those reclaimed with PM; although, neither the rate of respiration nor microbial biomass N in either reconstructed soil ever reached the levels observed in the natural reference soil, which was kept at its original *in situ* moisture content (McMillan 2007). In this project, it was concluded that differences in the soil microbial

composition between the reclaimed sites and the natural forest stands were not due to a moisture limitation, nor was the composition of the microbial communities primarily driven by soil moisture. Thus, the effects of moisture on the functional ability and efficiency of the soil microbial community requires further investigation. Further, when the soil microbial communities of both the reconstructed and natural soils were assessed together, the natural soil (from the aspen-dominated natural forest stand) appeared more resistant to changes in moisture content than the reconstructed soils. However, the extent to which the soil microbial community in the undisturbed boreal forest can resist changes in soil moisture is yet unknown. Moreover, how the reconstructed and natural soil microbial communities in the Athabasca Oil Sands Region respond to changes in temperatures has yet to be investigated.

Laboratory to field extrapolations are often not possible due to the complexity of factors that can potentially influence soil microbial communities (Lazzaro et al. 2010). This means that unlike research on reclaimed sites where soil properties are constructed and can therefore be controlled, the influence of individual abiotic factors on soil microbial communities in glacial environments are more difficult to study. For example, while previous work has included the analysis of abiotic factors such as soil nutrients, pH and coarse fragments (< 2 mm) in studies of soil microbial succession (Kastovska et al. 2005; Mavris et al. 2010; Tscherko et al. 2005) and a few studies have related soil microbial communities to soil properties (Kastovska et al. 2005; Kastovska et al. 2007; Tscherko et al. 2005), the influence

of individual factors, as well as the effects of single climatic variables are thus far unknown.

4.2.2 The influence of individual plant species

Previous work has found microbial functional diversity to increase with plant development and organic matter accumulation (Tscherko et al. 2003). Similarly, microbial biomass and enzymatic activity in rhizosphere soil samples are typically higher than in samples taken from the bulk soil (Miniaci et al. 2007; Tscherko et al. 2004). However, while Bardgett and Walker (2004) reported that in soils deglaciated 12-15 years prior, yellow mountain avens had no effect on the soil microbial community. The results from this research indicate that in the mid-successional stage (approximately 60 years since deglaciation) yellow mountain avens had a measurable effect on microbial biomass. Similarly, *Poa alpina* L. has been found to influence soil microbial biomass only in mid and late successional stages (Tscherko et al. 2004). Here we propose that the delay in the rhizosphere influence of yellow mountain avens is due to the mid-successional stage onset of nitrogen fixation in the root nodules of this plant (Kohls et al. 1994) or perhaps to "the home field advantage" a theory that states that the soil microbial community adapts to the substrates they most frequently encounter, and thus become more efficient in their use of these substrates (Gholz et al. 2000). Previous work has suggested that the "harsh soil environment" is the primary driver of soil microbial communities in the pioneer stages of succession, and it is not until later successional stages, when more favourable soil conditions are reached, that the

plant rhizosphere can have a measurable influence on these communities (Tscherko et al. 2004). These competing explanations bring to light the need for more research into plant-microbial relationships through time and successional development.

In the Athabasca Oil Sands Region, vegetation has been found to differ between sites reclaimed with PM and those reclaimed with FFM. Specifically, the application of FFM to reclaimed areas has been shown to provide a source of propagules, and result in a greater native revegetation response compared to sites reclaimed with PM (Mackenzie and Naeth 2006). This research assessed whether or not the greater presence of native plants promotes soil microbial communities more similar to those on natural forest sites, however, the influence of individual species on the soil microbes in these areas has not yet been investigated. The identification of specific species that promote "natural" or target microbial communities could influence and improve planting and management of vegetation on reclaimed sites.

4.3 Implications of results for reclamation and future research

The use of FFM as a soil organic amendment in the Athabasca Oil Sands Region has been shown to be advantageous in terms of native upland forest vegetation establishment and survival when reclaiming sites (AMEC Earth and Environmental 2003; AMEC Earth and Environmental 2006; Brown et al. 2003; Mackenzie and Naeth 2006; Mapfumo 2003). This research indicates that the use of FFM also promoted the development of soil microbial communities more

similar to those found on natural forest stands than did the use of organic materials salvaged from surrounding peatlands. Hence, the similarities in plant communities found between the plots reclaimed with FFM and the natural reference stand, combined with "the home field advantage" (Gholz et al. 2000) may explain the accelerated development of the "natural", or target, soil microbial communities observed on the FFM reclaimed plots.

Glacier retreat environments provide unique opportunities in which to study primary ecological succession (Tscherko et al. 2003). Understanding more about primary succession and glacial environments has implications for both global warming and reclamation. The majority of information available about microbial succession in glacier retreat environments is based upon empirical evidence and inference. Thus, as soil microbial community successional patterns have been shown to differ among glacier forefields (Sigler et al. 2002; Tscherko et al. 2003), it is essential that the information pertaining to microbial succession come from numerous globally distributed environments, in order to capture the entirety of this intricate phenomenon. In this research project, the potential peroxidase and phenol oxidase enzymatic activities of soil microbial communities along a glacial chronosequence were quantified for the first time and hence added to the body of knowledge about this subject. Together phenol oxidase and peroxidase are used by soil microbes for the attainment of nutrients, ontogeny and defense and are involved with a variety of ecosystem processes such as lignin degradation, humification and carbon mineralization (Sinsabaugh 2010).Thus, the activity of these enzymes may be indicative of biogeochemical cycling and soil organic

matter formation. Further, the results from this project indicate that plant-microbe relationships change with successional stage, which suggests that future research into microbial succession in glacier environments should focus on plant-microbe interactions and their shifts and development through time.

4.4 Conclusions

In summary, the use of FFM promoted the development of soil microbial communities more similar to those found on natural forest stands than did the use of organic materials salvaged from surrounding peatlands. Additionally, the vegetation on the FFM reclaimed plots is more similar to the natural reference stand than is the vegetation on the PM reclaimed plots. Thus, the accelerated development of the "natural", or target, soil microbial communities observed on the plots reclaimed with FFM, may be linked to the similarities in plant communities found between these plots and the natural forest reference stand.

In conclusion, plant-microbe interactions change with successional stage as biomass was not measurably influenced by yellow mountain avens in early successional stages, yet in the mid-successional stage biomass doubled in the plant rhizosphere as compared to the bulk soil. Low β-glucosidase activity rates and glucose respiration rates in the early successional stage soils indicated that the early successional stage soil microbial communities were not readily able to utilize glucose. Overall, increased microbial biomass, enzyme activity and substrate respiration along the chronosequence, as well as significant correlations between the microbial respiration of N-acetyl-glucosamine, protocatechuic acid,

glucose and percent soil N indicated that the soil microbial community was responding to changes in the soil environment. However, soil microbial succession along the Robson glacier is a process that extends well beyond 100 years.

4.4.1 Linking early successional stage soils and soil reconstruction

Reclamation is done with the objective of "kick starting" or accelerating ecosystem development beyond that which would naturally occur. By studying the natural development of ecosystems or the succession of specific environmental components in undisturbed landscapes, the realization of the aforementioned reclamation goal can be assessed. By 2010, total microbial biomass on the three reclaimed sites in the Athabsaca Oil Sands Region was on average 24% of that found on the natural reference stand. Microbial biomass on Site 1 (reclaimed 12 years prior) reached 36% of that found on the natural reference stand. In contrast, during the first 20 years of succession, microbial biomass in soils sampled at Stage 1 at Mount Robson Provincial Park was only 0.01% of that in the reference stand. Further, after 99 years, biomass along the Mount Robson chronosequence was only 9% of that of the reference stand. Environmental differences in elevation, climate and climax vegetation may influence the rate of development; however, microbial biomass in the reclaimed soils appears to be recovering at rate much faster than that observed at the Robson glacier perhaps attesting to reclamation success.

Vegetation was identified as an important influencing factor in soil microbial development in both the reclaimed plots and the glacial chronosequence. I was able to observe that microbial biomass, respiration and enzymatic activity along the glacial chronosequence, and microbial biomass and composition in the reclaimed plots increased or became more similar to the reference stands as vegetation developed. Thus, soil microbial community development and succession may be strongly linked to the development and succession of vegetative communities. Either "the home field advantage", or more complex interactions between soil, plants, microbes and carbon resources may be responsible for relationship between microbial community development and vegetation development noted in this research. However, these theories, as well as the influence of individual plant species on the soil microbial community in both reclaimed and glacial landscapes require further research. Finally, as neither the vegetative communities nor the microbial communities on the reclaimed plots and along the glacial chronosequence became analogous to their respective reference stands, it may be that microbial succession is completed only once the climax plant community is present.

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Appendix A: Seasonal *versus* Annual Variation of Soil Microbial Community Composition

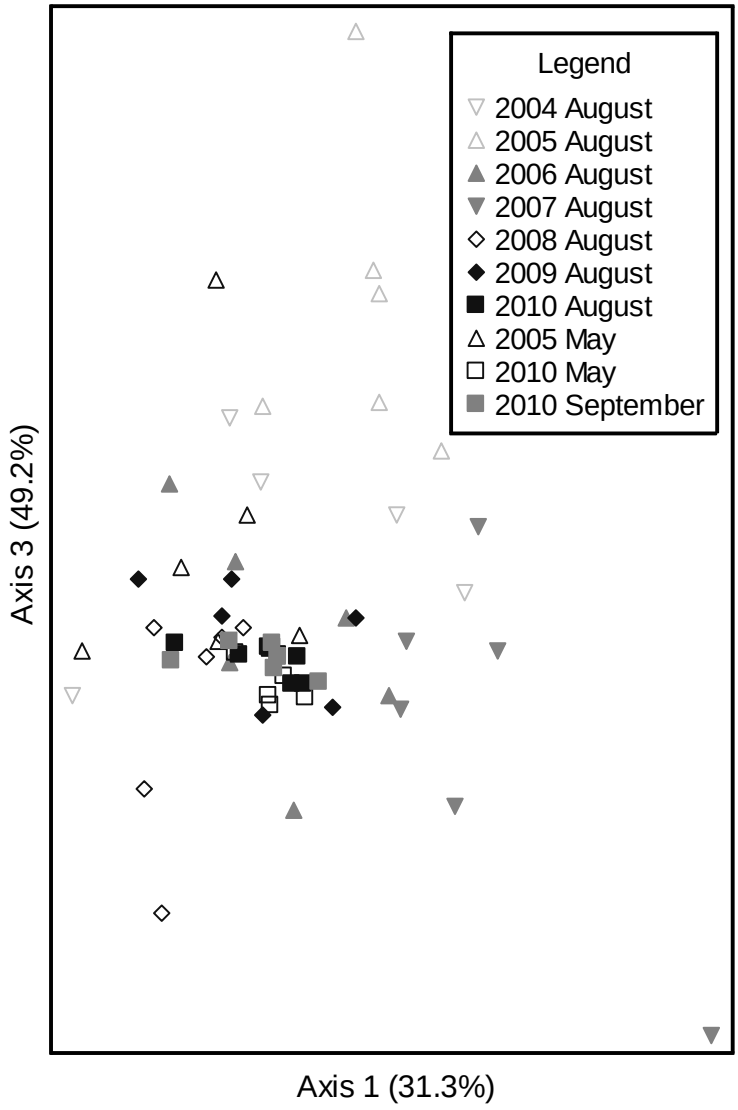

Figure A-1 NMDS ordination of seasonal versus annual variation of the soil microbial community composition as measured by phospholipid fatty acid analysis. Three-dimensional solution (axis 2 represents 21.5%) with a final stress of 11.3 after 114 iterations.