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Restoring Native Grassland Function in Urban Environment: Implications for Soil-Plant Relations

by

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Abstract

The area of rough fescue prairie has been reduced in Western Canada because of human activities including housing development. Urban development can impact natural ecosystems by eliminating native species and their habitat. Larch Park is an Edmonton residential development area to which land reclamation and restoration ecology have been applied in order to rebuild natural grassland instead of turf grasses. By using salvaged soil, planting native communities, and adding biochar as a fire surrogate to the soil, we expected ecosystem function and services in the reclaimed site to be more similar to the natural grassland site. A greenhouse study was also conducted to examine the effects of biochar and native species on soil processes. We examined ecosystem functions in the reclaimed and the natural grassland sites by measuring soil nitrogen availability using resin capsules, soil microbial biomass C and N by chloroform fumigation-extraction method, microbial respiration by alkali trap method, and microbial community structure with phospholipid fatty acid (PLFA) analysis. Disturbance followed by land reclamation at Larch Park caused drastic changes in soil processes. We found significant differences in soil properties including higher nitrogen availability, lower microbial biomass, and lower visual variability of microbial community structure in the reclaimed site compared to the natural grassland site. Greenhouse results showed stimulatory effects of native species on microbial biomass and respiration, and decreasing impact on nitrogen availability. The results also indicated that biochar had some significant interaction effect on soil-plant processes.

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Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

1.1 Overview of this study

In this study, land reclamation and restoration ecology principles have been applied in order to rebuild natural grassland instead of turf grass in an urban development area. By using salvaged soil, planting native communities with different reproduction abilities (sexual and vegetative), and adding biochar as a fire surrogate to the soil, we expected ecosystem function and services in the reclaimed site (Larch Park) to be more similar to a natural grassland site and require less maintenance such as watering, fertilization, and weed control. The level of similarity of a reclaimed site to a native grassland site, in terms of ecosystem functions and services, would determine the reclamation success.

In this chapter, native grassland will be described first and then interactions between plants, soil, and microorganism will be studied. Also plant reproduction processes will be reviewed followed by a discussion on fire and urbanization as natural and anthropogenic disturbances. Finally, the research questions and the objectives of this research will be put forward.

1.2 Native grassland

The largest vegetative province in North America is the native prairie (Sampson and Knopf 1994). Natural grasslands are the main habitat for many different organisms, including: song birds, small mammals, amphibians, insects, plants and microorganisms. These grasslands can also be considered as a traditional habitat for bison, deer and horses and they provide high quality forage

for wildlife and livestock (Bailey et al. 2010). Natural grasslands provide more ecological services and processes than high quality forage for ungulates. Ecological goods and services include essential services for living on earth such as plant photosynthesis which provides oxygen and energy for microorganism (Table 1- 1). Grassland topsoil is one of the most important carbon sinks, however the accumulation of carbon in grassland aboveground biomass is quite small compared to forest (Stypinski et al. 2006). Hypothetically grassland root production might provide more carbon to soil humus; therefore excess of CO_2 could be stored in the grassland roots, stolons and top soil in addition to the aboveground biomass (Stypinski et al. 2006). Therefore, the accumulation of organic matter in the Ah layer of grassland soils is described as carbon sequestration (Bailey et al. 2010).

The natural grasslands also provide a variety of ecological services including nitrogen fixation, oxygen production, biodiversity, water and watershed management, erosion management, and contaminants filtration (Bailey et al. 2010). These main ecological services are required for human societies to live on earth (Table 1- 1). Therefore, the reclamation of natural prairie grasslands is important (Bailey et al. 2010).

Alberta fescue prairie is divided into three ecoregion categories including Northern Fescue and Aspen Parkland Subregions, Montane Subregion, and Foothills Fescue Subregion. Northern Fescue and Aspen Parkland Subregions were conventionally dominated by *Festuca hallii* (Vasey) Piper (plains rough fescue) (Pavlick and Looman 1984, Sherritt 2012). Native rough fescue

grasslands currently occupy less than 10% of southern Alberta (roughly 11,000 km^2 of 112,000 km^2) (Adams et al. 2003, Bradley 2003, Sherritt 2012). *Festuca hallii* (plains rough fescue) grasslands are found primarily in central Alberta, Saskatchewan and southern Manitoba, and are associated with the development of black chernozemic soils (Holcroft Weerstra et al. 2003). Less than 5% of the original grassland dominated by *Festuca hallii* remains (Grilz et al. 1994).

Festuca hallii is a late-successional, long-lived perennial bunchgrass, with a rhizomatous growth form. *Festuca hallii* usually need three to four years for establishment (Anderson 2006). *Festuca hallii* is a cool season grass species adapted to short growing seasons (Anderson 2006). The area of rough fescue prairie has been reduced to remnants, because of human activities and land use practices (Holcroft Weerstra et al. 2003). Various human activities such as mining, pipelines, power lines, road construction, oil and gas exploration, urban development, and agricultural activities disturb natural grasslands and connected surface soil. Consequently, the reclamation of these disturbed sites is important (Bailey et al. 2010). It is also necessary to conserve the representatives of native vegetation communities for protecting the species and ecosystems biodiversity (Holcroft Weerstra et al. 2003).

1.3 Plant soil microorganism interactions

Western Canadian prairies soils are very young and developed over the last 10,000 years after the retreat of the last glaciation. Soil that has formed under natural prairies in cool temperate regions has high organic matter in the upper soil horizon (Ah). The natural grasslands soils are very fertile and they are called

Chernozems or if they have high sodium in the B horizon, they are called Solonets (Bailey et al. 2010). Grassland soil is the habitat of plant roots and microorganisms including bacteria and fungi. Soil microbial communities are main constituent of many ecosystem processes, thus the role of these communities has been studied extensively (Jackson et al. 2007, Strickland and Rousk 2010). Soil microbial communities are essential in determining soil organic matter turn over and biogeochemical cycles in soils (Card and Quideau 2010). Soil microorganisms have an important role in assisting soil formation, revegetation and soil organic matter transformation by acting as decomposers, N_2 fixer and nutrient recyclers. Soil microorganisms are very sensitive to environmental alteration; disturbance can cause significant degradation of the microbial community in terms of total biomass and species composition (Visser et al. 1983, Mummey et al. 2002).

Plants accumulate nutrients in their biomass and transfer them to the soil in the form of organic matter. Soil organic matter is an essential constituent of soil structure and is the main source of carbon for soil microorganisms (Bradshaw 2000). Consequently, soil microorganisms are responsible for decomposition of organic matter, from which they obtain carbon for building their biomass. Some carbon is sequestrated into the stabilized humic material and some nutrients are released for plant uptake as a result of organic matter consumption and mineralization (Bradshaw 2000, MacKenzie and Quideau 2010).

Plants require soil nutrients for their growth. Nitrogen is an essential, frequently limiting nutrient in terrestrial ecosystem (Vitousek and Melillo 1979,

Chapin et al. 1986). In terrestrial ecosystems, lack of nitrogen is the problem because atmospheric nitrogen is not plant available. However, disturbance can cause high nitrogen mineralization rates resulting in higher available nitrogen in disturbed lands compared to natural ecosystems.

Nutrient cycling includes localized interactions between plants and soil microorganisms. Plant roots can change nutrient dynamics of the rhizosphere by inputting large amount of carbon from root death (Chapin et al. 2002), and the root exudation of organic compounds into the soil (Chapin et al. 2002). Root exudation stimulates the bacterial growth by providing a labile carbon source; bacteria obtain their nitrogen by organic matter mineralization in the rhizosphere. This nitrogen turns into roots available nitrogen after protozoa grazed bacteria (Chapin et al. 2002).

All terrestrial ecosystems involve aboveground and belowground components, that have interaction to influence on one other and ecosystem processes and properties (Wardle et al. 2004). The interactions between plants and soil organisms are important in nutrient cycling and plant mineral nutrition (Richards 1987). A good review about the diversity of plant-microbes interactions in soil can be found in Reynolds et al.(2003).

Plants are responsible for providing the organic carbon needed for the decomposer subsystems and also the resources for obligate root-associated organisms such as symbiotic matualists and root herbivores (Wardle et al. 2004). The decomposers are responsible for breaking down the dead plant material and

also indirectly controlling plant growth and community composition by determining the available nutrients supply (Wardle et al. 2004).

Two microbial processes that could have main effects on plant community structure and dynamics are feedback dynamics between the plant-soil microorganisms and microbial mediation of niche differentiation in plant resources use (Reynolds et al. 2003). The theory for niche differentiation can be explained in this way: soil nutrients happen in different chemical forms, thus plants need different enzymes for having access to these nutrients and soil microorganisms are a major source of these enzymes (Reynolds et al. 2003).

The feedbacks dynamics between plant and soil microorganisms include positive and negative feedbacks. Soil communities play an essential role in plant-soil community interactions and also in plant community dynamics causing positive feedback on plant growth. Negative feedback plays an important role in plant community structures and also in grassland communities (Bever 2003). Negative feedback on plant growth and survival through their soil communities can be caused by an accumulation of particular pathogens (Burdon 1987). Accumulation of parasites, root herbivores and pathogens in rhizosphere can produce a negative feedback on plant growth, by eliminating carbon and nutrient directly from plant tissues and decreasing root uptake ability (Bever et al. 1997). In contrast, mycorrhizal fungi have a positive feedback on plant productivity by increasing access to limiting nutrients (Smith and Read 1997). Mycorrhizae are symbiotic relationships between plant roots and fungal hyphae, where the plant obtains nutrient from the fungus and in return it provides carbohydrate which is a

main carbon source for the fungus (Chapin et al. 2002). Therefore, the interaction between plants and soil organisms can be changed from mutually beneficial to pathogenic (Bever 2003).

Zak et al. (2003) reported that feedbacks between plant and microbial communities control ecosystem productivity and their results indicate that plant species richness is an essential factor effecting this biotic interaction. Their results indicate that microbial community composition and function have been altered by greater plant diversity (Zak et al. 2003).

1.4 Plant community recruitment and reproduction

Plants can regenerate both sexually and vegetatively. Sexual regeneration can happen by seed at specific times of year, while vegetative regeneration is a form of asexual reproduction in plants and it can happen when plants produce new shoots along rhizomes belowground or stolon aboveground. The vegetative reproduction drives the annual regeneration and conservation of plant community composition and aboveground plant populations on tall grass prairie rather than recruitment from seeds. In established prairie more than 99% of aboveground shoots were reproduced vegetatively (Benson and Hartnett 2006).

The underground competition for soil resources including water and mineral nutrients is usually stronger than aboveground competition for light (Casper and Jackson 1997, Cahill 2003). Root biomass can affect underground competition. Within a plant with higher root densities, competition can be higher. Belowground competition can impact individual plant growth and it is usually different among species (Cahill 2003). Belowground competition may be stronger

than aboveground competition in the native grassland site due to the vigorous development of grassland roots, while in the reclaimed site plant roots are currently developing and are not well established. Also, in rhizomatous community due to existence of more roots and rhizomes, competition may be higher than non-rhizomatous species.

Plant community composition has great effects on the community compositions of root-associated organisms (Yeates 1999). Individual plant species could have essential impacts on the soil biota components and plant-controlled processes, because plant species return resources with different quality and quantity into the soil. For instance, the microbial community composition around the roots of grassland species is different (Bardgett et al. 1999), which is helpful to describe why soil planted with different grass species can support different abundances of soil microbes and microbe-feeding fauna (Griffiths et al. 1992).

In microsite with soil disturbances, successful reproduction from seed may be more common (Platt 1975). Increasing soil disturbance resulted in decreasing the number and proportion of vegetative regenerated species, but no significant impact on species with no-vegetative reproduction. By increasing the frequency of disturbance, the abundance of seed reproducers increases compared with vegetative reproducers (McIntyre et al. 1995). Also seed regeneration could be more essential for weedy species for covering bare soil after disturbance.

All the grassland ecosystem components mentioned above including plant, soil micro-organisms and their interactions can be affected by disturbance, which will be discussed in the following sections.

1.5 Disturbance and plant community dynamics

Disturbance is a temporary alteration of ecosystem function, physical environment, and soil biological processes (Pickett 1985). It can be mediated by natural or anthropogenic processes. Natural disturbances such as fire and flooding are generally influenced by weather condition, climate, and location (Pickett 1985, Virginia H. Dale 2001). These conditions usually happen in a cyclic pattern and disturbances may be periodic with a specific time interval. However, disturbances caused by human activities such as harvesting, mining, and housing development can take place everywhere and they are not essentially following cyclic pattern (Pickett 1985, Rogers 1996, Virginia H. Dale 2001).

The role of natural disturbance in conserving species diversity is an important principle in ecology and recently the maintenance of proper disturbance regimes has become accepted as a practical approach in conservation biology (Harrison et al. 2003). Disturbance (natural or anthropogenic) was conventionally viewed as an incident that introduced primary or secondary succession (Johnson and Miyanishi 2007). Succession is the series of community changes that happen over time where plant communities substitute each other in sequence until a stable community is reached. Succession also describes the vegetation development in the absence of disturbance. A classic example of primary succession is that on glacial till left by the retreating glacier at Glacier Bay, Alaska (Johnson and Miyanishi 2007). In other words, when disturbance is very severe that there is no biological legacy (plant roots, seed, propagules and soil organic matter), the recovery process is called primary succession. On the other hand, the secondary

succession refers to the recovery process in which there is a considerable biological legacy after disturbance (Walker 2011).

Biotic succession can increase the number of plant and animal species after a disturbance in natural ecosystems (McKinney 2002). For example, natural primary succession on disturbed land includes a series of identifiable processes including primarily biological and primary physical processes (Bradshaw 2000). These are listed separately in Table 1- 2. The biological processes start with plants arrival and their establishment and creation of biological active soil development. These processes are helped by physical changes, mostly as a result of weathering of the initial mineral materials (Bradshaw 2000). Using natural processes for restoration will take decades or centuries (Table 1- 2); restoration of advanced communities could take thousand years or more, but this long time period can be overcome by artificial intervention, and if they use or mimic natural processes they may be very successful. So these intervention processes are the ecological restoration principle (Dobson et al. 1997).

As a natural disturbance, fire can cause alteration in physical and chemical properties of soils, vegetation dynamics and soil microorganisms, which is discussed in the following section.

1.5.1 Fire - natural disturbance

Fire is one of the most important agents of natural disturbance in western ecosystems, with the ability to change successional organization. Fire causes change in physicochemical and biological environment by heating and oxidation, and also creates new abiotic substrate for soil including charcoal (Hart et al.

2005). Reduced plant density post-fire can enhance solar penetration and soil temperature; therefore the soil microclimate can be changed (Neary et al. 1999, Hart et al. 2005). Fire increases soil organic matter oxidation, consequently changing its chemical composition (Fernandez et al. 1997), but the level of organic matter oxidation relies on fire temperature, fire duration and heat penetration (Hungerford et al. 1991).

Fire also has variable effects on grasslands. Grassland topsoil layer is one of the most important carbon sink on earth, due to high accumulation of organic matter (Bailey et al. 2010). Fire infrequently consumes the sequestered carbon in the grasslands because of high organic matter content. By contrast, during crown fire in forests, the aboveground carbon sink releases a lot of carbon into the atmosphere (Bailey et al. 2010). Usual responses to the fire include a flush of forb growth, flowering and temporarily increase in overall productivity as the litter removal increases the availability of space, light and nutrients (Harrison et al. 2003).

Main residue of fire is charcoal, charcoal is generated during natural fire in forest and prairie environments through the partial combustion of organic materials (Preston and Schmidt 2006). Charred carbon is an important component of Black Chernozemic soils in the native grasslands of the prairie region where it has been shown to contribute up to 45 % of the total carbon (Ponomarenko and Anderson 2001). Incidence of fires under cool and moist conditions can increase accumulation of char in prairie soils (Ponomarenko and Anderson 2001). Man-made charcoal is called biochar. Biochar is the carbon-rich solid created by

thermal degradation of organic materials in the absence of oxygen (pyrolysis) (Lehmann et al. 2011). Pyrogenic C is generally created as solid charred residues and its structures vary as a continuum from partially charred plant materials to charcoal, volatile soot and eventually graphite (Preston and Schmidt 2006).

The addition of carbon amendment to soils has been considered as a restoration technique to mitigate the negative impacts of anthropogenic nitrogen enrichment and reduce exotic species invasion (Morgan and Seastedt 1999, Spiegelberger et al. 2009). Therefore, there was some interests in using saw dust in grassland ecosystem as a potential tool to reduce nutrient availability and aboveground productivity (Morgan and Seastedt 1999, Spiegelberger et al. 2009). Saw dust is an inexpensive carbon source that can help counter invasion by non-native plants (Alpert and Maron 2000). Some studies, however, showed that the addition of carbon promoted N immobilization at the early stages of application. But this impact was short term, lasted only a couple of months and declined by the end of season (Morgan and Seastedt 1999, Bleier and Jackson 2007). Saw dust is also subject to more rapid microbial degradation, while charcoal or black carbon is chemically and biologically stable because of its polycyclic aromatic structure. Thus charcoal can be persisting in the environment for centuries (see Preston and Schmidt 2006 for a complete review). Oxidation throughout formation of charcoal creates carboxylic groups on the boundaries of the aromatic structure, consequently enhances the nutrient and water holding capacities of charcoal (Glaser et al. 2002). Biochar has the potential to affect soil fertility and possibly mitigate climate change through carbon sequestration (Woolf et al. 2010,

Lehmann et al. 2011). The importance of using biochar to mitigate climate change is based on its relative recalcitrance against microbial decay and its slower return of terrestrial organic C in form of CO_2 to the atmosphere (Lehmann 2007).

Biochar addition to soil can reduce soil bulk density, improve nutrient retention through cation adsorption (Lehmann et al. 2011), also change soil biological community composition and abundance (Lehmann et al. 2011). Nutrient availability can be affected by changes in cation exchange capacity (CEC), water holding capacity (WHC) and pH (Anderson et al. 2011). Biochar has been also reported to develop root growth by improving the chemical and physical characteristics of soil such as nutrient, pH, aeration and water holding capacity. Biochar has important impacts on microbial mediated nutrients transformation in soil (Lehmann et al. 2011). By adding biochar to the soil, microbial community structure and function might be shifted by altering physicochemical properties of the soil (Smith et al. 2010). Depending on biochar type, it may sometimes stimulate microbial activity and increase their abundance (Steiner et al. 2008). Steiner et al. (2008) indicated that biomass derived charcoal increases soil microbial biomass, growth and activity.

In addition to the natural disturbances, anthropogenic ones can also change the soils properties. In the next section, urbanization as an anthropogenic disturbance is described.

1.5.2 Urbanization - anthropogenic disturbance

Urban development is one of the human activities that cause habitat loss; it impacts natural ecosystems by eliminating the majority of native species and their

habitat. Urbanization is usually longer lasting than other kinds of habitat loss such as farming and logging (McKinney 2002). Urban development causes substituting the lost native species with wide-spread weedy non-native species. Urban-gradient studies indicate that, the number of non-native species for many taxa including plants, birds and butterflies increases near urban core, while the number of native species decreases. Much of the reduction in number of species (species richness) is apparently affected by the loss of vegetation (McKinney 2002).

Developers usually remove most vegetation and topsoil before constructing residential buildings to provide ready access for equipment to the construction sites and also to reduce the costs. Using heavy construction equipment has negative impacts on soil physical properties including soil compaction, reduced water infiltration and root growth. Therefore, active development areas tend to have low biodiversity and wildlife habitat, as habitats for almost all species are eliminated by paving some of the area and removing the total vegetated area. Consequently, the ecosystem function and services of the developed area will be less than that of the natural site (McKinney 2002, Gregory et al. 2006, Pitt et al. 2008). Therefore, soil development and plant reproduction in the developed area will take thousands of years. Rebuilding green spaces inside development borders can be considered as a land reclamation practice (Figure 1- 1).

In addition, the developed area requires high maintenance and weed control because the species planted after construction are usually made up of alien or horticultural varieties. Disturbance followed by reclamation at developed areas also causes extreme changes in soil processes including higher levels of nutrient

availability and lower microbial biomass in reclaimed sites compared to natural areas (McMillan et al. 2007).

Given that urban areas are expanding, developers should find techniques to preserve biodiversity by either modifying natural habitat or trying to restore it. The most effective conservation efforts would concentrate on protecting remnant natural habitat as much as possible (McKinney 2002). Studying the impacts of urban expansion on native ecosystems can help conservation practices in two main ways. One is by using ecological principles to reduce the effects of urban development on native ecosystems. For example conserving remnant natural habitat and re-establishing modified habitats in order to help conservation of native species. A second one is by assisting to improve a more ecologically educated and well informed public (McKinney 2002).

Restoration ecology is a young science that offers the scientific and practical frameworks to guide management and repair damaged ecosystems. Ecological restoration practice is progressively becoming an essential tool for human efforts to manage and repair the increasing environmental damage in ecosystems (Dobson et al. 1997, Young 2000, Hobbs and Cramer 2008). Strategic restoration efforts may reduce the effects of urban expansion on native ecosystem by protecting natural habitat and re-establishing modified habitat. In order to enhance native biodiversity in managed habitats, one restoration strategy is cultivation with native plant species, which will benefit native plant and animal populations (McKinney 2002).

Restoring native plant communities in urban development area is a novel practice. By using salvaged soil, planting native communities and adding biochar as a fire surrogate, this study has tried to re-establish ecosystem processes similar to those of natural sites in as short time as possible. In reclamation and restoration projects the principles for judging "success" is usually limited to distinct visual aboveground indicators such as plant diversity, coverage and wildlife use (Mummey et al. 2002, Card and Quideau 2010). Therefore, the reclamation success in this research depends on the level of similarity of the reclaimed site to the native grassland site, in terms of ecosystem functions and services.

1.6 Research questions and objectives

1) If we salvage soils and use native plants communities, will the soil processes created in a reclaimed environment be more like those of a native prairie ecosystem?
    a. The first objective was to determine how nitrogen availability changes in reclaimed and native grassland sites along transects and under one individual species (*Festuca hallii*).
    b. The second objective was to characterize soil microbial community function and structure along transects and under *Festuca hallii* at reclaimed site and compare these to benchmark properties in a native community.
2) Do additions of biochar to natural grassland and reclaimed soils influence the soil-plant processes and microbial dynamics under greenhouse conditions?

a. The first objective was to determine soil nitrogen availability with and without biochar.
b. The second objective was to measure soil microbial activity with and without biochar.

3) Do soil disturbance and biochar addition affect soil processes in reclaimed and natural grassland sites?
    a. The first objective was to measure nitrogen availability in disturbed plots with and without biochar.
    b. The second objective was to characterize soil microbial community function and structure in disturbed plots with and without biochar.

Tables and Figures

Table 1- 1: List of ecosystem processes, functions, and services

Ecosystem processes	Ecosystem functions	Ecosystem services
Nutrient cycling (C and N)	Plant photosynthesis	Carbon sequestration
Water cycling	Water filtration	Oxygen production
Organic matter decomposition		Water and air purification
Nitrogen mineralization		Nitrogen fixation
Microbial respiration		Detoxification of wastes

Table 1- 2: The time scales for biological and physical processes involved in the development of ecosystems on a newly produced bare area (Dobson et al. 1997, Bradshaw 2000).

Biological processes		Physical processes	
Time (year)	Process	Time (year)	Process
1–50	1. Immigration of appropriate plant species	1–100	1. Break up of compacted surfaces by frost or periodic drought
1–50	2. Establishment of appropriate plant species	1–1000	2. Accumulation of fine material by rock weathering
1–10	3. Surface stabilization and accumulation of fine mineral materials by plants	1–1000	3. Decomposition of soil minerals by weathering
1–100	4. Accumulation of nutrients by plants from soil minerals	1–100	4. Improvements of soil available water capacity
1–100	5. Accumulation of nitrogen by biological fixation and from atmospheric inputs	1–1000	5. Release of mineral nutrients from soil minerals
1–20	6. Immigration of soil flora and fauna supported by accumulating organic matter	10–10000	6. Leaching of mobile materials from surface to lower layers
1–20	7. Changes in soil structure and function due to plant, soil micro-organism and animal activities	100–10000	7. Formation of distinctive horizons in the soil profile
10–1000	8. Reduction in toxicities by accumulation of organic matter and leaching		

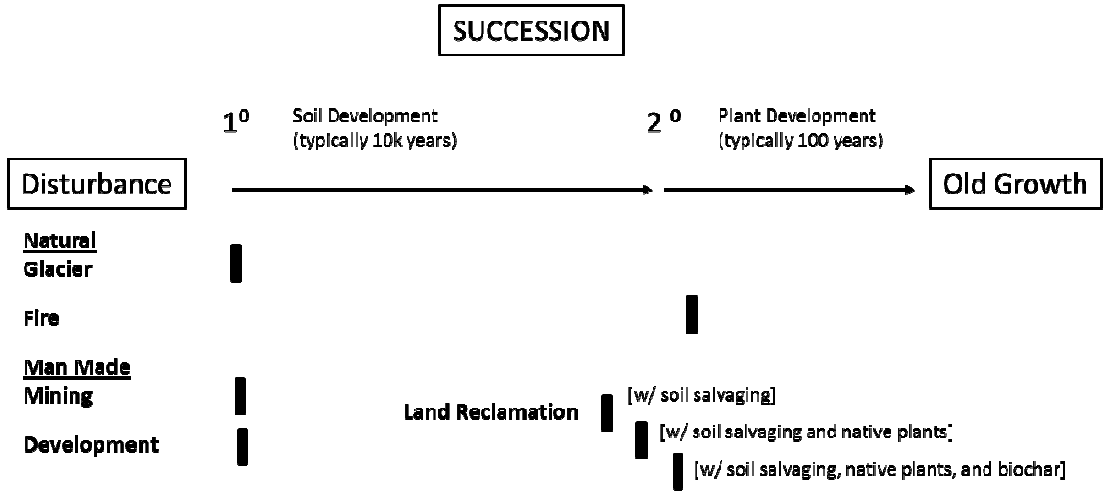

Figure 1- 1: Schematic diagram outlining the effect of different kinds of disturbance on soil and plant succession, along with theoretical ways of jump starting secondary succession with land reclamation.

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Chapter 2: Land Reclamation Following Urban Development: Effects on Nitrogen Availability and Microbial Dynamics

2.1 Introduction

Alberta fescue prairie is separated into three ecoregion categories including Northern Fescue and Aspen Parkland Subregions, Montane Subregion, and Foothills Fescue Subregion. Northern Fescue and Aspen Parkland Subregions were conventionally dominated by *Festuca hallii* (Vasey) Piper (plains rough fescue) (Pavlick and Looman 1984, Sherritt 2012). *Festuca hallii* (plains rough fescue) is a C_3 grass primarily found in central Alberta, Saskatchewan and southern Manitoba Aspen Parkland ecoregion (Pavlick and Looman 1984). *Festuca hallii* (plains rough fescue) grasslands are associated with the development of black chernozemic soils (Holcroft Weerstra et al. 2003). Less than 5% of the original grassland dominated by *Festuca hallii* remains (Grilz et al. 1994). The area of rough fescue prairie has been reduced because of various human activities such as mining, pipelines, power lines, road construction, oil and gas exploration, urban development and agricultural activities (Holcroft Weerstra et al. 2003, Bailey et al. 2010).

Urban development is an anthropogenic disturbance on the same order of magnitude as a glacier as it involves the removal of soils, aboveground biomass and surface geologic materials. It influences natural ecosystems by eliminating the majority of native species and their habitat. Urbanization is usually longer lasting than other kinds of habitat loss such as farming and logging (McKinney

2002). Urban development usually results in the replacing of lost native species with wide-spread weedy non-native species. Urban-gradient studies show that, the number of non-native species for many taxa including plants, birds and butterflies increases near urban core, while the number of native species decreases. Much of the reduction in number of species (species richness) is apparently affected by the loss of vegetation (McKinney 2002).

Developers usually remove most vegetation and topsoil before constructing residential buildings to provide ready access for equipment to the construction sites and also to reduce the costs. Using heavy construction equipment has negative impacts on soil physical properties including soil compaction, reduced water infiltration and root growth. Therefore, active development areas tend to have low biodiversity and wildlife habitat, as habitats for almost all species are eliminated by paving some of the area and removing the total vegetated area. Consequently, the ecosystem function and services of the developed area will be less than that of the natural area (McKinney 2002, Gregory et al. 2006, Pitt et al. 2008). Developed area also needs high maintenance and weed control because the species planted after construction are usually made up of alien or horticultural varieties. Disturbance followed by reclamation at developed areas also causes extreme changes in soil processes including higher levels of nutrient availability and lower microbial biomass in reclaimed sites compared to natural areas (McMillan et al. 2007).

Given that urban areas are expanding, developers should find techniques to preserve biodiversity by either modifying natural habitat or trying to restore it.

The most effective conservation efforts would concentrate on protecting remnant natural habitat as much as possible (McKinney 2002). Studying the effects of urban expansion on native ecosystems can help conservation practices in two main ways. One is by using ecological principles to reduce the effects of urban development on native ecosystems. For instance conserving remnant natural habitat and re-establishing modified habitats in order to help conservation of native species. A second one is by assisting to develop a more ecologically educated and well informed public (McKinney 2002).

Restoration ecology is a young science that offers the scientific and practical frameworks to guide management and repair damaged ecosystems. Ecological restoration practice is progressively becoming an essential tool for human efforts to manage and repair the increasing environmental damage in ecosystems (Dobson et al. 1997, Young 2000, Hobbs and Cramer 2008). Strategic restoration efforts may reduce the effects of urban expansion on native ecosystem by protecting natural habitat and re-establishing modified habitat. In order to enhance native biodiversity in managed habitats, one restoration strategy is cultivation with native plant species, which will benefit native plant and animal populations (McKinney 2002).

By using salvaged soil and planting native communities, this research project has tried to re-establish ecosystem processes similar to those of natural sites in as short time as possible. In reclamation and restoration projects the principles for judging "success" is usually limited to distinct visual aboveground indicators such as plant diversity, coverage, and wildlife use (Mummey et al.

2002, Card and Quideau 2010). Therefore, the reclamation success in this research depends on the level of similarity of the reclaimed site to the native grassland site in terms of ecosystem functions and services.

In this study, ecosystem processes can be characterized by measuring microbial community dynamics and nitrogen availability in natural and reclaimed sites. Soil microbial communities are main constituent of many ecosystem processes, thus the role of these communities has been studied extensively (Jackson et al. 2007, Strickland and Rousk 2010). Soil microbial community is essential in determining soil organic matter turn over and biogeochemical cycles in soils (Card and Quideau 2010). Soil microorganisms have an important role in assisting soil formation, revegatation and soil organic matter transformation by acting as decomposers, N_2 fixer, and nutrient recyclers. Soil microorganisms are very sensitive to environmental alteration; therefore disturbance can cause significant degradation of the microbial community in terms of total biomass and species composition (Visser et al. 1983, Mummey et al. 2002).

Plants accumulate nutrients in their biomass and transfer them to the soil in the form of organic matter. Soil organic matter is an essential constituent of soil structure and is the main source of carbon for soil microorganisms (Bradshaw 2000). Consequently, soil microorganisms are responsible for decomposing soil organic matter, from which they obtain carbon for building their biomass. Some carbon is sequestrated into the stabilized humic material and some nutrients are released for plant uptake as a result of organic matter consumption and mineralization (Bradshaw 2000, MacKenzie and Quideau 2010).

Plants require soil nutrients for their growth. Nitrogen is an essential, frequently limiting nutrient in terrestrial ecosystems (Vitousek and Melillo 1979, Chapin et al. 1986). However disturbance can cause high nitrogen mineralization rates resulting in higher available nitrogen in disturbed lands compared to natural ecosystems. Nutrient cycling and plant mineral nutrition can be affected by the plants-soil microorganisms interactions (Richards 1987).

In this study, native plant communities with different regeneration abilities (sexual and vegetative) were planted at the reclaimed site to stimulate the soil microorganisms-plants connection. Sexual regeneration can happen by seed at specific times of year, while vegetative regeneration is a form of asexual reproduction in plants and it can happen when plants produce new shoots along rhizomes belowground or stolon aboveground. The vegetative reproduction drives the annual regeneration and conservation of plant community composition and aboveground plant populations on tall grass prairie rather than recruitment from seeds. In established prairie more than 99% of aboveground shoots were reproduced vegetatively (Benson and Hartnett 2006).

Disturbance was conventionally viewed as an incident that introduced primary or secondary succession (Johnson and Miyanishi 2007). When disturbance is very severe that there is no biological legacy (plant roots, seed, propagules and soil organic matter), the recovery process is called primary succession. On the other hand, the secondary succession refers to the recovery process in which there is a considerable biological legacy after disturbance (Walker 2011). Therefore, in microsite with soil disturbances, successful

reproduction from seed may be more common (Platt 1975). Increasing soil disturbance resulted in decreasing the number and proportion of vegetative regenerated species, but no significant impact on species with no-vegetative reproduction. By increasing the frequency of disturbance, the abundance of seed reproducers increases compared with vegetative reproducers (McIntyre et al. 1995). Also seed regeneration could be more essential for weedy species for covering bare soil after disturbance.

2.2 Research questions and objectives

If we salvage soils and use native plants communities, will the soil processes created in a reclaimed environment be more like those of a native prairie ecosystem?

1. The first objective was to determine how nitrogen availability changes in reclaimed and native grassland sites along transects and under one individual species (*Festuca hallii)*.
2. The second objective was to characterize soil microbial community function and structure along transects and under *Festuca hallii* at reclaimed site and compare these to benchmark properties in a native community.

2.3 Materials and methods

2.3.1 Study area

The Larch Park Storm Water Management Facility (SWMF) is an 8,800 m^2 urban development site and was the focus of this study. It was the first native

ecosystem SWMF developed in Edmonton and as such is unique. Both wetland (1200m^2) and terrestrial (6600m^2) areas were rebuilt with salvaged soils and native plant species to emulate Rough Fescue Prairie, Aspen Parkland and the urban wetlands nearby. Over 77 graminoid, forb, shrub and tree species were planted across the site based on different environmental conditions and representing the complexity of healthy native ecosystems. Before development, the site was degraded agricultural land that had been tilled for the last four years, but had not had a crop. Its seed bank was mostly agricultural weeds, mainly wild oat, thistles and canola. Larch Park is located in southwest Edmonton, next to the Magrath neighborhood and Blackmud Creek Ravine.

Larch Park was compared to a native rough fescue grassland to have a measurement of reclamation success. The native grassland site was located at the University of Alberta Research Ranch, near Kinsella, Alberta (53.09º N, 111.55º W). Kinsella ranch has lots of *Festuca hallii* grasslands, which are considered as a main historical vegetation in the Aspen Parkland natural ecoregion in Alberta (Sims and Risser 2000, Lamb et al. 2007, Attaeian 2010), a savanna type ecosystem with a combination of trembling aspen stands (*Populus tremuloides*) and rough fescue prairie (*Festuca hallii*) (Lamb et al. 2007, Shore 2009). These natural grasslands can be classified as part of the northern fescue natural subregion and they have quite high plant diversity which can discriminate this site from other grasslands of this area (Natural Regions Committee 2006, Attaeian 2010). In this site 72% of plant biomass is dominated by grasses and 70% of plant species diversity is dominated by forbs (Coupe et al. 2009, Shore 2009, Attaeian

2010). Main grass species included *Festuca hallii*, *Hesperostipa curtiseta*, *Poa pratensis*, and *Koeleria macrantha* (Shore 2009). Common forbs included *Achillea millefolium*, *Solidago missouriensis*, *Artemisia frigida*, and *Comandra umbellata* (Shore 2009). Soils at the site are classified as orthic black chernozems or grassland soils with thick organic matter enriched surface horizons, over glacial till. Parent geologic materials consist of cretaceous sediments, non-marine sandstone, composed of marine shales, and mudstones (Natural Regions Committee 2006, Attaeian 2010). Soil texture is sandy clay loam in the upper 5 cm and loam to sandy loam below (Howitt 1988, Naeth et al. 1990, Soil Classification Working Group 1998). The area has a continental climate with dry sub-humid condition. The mean annual temperature is 2.4ºC and the mean annual precipitation is 431.3 mm (Attaeian 2010). The climate in the Northern Fescue Natural Subregion refers to a transition between the dry Mixedgrass Natural Subregion and the northern Central Parkland Natural Subregion (Natural Regions Committee 2006, Attaeian 2010). This site has a long history of grazing by cattle, but it has never been tilled (Cahill 2003, Shore 2009, Clark 2010). Nitrogen and water availability are the major limitation for plant growth (Lamb et al. 2007).

The plant community and climate at Kinsella were historically similar to Edmonton. However, Edmonton has higher precipitation (with mean annual precipitation of 476.9 mm (Environment Canada)) and so higher probability of growing trees in Larch Park compared to Kinsella (which is a natural grassland). Even though Larch Park and Kinsella are not quite similar, we desired to establish native grassland around the storm water management facility at Larch Park to

have a natural grassland ecosystem similar to Kinsella. Kinsella was chosen for this research since the grasslands in Kinsella ranch have been studied extensively and valuable site background information, important plant community, and site characteristic data are available (Shore 2009, Attaeian 2010).

2.3.2 Field assessment and soil sampling

Soils were rebuilt at Larch Park to have soil layers resembling the natural soil horizons present before development and to ensure that rebuilt soils are as similar to native soils as possible. The three soil horizons of chernozemic soil, Ah (15 cm), Bt_1 (20 cm) and Bt_2 (20 cm), were removed and stored separately on the site. During reclamation, soil profiles were created by replacing lower soil (LS), upper soil (US), and top soil (TS) from bottom to the surface, respectively (www.gov.ab.ca/env/).

2.3.2.1 Transect

Two transects were placed at Larch Park and Kinsella on October 2010, one in grass and one in tree dominated vegetation, for examining the effects of disturbance on nitrogen availability and microbial activity at the reclaimed and native grassland sites. Each transect included two 50-meter long rows of 5 points (with 10 meter spacing), totaling 10 sampling points along each grass and tree transect (Figure 2- 1).

Ion exchange resin (IER)

Soil nitrogen availability was measured using ion exchange resin (IER) capsule (Dobermann et al. 1997). Using ion exchange resin-based techniques are becoming more popular for determining soil nutrient availability compare to the

traditional soil sampling measurement. IER-based techniques have advantages over the traditional soil sampling method by causing minimal disturbance in the soil and allowing for re-measurement of specific points in the soil over time (Johnson et al. 2005). Soil moisture and temperature are significant factors influencing ion supply rate and resin adsorption. Variations in the soil moisture can be an important source of differences in ion supply rates measured by resin capsules in the field (Qian and Schoenau 2002). Qian and Schoenau (1996) reported that the amount of nitrate-N and phosphate-P removed by resin capsules from two chernozemic soils declined significantly by decreasing soil moisture content, this decrease represents the relationship between diffusive flux of nutrient ions and soil moisture content. Effects of temperature on the diffusion of ion to resins is relatively less than the effects of moisture (Qian and Schoenau 1996). Increase in temperature causes an increased nutrient accumulation rate, consequently increasing adsorb rate of resin (Qian and Schoenau 2002).

Comparing field measurements of IERs with traditional soil measurements are challenging because IER measurements' values cannot be associated to specific amount of soil; they are expressing as weights or moles of nutrient per unit weight or surface area of resin capsules rather than in weights or moles of nutrient per unit weight of soil (Johnson et al. 2005).

In this study, resin capsules were used to compare nitrogen availability between the reclaimed and native grassland sites. They were inserted in the soil on October 2010, at each sample point along the grass and tree transects at Larch Park and Kinsella (twenty resin capsules at each site). Resin capsules stayed in the

soil during fall and winter, then they were collected and replaced on May 2011. The new resins remained in the soil during spring and summer and were collected at the end of August 2011 at the same time as soil samples were collected. See laboratory analyses section for more details on resin capsules.

Soil sampling

Twenty soil samples were collected from Larch Park and 20 from Kinsella at the end of August 2011. The soil samples were taken from 0 to 15 cm depth at each sample point along the grass and tree transects using a metal soil probe 2.5 cm in diameter. There was no organic layer at the sampling points of grass transect, but in the tree transect samples were collected from organic and mineral layers due to the presence of some forest floor at the sampling points. The samples were kept cold in coolers with ice packs until they were brought back to the laboratory where they were stored in fridge at 4 °C. The collected samples were used for measuring soil microbial biomass carbon and nitrogen, moisture content, respiration, total carbon and total nitrogen.

2.3.2.2 Phytometer (Festuca hallii)

Twenty monitoring blocks were installed at Larch Park in summer 2010 using a randomized complete block design across a soil depth gradient. Blocks were located at three different soil depth classes: seven blocks with shallow-depth soil (10-50 cm), seven blocks with medium-depth soil (51-90 cm), and six blocks with deep soil (91-140 cm). Each block had two plots with different plant communities of rhizomatous and non-rhizomatous species. The rhizomatous

community consisted of plants that regenerate vegetatively and by seed, and the non-rhizomatous community consisted of plants that regenerate only by seed.

There was a late successional species; *Festuca hallii* (Plain Rough Fescue) used in all rhizomatous and non-rhizomatous plots as a phytometer to study nitrogen availability changes and microbial community dynamics under one individual species as a bio-indicator of restoration success. Also, eighteen *Festuca hallii* were randomly selected at Kinsella at three different soil depth classes; shallow, medium and deep soil to compare Larch Park with Kinsella and study the differences between these sites. By selecting the same species (*Festuca hallii)* as a phytometer in the natural and reclaimed sites, the kind of carbon input into the rhizosphere would be just due to this species.

Ion exchange resin (IER)

To measure nitrogen availability under *Festuca hallii* and study the effect of different plant communities (rhizomatous and non-rhizomatous) on available nitrogen, resin capsules were inserted under *Festuca hallii* at Kinsella and Larch Park on June 2011. Resin capsules were placed at Larch Park under Festuca in both rhizomatous and non-rhizomatous plots (in all monitoring blocks), also they were inserted at Kinsella under eighteen randomly selected Festuca. Resin capsules were collected at the end of August, 2011 with soil samples. More detail about resin capsules can be found in the laboratory analyses section.

Soil sampling

Soil samples were collected from 0 to 15 cm depth under *Festuca hallii* in both the rhizomatous and non-rhizomatous communities at Larch Park and also

under the eighteen randomly selected Festuca at Kinsella, at the end of August 2011. Microbial community structure (PLFA), moisture content, pH, respiration, total carbon and total nitrogen were measured on collected samples. Soil samples were taken using a metal soil probe 2.5 cm in diameter. All sampling equipment was washed with 70% ethanol between samples to avoid phospholipid contamination. Samples were put into separate sterile Whirl-Pak® Sampling Bags (Nasco, USA). The samples were placed in a cooler and kept cold with ice packs until they were transported back to the laboratory, then they were stored at 4 °C, except for PLFA samples, which immediately stored at -80 °C and freeze-dried before performing PLFA extraction.

2.3.3 Laboratory analyses

Moisture content was calculated by weighing soil samples before and after oven drying at 105°C for 24 hours (Kalra and Maynard 1991). PH of soil was measured by adding 0.01M calcium chloride to oven dried soil (not sieved) using a 1: 2 soil-to-solution ratio. More detail can be found at Kalra and Maynard (1991).

Soil microbial biomass carbon and nitrogen (MBC/MBN) were measured using the chloroform fumigation-extraction method in which C and N were extracted by 0.5 M K_2SO_4 as fully described by Brookes et al. (1985). Briefly, 25 g field fresh soil was placed in a 50 ml beaker and fumigated with chloroform for 4 days. Chloroform fumigation was repeated after 2 days. Then samples were extracted with 50 mL of 0.5 M K_2SO_4 (1:2 soil-to-solution ratio), shaken for 1 hr., and vacuum filtered with Whatman P2 filter paper. The concentration of dissolved

organic carbon (DOC) and dissolved organic nitrogen (DON) in the extracted solution were measured using a Shimadzu TOC-VTN instrument (Mandel Scientific Company Inc., ON, Canada). The difference in DOC and DON concentrations between fumigated and unfumigated samples was calculated to determine microbial biomass carbon and microbial biomass nitrogen (Swallow et al. 2009).

For measuring total carbon and nitrogen (TC/TN), 1-5 g dry soil was ground with Retsch MM200 ball mill grinder. Then approximately 10 mg of ground sample were tested for TC and TN by dry combustion with a thermocouple sensor (Costech Analytical Technologies Inc., Valencia, CA, USA) (Norris et al. 2011, Hahn and Quideau 2012).

Phospholipid fatty acid (PLFA) analysis was performed to characterize the soil microbial community structure. PLFA samples were freeze dried before phospholipid fatty acid extraction. Polar lipids were extracted from the freeze-dried soil samples (2 g) with a modified Bligh and Dyer extraction (Bligh and Dyer 1959, Frostegård and Bååth 1996). The extracted solutions were filtered by pre-packed silicic acid columns (Agilent Technologies, Wilmington, DE), they were then subjected to a mild alkaline methanolysis to form fatty acid methyl esters (FAMEs). An Agilent 6890 Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE) with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column was used to separate the FAMEs. The individual fatty acids were identified by MIDI peak identification software (MIDI, Inc., Newark, DE) (Hannam et al. 2006, Swallow et al. 2009, Hahn and Quideau 2012).

Soil microbial respiration was measured using an alkali trap method, where evolved CO_2 was trapped in 0.5 M sodium hydroxide (NaOH), forming sodium carbonate (Na_2CO_3). In this method, 50 g field fresh soil was weighed and placed in a 1L glass jar. An open cap scintillation vial containing 20 ml of 0.5 M NaOH was carefully placed into the soil inside the glass jar. The jar was sealed and incubated at room temperature (25°C) for 7 days. After incubation completed, the scintillation vial was taken out and capped immediately, then the solution was titrated with 0.5 M hydrochloric acid (HCl) to a clear end point (Zibilske 1994, Hopkins 2007).

In addition, a second set of respiration test was performed on re-wet soil samples. Soil respiration was normalized by adding water to the soil samples to bring them all to a moisture content of 20%. First, the moisture content of the field fresh soil samples from Kinsella and Larch Park was measured. Then certain amount of water was added to each sample (due to its moisture content) to gain moisture content of 20%.

Soil nitrogen availability was measured using ion exchange resin (IER) capsule (Dobermann et al. 1997). Resin capsules were inserted into the soil at Larch Park and Kinsella for a specific time interval, then they were retrieved and stored separately in plastic bags. Resin capsules were kept cold in coolers with ice packs until arriving at the lab where they were extracted with 2M KCl. Briefly, collected resin was rinsed with deionized water to remove soil particles adhering to its surface and then 20 mL of 2M KCl was added into centrifuge tube containing a resin and shaken for half an hour. Then, 15 mL of solution was

decanted into new centrifuge tube with the same label. This process was repeated for 3 times to get 45 mL extracted solution at the end. Extracted solutions were then analyzed for ammonium and nitrate. Concentrations of ammonium and nitrate were measured colorimetrically on the extracted solutions by the sodium salicylate/nitroprusside method for ammonium (Mulvaney 1996) and the cadmium reduction method for nitrate (Mulvaney 1996) using Smart Chem (Westco Scientific Instruments, Inc.).

The ratio of soil basal respiration to microbial biomass carbon was used as a relative measure of the microbial metabolic quotient (Anderson and Domsch 1985), which can be used as a measure of microbial efficiency (Wardle et al. 1998).

2.3.4 Statistical analyses

Two sample t-tests were used for direct comparison of transects and phytometer data from two sites. This statistical analysis was applied to total carbon and nitrogen, total inorganic nitrogen, microbial biomass carbon and nitrogen, respiration (field fresh and re-wet soil samples) and metabolic quotient data from Larch Park and Kinsella. The SAS software (Version 9.2) was used to check normality, equality of variance and to perform the test. An alpha of 0.05 was used for all statistical tests.

Transect: MBN data at Larch Park and Kinsella and soil respiration data for field fresh samples in the grass transect at Larch Park were not normally distributed, and were therefore log transformed. Also, non-parametric test (Wilcoxon-Man-Whitney two sample test) was used for analyzing the total

inorganic nitrogen data, respiration data (re-wet samples), and metabolic quotient data for the grass transect because these data did not meet the assumption of normality regardless of transformation, so were analyzed by non-parametric test.

Phytometer: Total inorganic N and respiration for field fresh samples were not normally distributed at Larch Park. Also respiration data for re-wet samples was no normal at Kinsella. Thus, these data were log transformed to meet the assumption of normality.

Non metric multidimensional scaling (NMS) was used to analyze microbial community structure (PLFA data) under *Festuca hallii* using PC-ORD (Version 6, MjM Software Design). NMS is an iterative method that organizes multivariate data in a reduced number of dimensions based on distances between data points. Therefore, the distances in the ordination graph represent the similarities or dissimilarities in community structure of the original data. The Sorensen (Bray–Curtis) distance measurement was used in the ordination. NMS is not assuming a linear relationship between variables, so this method can be considered more appropriate than many other ordination methods (Hannam et al. 2006, Norris et al. 2011). The main matrix was included PLFA biomarkers measured and expressed on a nmol g^{-1} basis, relativized by row and transformed using the arcsine square-root function. The second matrix contained selected soil variables, site parameters, and bio-indicators including calculated microbial indices.

2.4 Results

2.4.1 Transect

Significant differences were observed between the natural and reclaimed sites for total carbon in tree dominated vegetation and also for total nitrogen in both grass and tree dominated areas; total carbon was similar between Larch Park and Kinsella under grass dominated vegetation (P = 0.0649), while in tree dominated areas, the level of total carbon was significantly higher at Kinsella compared with Larch Park (P = 0.0003) (Figure 2- 2). In addition, total nitrogen was significantly higher at Kinsella than Larch Park in both grass (P = 0.0148) and tree dominated vegetation (P = 0.0007) (Figure 2- 3).

The total inorganic nitrogen measured on resin capsules was significantly higher at Larch Park than the native grassland site in both grass (P = 0.0027) and tree transects (P = 0.0003) (Figure 2- 4).

Microbial biomass carbon at Kinsella was significantly higher than Larch Park in both grass (P <.0001) and tree transects (P = 0.0036) (Figure 2- 5), while microbial biomass nitrogen was similar between Kinsella and Larch Park in both grass (P = 0.1830) and tree transects (P = 0.4116) (data not shown).

Soil basal respiration for field fresh soil samples at native grassland site was significantly higher than Larch Park in both grass (P = 0.0018) and tree transects (P = 0.0002) (Figure 2- 6). Also, soil respiration for re-wet soil samples at Kinsella was significantly higher than Larch Park in both grass (P = 0.0035) and tree dominated vegetation (P = 0.0003) (Figure 2- 7), with a greater differences

between Kinsella and Larch Park in grass transect compared to the field fresh samples.

Metabolic quotient was similar between Kinsella and Larch Park in grass transect (P= 0.3862), while in tree transect metabolic quotient was higher at Kinsella compared to Larch Park (P= 0.0004) (Figure 2- 8).

2.4.2 Phytometer (*Festuca hallii*)

No significant differences were found between rhizomatous and non-rhizomatous communities in all soil properties measured (data not shown here). For this reason we consider the rhizomatous and non-rhizomatous data together to compare soil processes under *Festuca hallii* at Larch Park and Kinsella.

Total carbon and total nitrogen under *Festuca hallii* at native grassland site were significantly higher than Larch Park (P = 0.0023 and 0.0004, respectively) (Figure 2- 9 and Figure 2- 10).

Total inorganic nitrogen under *Festuca hallii* at Larch Park was significantly higher than *F. hallii* found in native grassland site (P = 0.0002) (Figure 2- 11), which is similar to the result from transect.

Soil basal respiration from under *Festuca hallii* at native grassland site was significantly higher than Larch Park for both field fresh soil (P = 0.0124) and re-wet soil samples (P <.0001) (Figure 2- 12 and Figure 2- 13). Microbial biomass from the PLFA data indicated that Kinsella had higher biomass than Larch Park (data not shown here).

The NMS ordination of the soil microbial communities produced a two-dimensional ordination solution with a final stress of 7.83 after 45 iterations, and

explained 97% of the variation in the data set (Figure 2- 14). Microbial community structure under Festuca at Larch Park and Kinsella were different. The variability of microbial community at Kinsella was higher than Larch Park based on the spread of data in the ordination graph; data were more distributed at Kinsella compared to the Larch Park. The microbial community at undisturbed site (Kinsella) under *Festuca hallii* was dominated by fungi and total biomass, while at the reclaimed site (Larch Park) was dominated by actinobacteria and arbuscular mycorrhizal fungi (AFM) (Figure 2- 14).

2.5 Discussion

2.5.1 The effects of disturbance on soil processes

Using heavy construction equipment and stockpiling soil before starting land reclamation can have negative impacts on soil properties such as soil bulk density, organic matter content and microbial biomass (McMillan et al. 2007). Soil microbial function is essential to control soil ecosystem level processes such as soil organic matter decomposition and nutrient cycling (Grayston et al. 2004). In this study, we have found significant differences in soil properties between the natural and reclaimed sites in many cases which can be due to the fact that disturbance followed by construction activities at developed area causes extreme changes in soil processes.

The results from transects indicated higher nitrogen availability (Figure 2- 4) and lower microbial biomass carbon (Figure 2- 5) at reclaimed site compared to native grassland site. We also found higher total inorganic nitrogen under *Festuca hallii* at Larch Park compared to Kinsella (Figure 2- 11). Using resin

capsule in situ allows us to check the bioavailability of soil nutrient in field condition. It also minimizes physical and chemical disturbance of the soil and provides a more precise measurement of soil nutrient and its temporal and spatial variability under field conditions (Dobermann et al. 1997, Qian and Schoenau 2002). Soil temperature and moisture are significant factors influencing resin adsorption (Qian and Schoenau 2002). By collecting soil samples in the traditional soil sampling method, samples will be disconnected from the ecosystem, thus the effects of temperature, moisture and plant root will be eliminated from the nitrogen availability measurement. But by using resin analysis, we can avoid these problems.

Resin data have shown higher levels of NO_3^- than NH_4^+ at the disturbed site and we also found that the level of NO_3^- was higher on reclaimed sites compared to natural grassland site (data not shown), which implies high nitrification rates. Higher nitrification and lower microbial biomass are generally representative of highly disturbed sites that have experienced loss of ecosystem function and disconnected soil-plant relations. These results are not unexpected as housing development and storm water pond construction are similar to land reclamation in many ways and research from these environments has produced similar findings (McMillan et al. 2007, MacKenzie and Quideau 2010).

Nutrient profiles are influenced by several factors including microbial community structure, organic matter content, plant community dynamic and time since disturbance (MacKenzie and Quideau 2010). Nutrients are transferred from plants to soil in the form of litter, after this movement nutrients are accessible for

mineralization and uptake by microbes or plants, or they can be lost from the ecosystem (Chapin et al. 2002). Given that there has never been any ground disturbance at the native grassland site there is a tight connection between microorganisms and plants, consequently released NH_4^+ is usually taken-up immediately by plants. Thus, the level of total inorganic nitrogen at the native grassland site was significantly lower than Larch Park.

In addition, disturbance enhances available nitrogen through increasing nitrogen mineralization; it can be due to the fact that disturbance changes soil temperature, moisture, structure, aeration and the exposure of soil organic matter (Likens et al. 1970, Vitousek and Melillo 1979). It also decreases plant nitrogen uptake by removing vegetation and creating a situation wherein nitrogen mineralization is much greater than nitrogen uptake by the vegetation (Vitousek and Melillo 1979). One theory is that a disturbed ecosystem has disconnected soil-plant relations so the concentration of NH_4^+ and NO_3^- is expected to be high due to the loose N cycle and reduced plant uptake and microbial immobilization at Larch Park.

We found lower respiration, total carbon and total nitrogen at Larch Park compared to Kinsella from both transect and phytometer data, which is similar to the findings of a previous study (McMillan et al. 2007). McMillan et al. (2007) reported that total carbon and nitrogen were significantly lower in reclaimed treatments compared to natural forest site. They also found that MBC and MBN were significantly lower in reclaimed site compared to natural forest site. Total carbon and nitrogen data are not showing the same pattern as mineralized carbon

and mineralized nitrogen at Kinsella and Larch Park. The results indicate that Larch Park had higher total inorganic nitrogen (mineralized N) and lower total nitrogen compared to Kinsella. Due to the tight connection between plant and soil microorganisms at natural site, available nitrogen will be up taken by plant immediately after soil organic matter decomposition. Consistent with this, our result showed that the level of total inorganic nitrogen at Kinsella was significantly lower than Larch Park. In addition, high microbial biomass carbon and high respiration, implying active microbial community, were found at Kinsella while Larch Park had lower respiration (mineralized carbon) and lower total carbon which must be explained by the negative impact of disturbance on soil microbial dynamics at Larch Park.

The NMS ordination of PLFA data, as plotted in Figure 2- 14, showed that the soil microbial community at the rebuilt site was different from the natural grassland site, which has been cited previously (Card and Quideau 2010). Card and Quideau (2010) found that the microbial community of younger restored soils differed significantly from the reference soils, where reference soils had higher microbial biomass, evenness and diversity.

The variability of microbial community at Kinsella was higher than Larch Park because disturbance had significant effect on microbial community composition including fungi. Previous study has reported that soil disturbance has negative effects on fungal growth as shown by reduced fungal biomarkers and increased bacterial dominance (Mummey et al. 2002). In addition, soil microbial communities of undisturbed terrestrial ecosystems have a tendency towards

fungal microbial biomass dominance, which contributes to a high FBR (Mummey et al. 2002). Our finding is in agreement with these studies. Also, plant species have been indicated to have a main selective impact on microbial communities in their rhizospheres (Grayston et al. 2004). It can be assumed that greater readily available carbon is released into the grasslands rhizosphere causing in higher carbon consumption by bacterial communities, but the greater recalcitrant compounds resulting in the higher fungal number and lower metabolic activity. Therefore, the presence of more readily utilizable carbon in the rhizosphere of grasslands causing in higher total carbon utilization by microbial communities (Grayston et al. 2004).

The ordination of PLFA data under *Festuca hallii* indicated that the microbial community at Kinsella was dominated by fungi and total biomass, while at Larch Park it was dominated by actinobacteria and arbuscular mycorrhizal fungi (AFM) (see Figure 2- 14). Increased relative abundance of actinobacteria in the Larch Park soil with high available nitrogen is similar to a study that showed that fertilization enhanced the relative abundance of actinobacteria PLFAs (Mach 2010).

Mycorrhizae are symbiotic relationships between plant roots and fungal hyphae, where the plant obtains nutrient from the fungus and in return it provides carbohydrate which is a main carbon source for the fungus (Chapin et al. 2002). Therefore, we have expected to have higher AMF at Kinsella where there is a tight competition between plant and microbial communities for using limited resources, but our data have shown that Larch Park with low vegetation

development is associated with AMF, which seems counterintuitive. The level of available N is very high at Larch Park and plant does not need increased access to the nitrogen. Thus, presence of AMF at this site may be due to the fact that water or other source of nutrient including P is limited at Larch Park. In addition, our result is contrary to some previous study in the literature, which reports that high N supply can have negative impact on the mutualistic relationships between plants and arbuscular mycorrhizal fungal communities (Bradley et al. 2006).

Mach (2010) also reported that PLFA 16:1ω5 was identified in both organic and mineral soil samples, but no mycorrhizal infection of plant roots was found. Thus, he has suggested that using the PLFA 16:1ω5 can be deceptive for identifying AMF. In most AMF, the PLFA 16:1ω5 is found to be a large proportion of total fatty acids and is usually not detected in other fungi. It is, however, a component in gram negative bacteria, so there is no absolutely particular fatty acid marker for AMF (Olsson 1999).

Plants show different reactions to colonization that are dependent on several parameters including environmental factors and plant type (Sherritt 2012). Sherritt (2012) also found that there was no significant relation between mycorrhizae on rough fescue roots and growth response variables. Many studies have also reported that a variety of C_3 grasses are generally insensitive and less responsive to AMF colonization (Hetrick et al. 1990, Bentivenga and Hetrick 1991). Thus, based on literature (Shore 2009, Sherritt 2012), we can suggest that Festuca does not have a significant impact on formation of AMF.

2.5.2 The effects of plant community (rhizomatous and non-rhizomatous) on soil-plant processes

We did not notice any significant differences between the rhizomatous and non-rhizomatous communities in all soil processes. However, plant data from 2010 and 2011 indicated that the survivorship of the rhizomatous species was higher than the non-rhizomatous species and the rhizomatous species produced more belowground biomass than the non-rhizomatous communities (data not shown). Also, introduced species including weeds and invasive species were higher in the rhizomatous species compared to the non-rhizomatous species. Plant communities have shifted from low percent cover native species communities in 2010 to more established and large biomass communities in fall 2011 (plant data not shown here).

2.5.3 Comparing soil respiration at field fresh and re-wet soil samples after normalizing respiration

Respiration could be controlled by four different factors including temperature, moisture, types of microorganisms and types of carbon (such as soil organic matter and biochar). In this study soil respiration was normalized in the laboratory environment; the effects of moisture and temperature were controlled by adding water to the soil samples to bring them all to a moisture content of 20% and controlling room temperature. In this case the differences between respiration results would be just due to the type of carbon and type of microorganisms.

By comparing respiration in grass transect at Larch Park and Kinsella, we noticed that after adding water the significant differences between Larch Park and

Kinsella became greater and it could be due to the fact that soil microbes responded differently after being wet. For example, at Kinsella the mg $C\text{-}CO_2$/g dry soil doubled in rewet soil sample, while at Larch Park after adding water the mg $C\text{-}CO_2$/g dry soil stayed at the same level. Also, the respiration data from under *Festuca hallii* at Larch Park and Kinsella showed that after adding water the mg $C\text{-}CO_2$/g dry soil at Kinsella increased significantly, but it remained at the same level for Larch Park. It could be explained by the differences of soil moisture content at Larch Park and Kinsella. Moisture content at Kinsella was generally lower than Larch Park and microbial community at this site was ready for having benefits of adding water. Consequently, adding water had a significant impact on soil microbial activity at Kinsella.

Figures

Figure 2- 1: Pattern of transect in tree and grass dominated vegetation, for collecting soil samples and installing resin capsules.

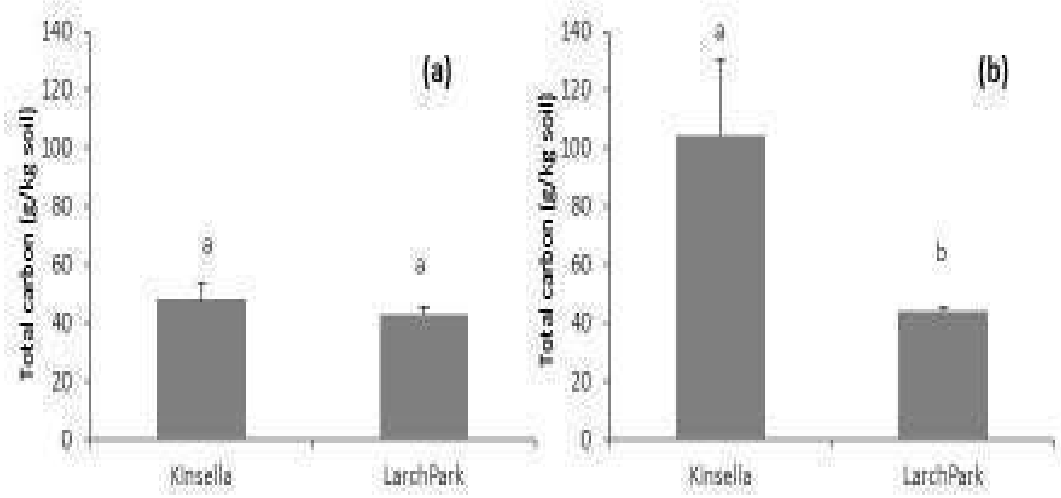

Figure 2- 2: Total carbon (g/kg soil), at Kinsella and Larch Park, for a) grass dominated vegetation, and b) tree dominated vegetation.

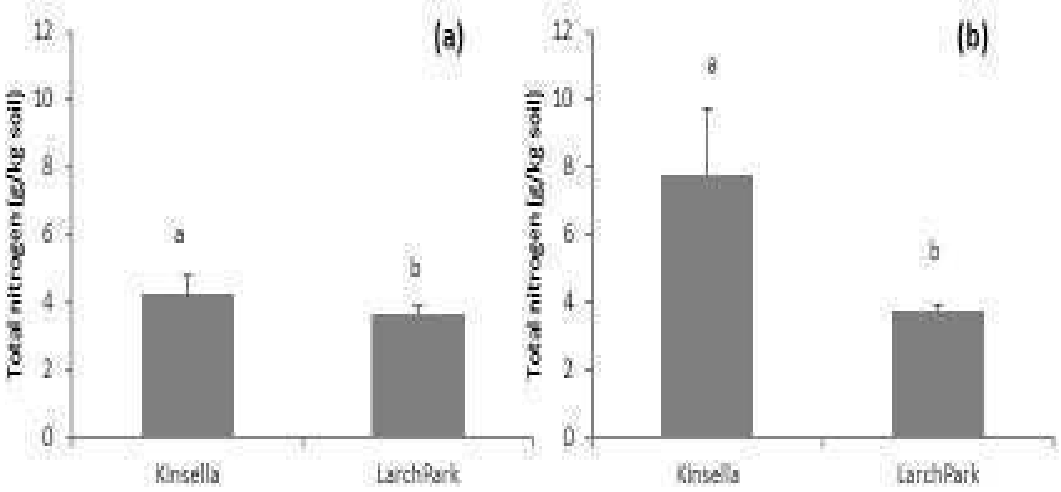

Figure 2- 3: Total nitrogen (g/kg soil), at Kinsella and Larch Park, for a) grass dominated vegetation, and b) tree dominated vegetation.

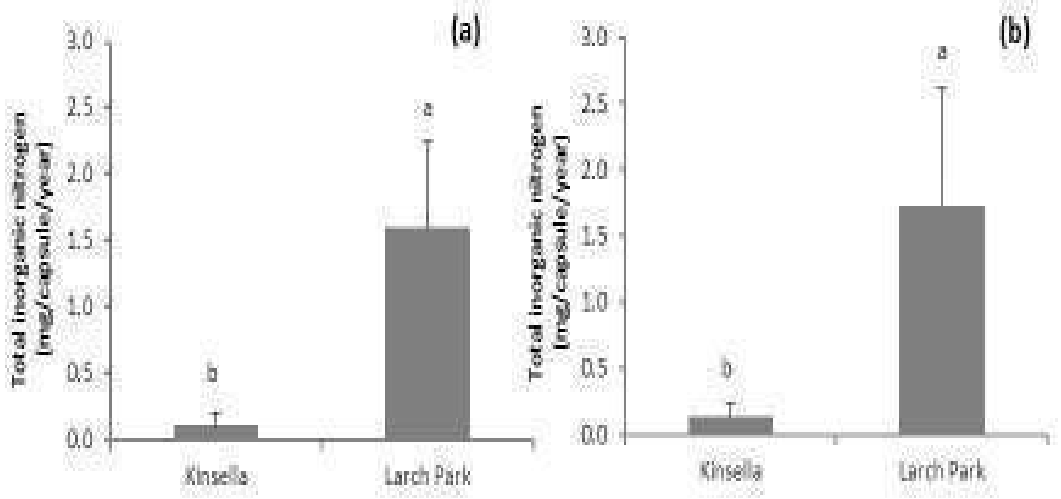

Figure 2- 4: Total inorganic nitrogen (mg/capsule/year), at Kinsella and Larch Park, for a) grass dominated vegetation, and b) tree dominated vegetation.

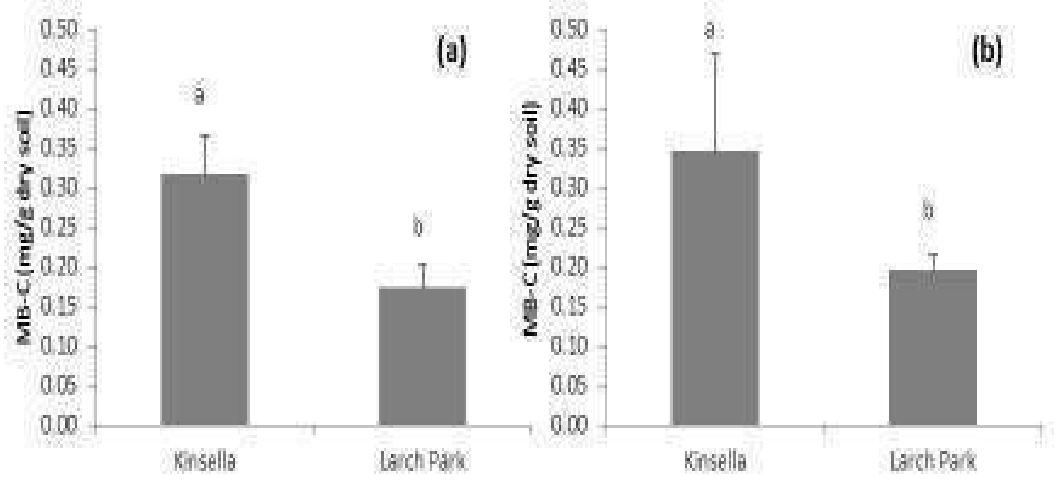

Figure 2- 5: Microbial biomass carbon (mg/g dry soil), at Kinsella and Larch Park, for a) grass dominated vegetation, and b) tree dominated vegetation.

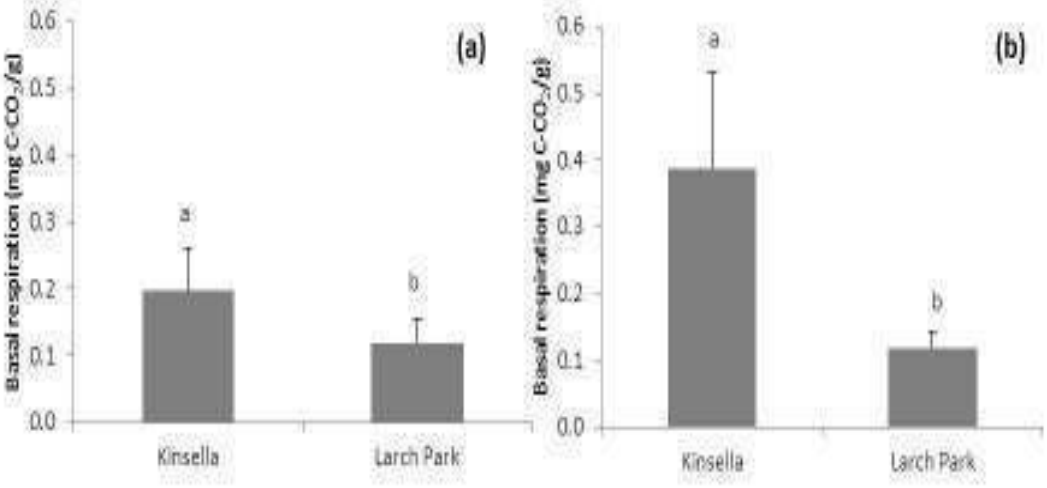

Figure 2- 6: Respiration (mg C-CO_2/g dry soil), at Kinsella and Larch Park, for a) grass dominated vegetation, and b) tree dominated vegetation. Experiment performed on field fresh soil samples.

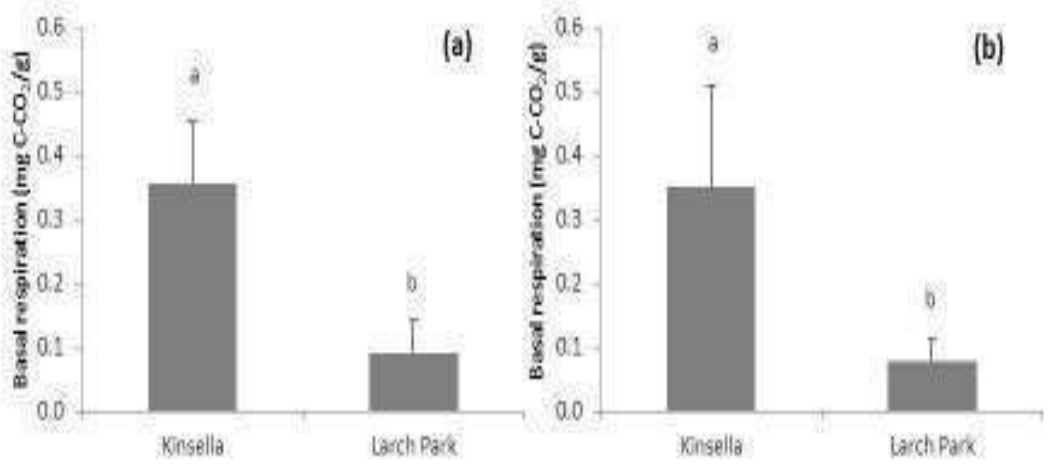

Figure 2- 7: Respiration (mg C-CO_2/g dry soil), at Kinsella and Larch Park, for a) grass dominated vegetation, and b) tree dominated vegetation. Experiment performed on re-wet soil samples.

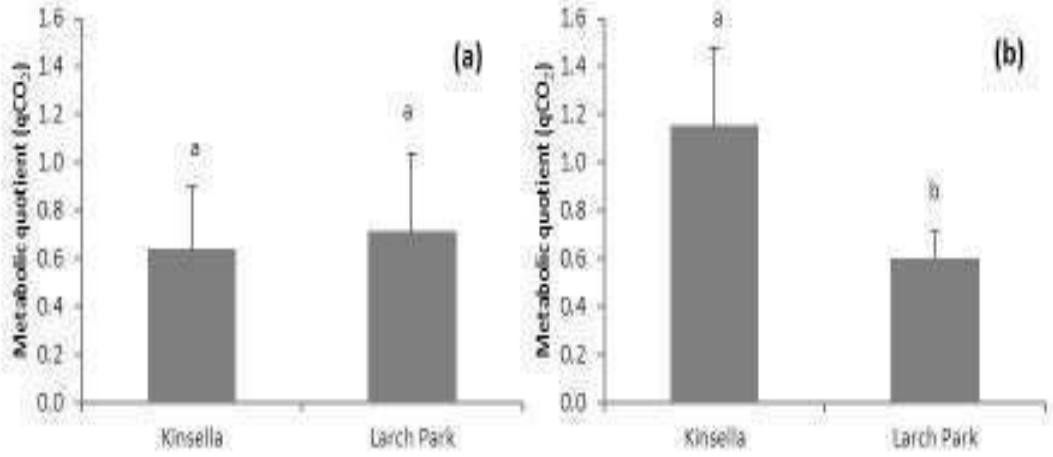

Figure 2- 8: Metabolic quotient (qCO_2), at Kinsella and Larch Park, for a) grass dominated vegetation, and b) tree dominated vegetation.

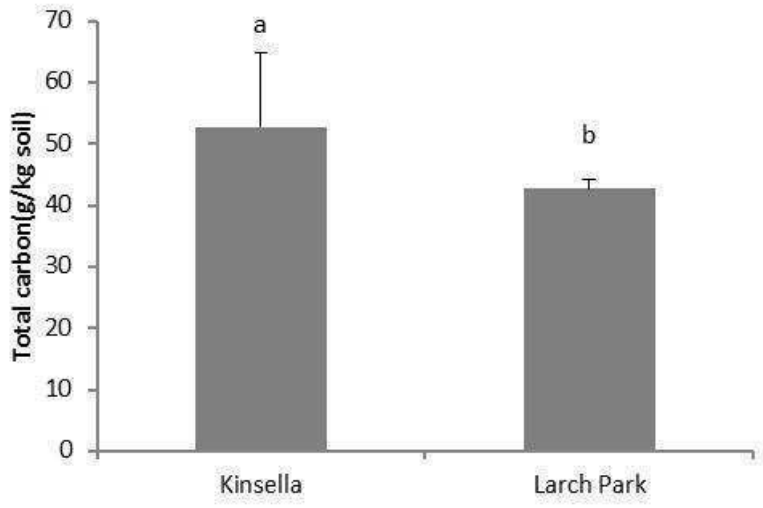

Figure 2- 9: Total carbon (g/kg soil), under *Festuca hallii* at Kinsella and Larch Park from two different plant communities.

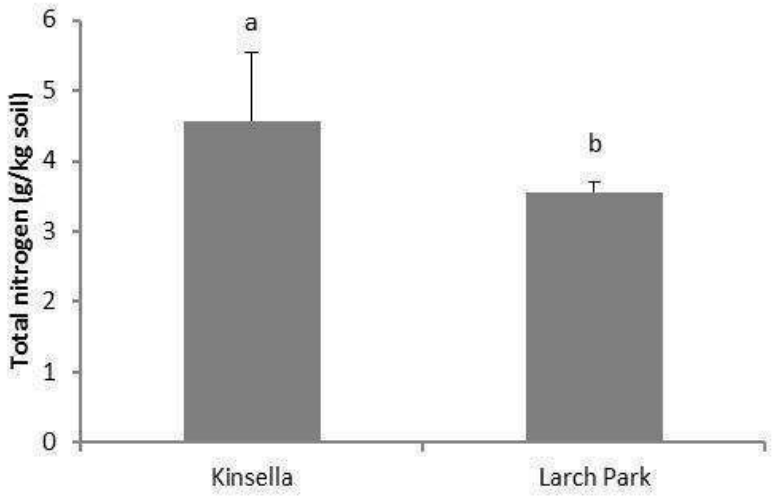

Figure 2- 10: Total nitrogen (g/kg soil), under *Festuca hallii* at Kinsella and Larch Park from two different plant communities.

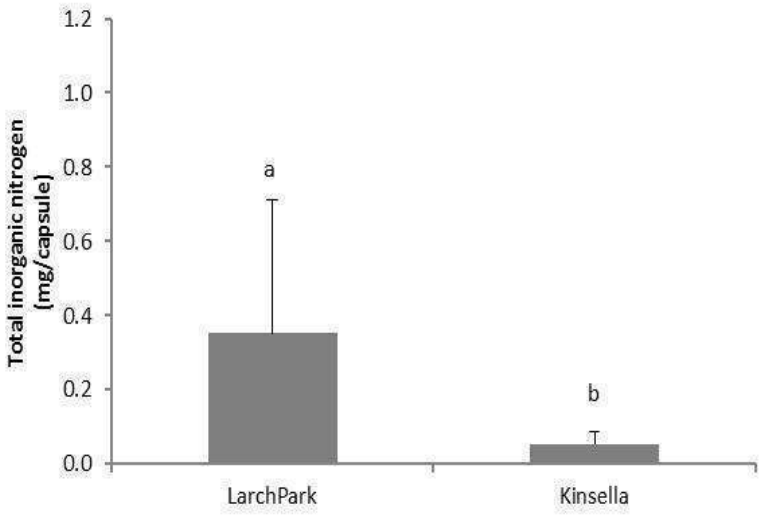

Figure 2- 11: Total inorganic nitrogen (mg/capsule), under *Festuca hallii* at Kinsella and Larch Park from two different plant communities, summer resin sampling.

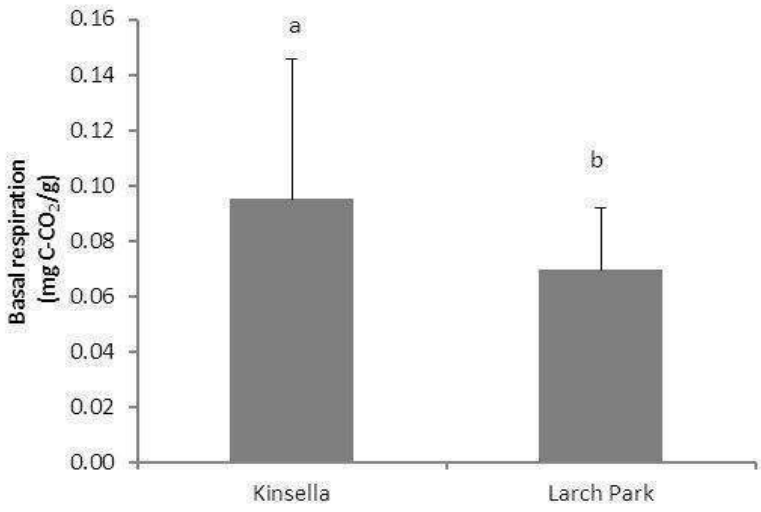

Figure 2- 12: Respiration (mg C-CO_2/g dry soil), under *Festuca hallii* at Kinsella and Larch Park from two different plant communities. Experiment performed on field fresh soil samples.

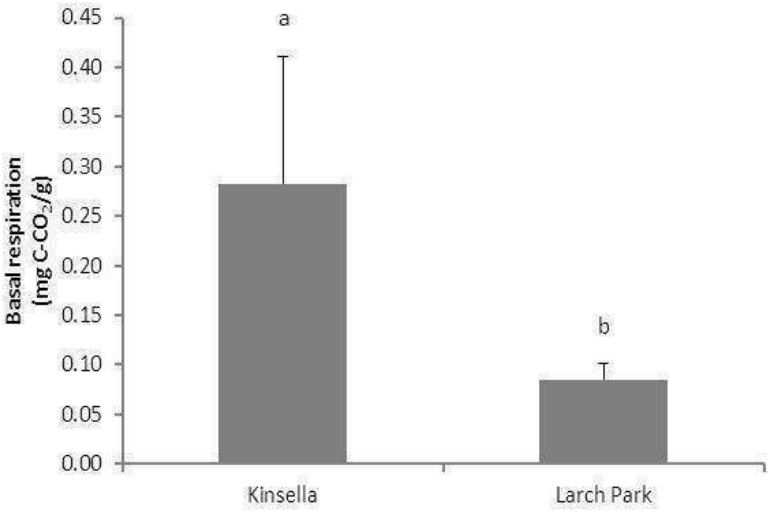

Figure 2- 13: Respiration (mg C-CO_2/g dry soil), under *Festuca hallii* at Kinsella and Larch Park from two different plant communities. Experiment performed on re-wet soil samples.

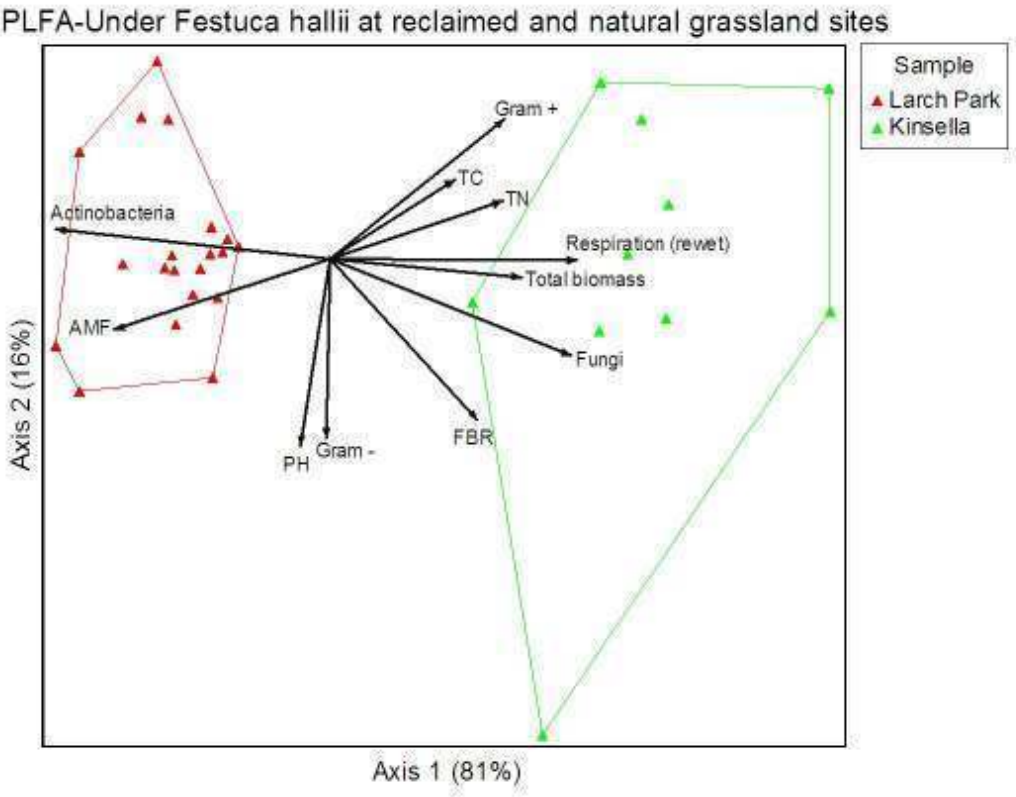

Figure 2- 14: Non-metric multidimensional scaling ordination of microbial phospholipids fatty acid (PLFA) data under *Festuca hallii* at Larch Park and Kinsella. The proportion of variance explained by each axis is based on the correlation between distance in the ordination space and distance in the original space, and is reported after each axis heading. The variability of microbial community at native grassland site (Kinsella) was higher than reclaimed site (Larch Park) based on the spread of data in the ordination graph; data were more distributed at Kinsella compared to Larch Park. Kinsella was dominated by fungi and total biomass, while Larch Park was dominated by actinobacteria and arbuscular mycorrhizal fungi (AMF).

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Chapter 3: Studying the Effects of Biochar on Soil-Plant Processes in Greenhouse Experiment

3.1 Introduction

Fire is one of the most important agents of natural disturbance in western ecosystems, with the ability to change successional organization. Fire causes change in physicochemical and biological environment by heating and oxidation, and also creates new abiotic substrate for soil including charcoal (Hart et al. 2005). Fire also causes alteration in vegetation dynamics, physical and chemical properties of soils and also soil microorganisms (Hart et al. 2005). Main residue of fire is charcoal, charcoal is generated during natural fire in forest and prairie environments through the partial combustion of organic materials (Preston and Schmidt 2006). Charred carbon is an important component of Black Chernozemic soils in the native grasslands of the prairie region where it has been shown to contribute up to 45 % of the total carbon (Ponomarenko and Anderson 2001). Incidence of fires under cool and moist conditions can increase accumulation of char in prairie soils (Ponomarenko and Anderson 2001). Man-made charcoal is called biochar. Biochar is the carbon-rich solid created by thermal degradation of organic materials in the absence of oxygen (pyrolysis) (Lehmann et al. 2011). Pyrogenic C is generally created as solid charred residues and its structures vary as a continuum from partially charred plant materials to charcoal, volatile soot and eventually graphite (Preston and Schmidt 2006).

Charcoal or black carbon is chemically and biologically stable because of its polycyclic aromatic structure, thus charcoal can be persisting in the environment for centuries (see Preston and Schmidt 2006 for a complete review). Oxidation throughout formation of charcoal creates carboxylic groups on the boundaries of the aromatic structure, consequently enhances the nutrient and water holding capacities of charcoal (Glaser et al. 2002).

Biochar can be used as a soil modifier (Lehmann et al. 2011), and it has the potential to affect soil fertility and possibly mitigate climate change through carbon sequestration (Woolf et al. 2010, Lehmann et al. 2011). The importance of using biochar to mitigate climate change is based on its relative recalcitrance against microbial decay and its slower return of terrestrial organic C in form of CO_2 to the atmosphere (Lehmann 2007). The potential impacts of adding biochar to soil include changing pH, nutrient retention and soil moisture retention, enhanced soil structure, declines in N_2O emissions, and reductions in leaching of inorganic N (Anderson et al. 2011). By adding biochar to the soil, microbial community structure and function might be shifted by altering physicochemical properties of the soil (Smith et al. 2010). Depending on biochar type, it may sometimes stimulate microbial activity and increase their abundance (Steiner et al. 2008). Steiner et al. (2008) showed that biomass derived charcoal enhances soil microbial biomass, growth and activity.

As discussed in chapter two, the concentration of available nitrogen was higher in the reclaimed soil compared to the natural grassland soil, which is an important issue and need to be controlled. The excess nitrogen can be lost,

leached out into the ground water (causing water contamination), denitrified, or it can increase the percentage of weedy species. By adding biochar to the soil we expected that the high level of nitrogen in the reclaimed site could be controlled and also biochar could have stimulatory impact on soil microbial activity.

3.2 Research questions and objectives

Do additions of biochar to natural grassland and reclaimed soils influence the soil-plant processes and microbial dynamics under greenhouse conditions?

1. The first objective was to determine soil nitrogen availability with and without biochar.
2. The second objective was to measure soil microbial activity with and without biochar.

3.3 Material and methods

3.3.1 Soil sampling and experimental design

A greenhouse experiment was conducted to study the effect of biochar addition on soil-plant processes and microbial dynamics under greenhouse conditions on field collected samples. The greenhouse environment offers absolute control for plant growth by removing moisture and temperature limitations and reducing competition.

Soils used for the greenhouse experiment were sampled from Larch Park and Kinsella in May 2011. Soils were mixed with sand in a 1:1 ratio in order to moderate soil texture, increase porosity, and improve soil tithe. Sand does not have significant impacts on soil nutrient and microbial community.

Before setting up the experiment, Larch Park and Kinsella soils were incubated at field capacity in the greenhouse condition for one week to acclimatize the soil microorganisms to the greenhouse condition. Each soil type had four treatments including: soil (S), soil with biochar (SB), soil with plant (SP), and soil with biochar and plant (SBP). Each treatment had 5 replicates. After one week of incubation, the treatments were set-up and the experiment was started in June 2011. Treatment soils were placed in 10 x 10 x 5 cm pots. 15% of the soil total carbon was added to some treatments as biochar carbon and it was mixed thoroughly with the soils. The biochar, produced from wheat straw, had 83% moisture content and 65.6 % carbon content. Also, *Festuca hallii* seeds were added to the soil surface of some treatments with and without biochar. For measuring nitrogen availability, a resin capsule was inserted into each pot.

The pots were randomly placed at five different trays; eight pots each, on the bench. Trays were covered by plastic lids with enough space between trays and lids for air circulation. Each pot was maintained at field capacity by adding water to the soil on regular basis. Above each tray there was a UV-VIS spectrometer for providing enough light for growing Festuca seeds.

After one month *Festuca hallii* survival and growth were less than 5%, therefore the experiment was taken down and resin capsules were analyzed as a "no plant" treatment and nitrogen availability was measured for two soil types (Larch Park and Kinsella) with and without biochar with increased replication.

At the end of July, a new experiment was set up using the pervious experimental design and a new Festuca species. *Festuca saximontana* Rydb.

(Rocky Mountain fescue) seeds, which were relatively large and easy to handle, were placed on the soil surface for germination. This native fescue grew very well during the experiment. A new resin capsule was also inserted into each pot for measuring nitrogen availability.

During the experiment plants were watered as needed. At first week of January 2012 the experiment was taken down and resin capsules were analyzed as a "with plant" experiment. Soil and resin capsules were collected separately in plastic bags and then brought back to the lab for analyses.

Plant biomass is an essential component for studying functional plant ecology and growth analysis. It is also the basis for net primary production and growth rate calculation (Tackenberg 2007, Golzarian et al. 2011). Therefore, plants were removed and collected from the pots for measuring plant biomass.

3.3.2 Laboratory analyses

Soil was dried at105°C for 24 hours to determine gravimetric moisture content (Kalra and Maynard 1991). Total plant biomass was measured by collecting, washing and oven-drying Festuca stems and roots, and weighing the biomass.

Soil microbial biomass carbon and nitrogen (MBC/MBN) were measured using the chloroform fumigation-extraction method in which C and N were extracted by 0.5 M K_2SO_4 as fully described by Brookes et al. (1985). Briefly, 25 g field fresh soil was placed in a 50 ml beaker and fumigated with chloroform for 4 days with the fumigation repeated after 2 days. Then samples were extracted with 50 mL of 0.5 M K_2SO_4 (1:2 soil-to-solution ratio), shaken for 1 hr., and

vacuum filtered with Whatman P2 filter paper. The concentration of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in the extracted solution were measured using a Shimadzu TOC-VTN instrument (Mandel Scientific Company Inc., ON, Canada). Then the difference in DOC and DON concentrations between fumigated and unfumigated samples was calculated to determine microbial biomass carbon and microbial biomass nitrogen (Swallow et al. 2009).

Soil microbial respiration was measured using alkali trap method, where evolved CO_2 was trapped in 0.5 M sodium hydroxide (NaOH), forming sodium carbonate (Na_2CO_3). In this method, 50 g field fresh soil was weighed and placed in a 1L glass jar. An open cap scintillation vial containing 20 ml of 0.5 M sodium hydroxide (NaOH) was carefully placed into the soil inside the glass jar. The jar was sealed and incubated at room temperature (25°C) for 7 days. After incubation completed, the scintillation vial was taken out and capped immediately, then the solution was titrated with 0.5 M hydrochloric acid (HCl) to a clear end point (Zibilske 1994, Hopkins 2007).

Ion exchange resin (IER) capsule were used for measuring soil nitrogen availability (Dobermann et al. 1997). Resin capsules were inserted into each pot, and at the end of experiment they were collected and stored separately in plastic bags. They were kept cold in coolers with ice packs until their arrival at the laboratory where they were extracted with 2M KCl. Extracted solutions were then measured for ammonium and nitrate.

The ratio of soil basal respiration to microbial biomass carbon was used as a relative measure of the microbial metabolic quotient (Anderson and Domsch 1985), which can be used as a measure of microbial efficiency (Wardle et al. 1998).

3.3.3 Statistical analyses

The statistical analyses performed included two-way ANOVA and multi-way ANOVA; the former for total inorganic nitrogen data in the first experiment (no plant treatment) and plant biomass data, and the latter for microbial biomass carbon and nitrogen, total inorganic nitrogen, respiration and metabolic quotient in the second experiment (with plant). The SAS software version 9.2 was used to check the assumptions of ANOVA (normality and homogeneity of the variance) and perform two-way ANOVA and multi-way ANOVA. An alpha of 0.05 was used for all statistical tests.

Total inorganic nitrogen data in "no plant" treatment was not normally distributed at Kinsella, so square root transformation was applied to meet the assumption of normality. MBC data was not normal at Larch Park and total inorganic N in the "with plant" experiment was not normal at Kinsella, so they were log transformed to conform to the normal distribution. Metabolic quotient data was not normally distributed at Larch Park, biochar and plant variables. Thus, they were square root transformed to become normal.

3.4 Results and discussion

In this study, the effects of biochar and native plant species on soil processes have been focused under greenhouse condition. The greenhouse

environment is ideal for minimizing moisture and temperature limitation, while field environment has some restricted conditions such as high temperature and low moisture during days and very low temperature during nights.

Resin data from the first greenhouse experiment (no plant treatment) indicated that there was a significant effect of soil type on total inorganic nitrogen ($P < .0001$). Larch Park soil had higher total inorganic nitrogen compare to Kinsella soil, most likely because of disturbance followed by land reclamation. No significant effect of biochar ($P = 0.2647$) and no significant interaction between soil type and biochar ($P = 0.9610$) were found. However, the level of total inorganic nitrogen in soil with biochar treatments (SB) was slightly lower than the soil without biochar (S), but this difference was not significant (Figure 3-1).

The resin data from the second experiment (with plant) showed that there was a significant effect of plant on total inorganic nitrogen ($P < .0001$); the level of total inorganic nitrogen in SP treatments was significantly lower than S treatments because plants uptake inorganic nitrogen for growth. But no significant effects of soil type ($P = 0.0770$) and biochar ($P = 0.4333$) were found. However, the level of total inorganic nitrogen in the SB treatments was lower than S treatments to some extent, as biochar has the ability to absorb inorganic nitrogen (Clough and Condron 2010), but this difference was not significant. Also there was significant interaction between soil type, biochar and plant ($P = 0.0018$); total inorganic nitrogen concentration in SBP treatments was significantly lower than S treatments (Figure 3- 2). Reduced available nitrogen with biochar addition and

planting native species in the reclaimed soil is beneficial in the context of the reclaimed site where there was very high available nitrogen as discussed in chapter 2.

For both "no plant" and "with plant" treatments resin data showed higher level of NO_3^- than NH_4^+ (data not shown), but there was no significant trend for any of NO_3^- or NH_4^+ data separately. Therefore, we summed NO_3^- and NH_4^+ data up and analyzed them as a total inorganic nitrogen data.

Microbial biomass carbon in the native grassland soil was significantly higher than Larch Park soil ($P < .0001$). The microbial community at Kinsella has been stabled for a long time having never experienced tillage (Clark 2010), while disturbance followed by reclamation at Larch Park has had drastic effects on soil microbial community (Figure 3- 3), which is similar to our finding in chapter 2. In addition, by planting native species microbial biomass carbon has been increased significantly in SP treatments compared to S treatments ($P = 0.0037$). But no significant effect of biochar ($P = 0.4268$), and no significant interaction between these variables ($P = 0.3608$) were found on microbial biomass carbon (Figure 3-3).

Microbial biomass nitrogen in Kinsella soil was significantly higher than Larch Park soil ($P < .0001$), and SP treatments had higher microbial biomass nitrogen compared to S treatments ($P = 0.0003$). But there was no significant effect of biochar ($P = 0.1362$) and no significant interaction between them ($P = 0.4005$) (Figure 3- 4).

The higher nitrogen availability and lower microbial biomass of Larch Park soil compared to Kinsella's generally indicates that Larch Park soil is highly disturbed and has experienced loss of ecosystem function and disconnected soil-plant relations as was discovered in chapter 2. These results are similar to the findings of previous studies on land reclamation (McMillan et al. 2007, MacKenzie and Quideau 2010).

There was a significant effect of plant on soil basal respiration ($P = 0.0028$). Plants can stimulate microbial respiration by contributing labile C to the soil (Rees et al. 2005). It's not surprising so soil basal respiration in SP treatments was significantly higher than S treatments. But there were no significant effects of soil type ($P = 0.7447$), biochar ($P = 0.0707$) and no significant interaction between them ($P = 0.4596$). However the graph showed that soil basal respiration in SB treatments was slightly higher than S treatments, but it was not significant (Figure 3- 5).

Metabolic quotient in Larch Park soil was significantly higher than Kinsella soil ($P<.0001$). But no significant effects of biochar ($P = 0.2731$), plant ($P = 0.1507$) and no significant interaction ($P = 0.6683$) were found (Figure 3- 6). Based on these results and those of chapter 2, we have found higher available nitrogen, lower microbial biomass and lower visual variability of microbial community structure at Larch Park soil compared to Kinsella soil. On the other hand, we have noticed higher metabolic quotient at Larch Park meaning that there is more respiration per unit of biomass. This may be due to the fact that microbes

are more active at Larch Park or soil organic matter is more recalcitrant at this site, therefore microbes are working harder to eat organic matter at Larch Park.

After measuring total plant biomass, we found no significant effects of soil type and biochar addition on plant biomass. But a significant interaction between soil type and biochar was found on total plant biomass ($P = 0.0184$) (Figure 3- 7). The results indicated that biochar addition had no significant impact on plant growth at Kinsella soil, but there was a significant reduction in plant growth with biochar at Larch Park soil. Also, plant growth at Larch Park soil without biochar was at the same level as Kinsella soil. It was difficult to explain the plant biomass results according to the nitrogen availability data. Total inorganic nitrogen reduced in the SBP treatment compared to the SP treatment at Kinsella soil (Figure 3- 2), while we found similar plant growth in these treatments (Figure 3- 7). In addition, total inorganic nitrogen increased with plant and biochar at Larch Park soil in the SBP treatment compared to the SP treatment as shown in Figure 3- 2, but we noticed a significant reduction in plant growth with biochar at Larch Park soil (Figure 3- 7). We expected to have higher plant growth and biomass by increasing available nitrogen, while the result was contrary to our expectation. Therefore, further research is required to explain the relation between nitrogen availability data and plant biomass.

Festuca hallii was considered to be a proper species for reclamation practices because it was expected to be a dominant species in Alberta after fire and grazing. Therefore, *Festuca hallii* seeds were initially used in this study for germination under greenhouse condition. But after one month we noticed that

Festuca hallii growth was very low, which could be due to the fact that *Festuca hallii* is a late-successional, long-lived perennial bunchgrass with a rhizomatous growth form, and it usually needs three to four years for establishment (Anderson 2006). Also, restoring *Festuca hallii* after disturbance may be difficult in the absence of regular fire, and this species has been reduced as a result of over grazing (Looman 1969, 1983). Therefore, it was decided to use a different type of Festuca for germination in greenhouse condition. This time *Festuca saximontana* (Rocky Mountain fescue) was selected, which is a proper species to use in revegatation for reclamation practices at high elevations, and it showed a good survival and growth rate. *Festuca saximontana* is found north to Alaska and south to California, Arizona and New Mexico. It grows in grasslands, meadows, open forests and sand dune of the northern plains, boreal and mountain areas and it offers good forage for livestock (Barkworth et al. 2007).

The results from this study indicated that the native plant species (*Festuca saximontana*) had a stimulatory effect on soil respiration, decreasing impact on total inorganic nitrogen concentration and increasing influence on microbial biomass carbon and nitrogen. Also we found non-significant decreasing effect of biochar on total inorganic nitrogen and non-significant stimulatory impact on soil respiration.

After performing the greenhouse experiment and finding some interesting results about the effects of native species and biochar amendment, we tried to apply biochar into the Larch Park and Kinsella soils under field condition in order to study the effects of biochar on soil-plant processes.

Figures

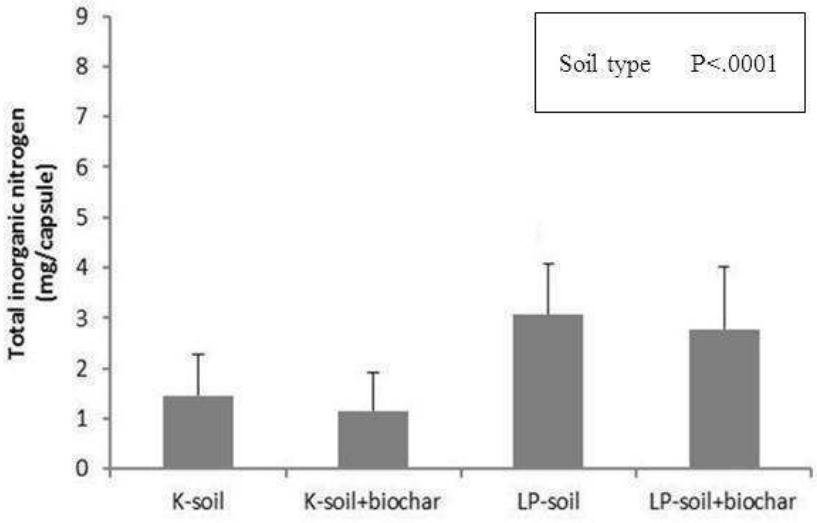

Figure 3- 1: Total inorganic nitrogen (mg/capsule) for Kinsella and Larch Park soils with and without biochar (no plant treatment), under greenhouse condition.

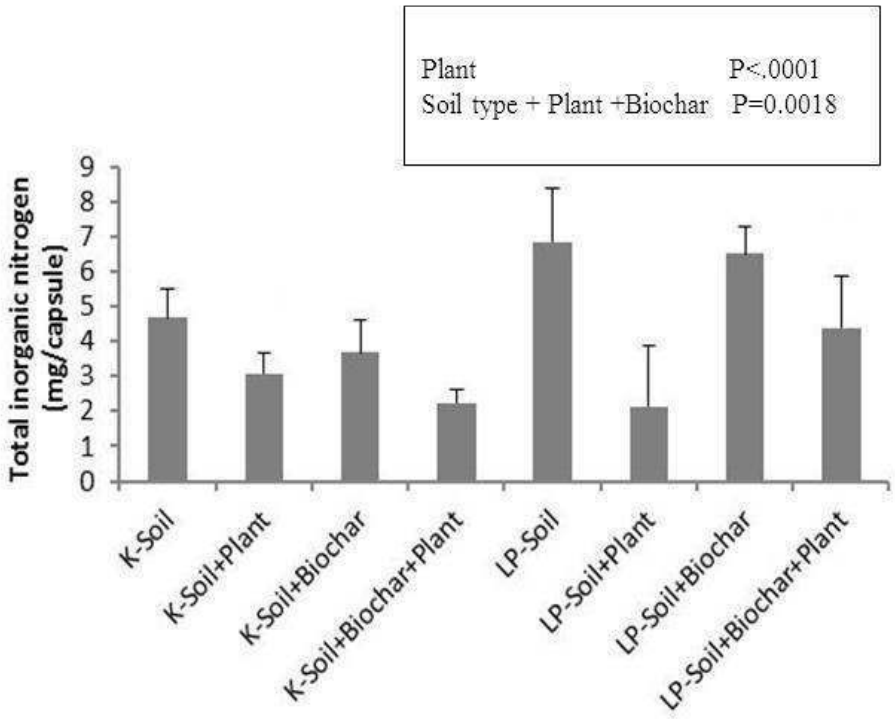

Figure 3- 2: Total inorganic nitrogen (mg/capsule) for Kinsella and Larch Park soils with and without biochar (with plant treatment), under greenhouse condition.

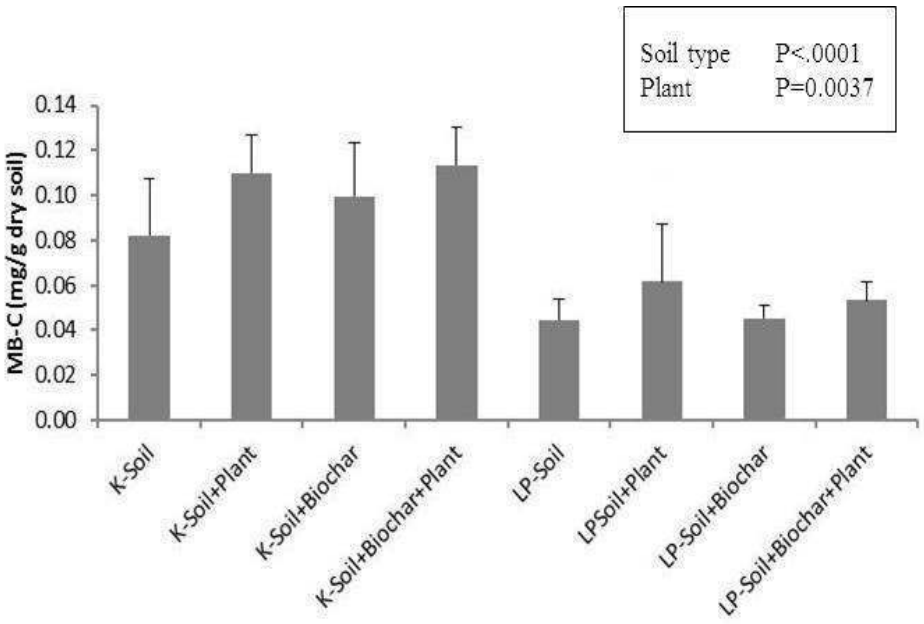

Figure 3- 3: Microbial biomass carbon (mg/g dry soil), at different treatments of Kinsella and Larch Park soils at greenhouse condition.

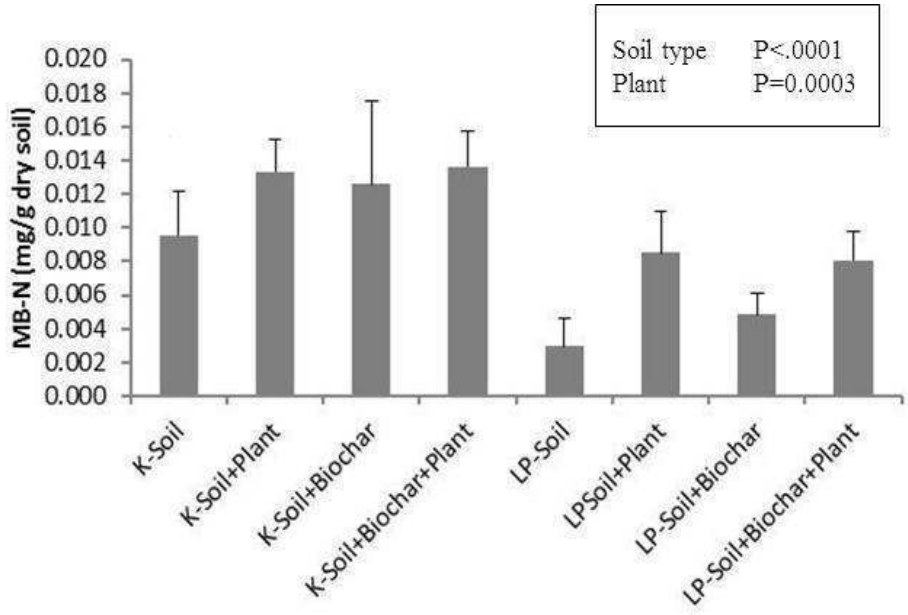

Figure 3- 4: Microbial biomass nitrogen (mg/g dry soil), at different treatments of Kinsella and Larch Park soils at greenhouse condition.

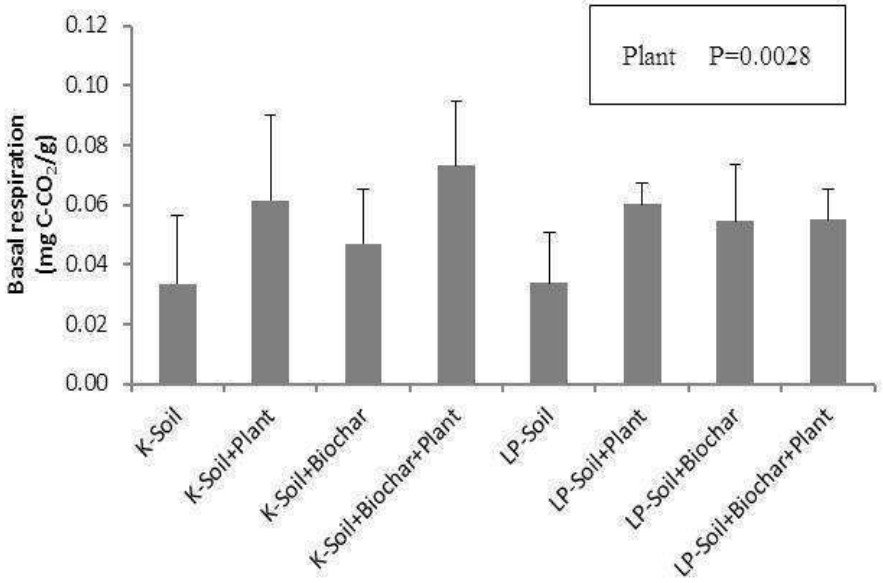

Figure 3- 5: Soil basal respiration (mg C-CO_2/g dry soil), at different treatments of Kinsella and Larch Park soils at greenhouse condition.

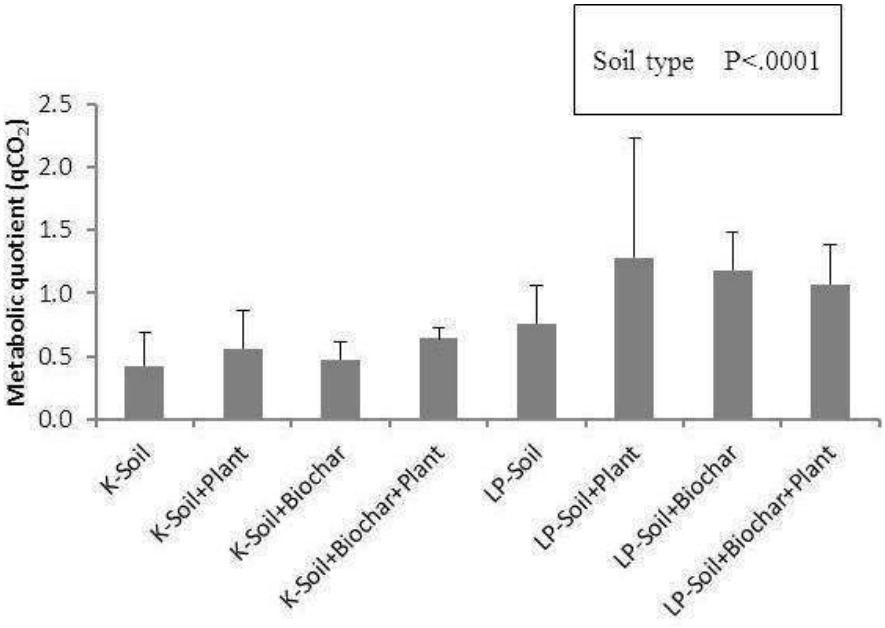

Figure 3- 6: Metabolic quotient (qCO_2), at different treatments of Kinsella and Larch Park soils at greenhouse condition.

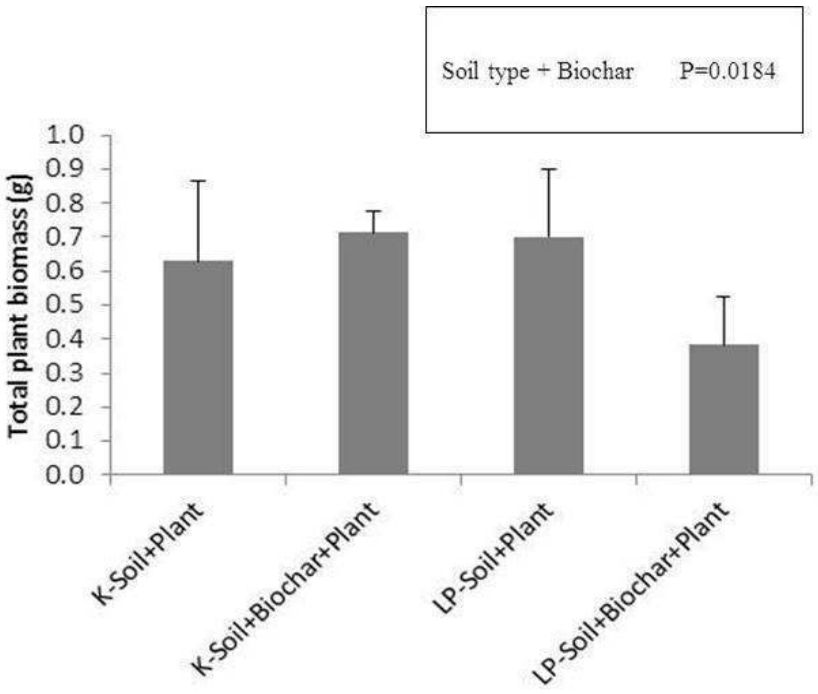

Figure 3- 7: Total plant biomass (g), for Kinsella and Larch Park soils with and without biochar at greenhouse condition.

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Chapter 4: Studying the Effects of Disturbance and Biochar Addition on Soil Processes

4.1 Introduction

Disturbance is a temporary alteration of ecosystem function, physical environment, and soil biological processes (Pickett 1985). It can be mediated by natural or anthropogenic processes. Natural disturbances such as fire and flooding are generally influenced by weather condition, climate, and location (Pickett 1985, Virginia H. Dale 2001). These conditions usually happen in a cyclic pattern and disturbances may be periodic with a specific time interval. However, disturbances caused by human activities such as harvesting, mining, and housing development can take place everywhere and they are not essentially following cyclic pattern (Pickett 1985, Rogers 1996, Virginia H. Dale 2001). The role of natural disturbance in conserving species diversity is an important principle in ecology and recently the maintenance of proper disturbance regimes has become accepted as a practical approach in conservation biology (Harrison et al. 2003).

One of the most important agents of natural disturbance in western ecosystems is fire, with the ability to change successional organization (Neary et al. 1999, Hart et al. 2005). Reduced plant density post-fire can enhance solar penetration and soil temperature; therefore the soil microclimate can be changed (Neary et al. 1999, Hart et al. 2005). Fire increases soil organic matter oxidation, consequently changing its chemical composition (Fernandez et al. 1997), but the level of organic matter oxidation relies on fire temperature, fire duration, and heat

penetration (Hungerford et al. 1991). Fire may modify soil communities over the long term by changing plant community composition through plant-based alterations in the soil environment (Hart et al. 2005).

Fire also has variable effects on grasslands. Grassland topsoil layer is one of the most important carbon sink on earth, because of high accumulation of organic matter (Bailey et al. 2010). Fire infrequently consumes the sequestered carbon in the grasslands due to high organic matter content. By contrast, during crown fire in forests, the aboveground carbon sink releases a lot of carbon into the atmosphere (Bailey et al. 2010).

Main residue of fire is charcoal, charcoal is generated during natural fire in forest and prairie environments through the partial combustion of organic materials (Preston and Schmidt 2006). Charred carbon is an important component of Black Chernozemic soils in the native grasslands of the prairie region where it has been shown to contribute up to 45 % of the total carbon (Ponomarenko and Anderson 2001). Incidence of fires under cool and moist conditions can increase accumulation of char in prairie soils (Ponomarenko and Anderson 2001). Man-made charcoal is called biochar. Biochar is the carbon-rich solid created through pyrolysis, a thermal degradation of organic materials in the absence of oxygen (Lehmann et al. 2011). Charcoal or black carbon is chemically and biologically stable because of its polycyclic aromatic structure, thus charcoal may be persisting in the environment for centuries (see Preston and Schmidt 2006 for a complete review). Biochar can be used as a soil conditioner (Lehmann et al. 2011), and it has the potential to mitigate climate change through carbon

sequestration (Woolf et al. 2010, Lehmann et al. 2011). In addition, biochar has been reported to develop root growth by improving the chemical and physical characteristics of soil such as nutrient, pH, aeration and water holding capacity. Adding biochar to soil can reduce soil bulk density, improve nutrient retention through cation adsorption and may also change soil biological community composition and abundance (Lehmann et al. 2011). By adding biochar to the soil, microbial community structure and function may be shifted by altering physicochemical properties of the soil (Smith et al. 2010). More detail on biochar can be found on chapter 3.

4.2 Research questions and objectives

Do soil disturbance and biochar addition affect soil processes in reclaimed and natural grassland sites?

1. The first objective was to measure nitrogen availability in disturbed plots with and without biochar.
2. The second objective was to characterize soil microbial community function and structure in disturbed plots with and without biochar.

4.3 Materials and methods

4.3.1 Experimental design

The effects of disturbance and biochar addition to soil processes including nitrogen availability and microbial community dynamics were measured by creating experimental plots at the reclaimed and the natural grassland sites in June 2011. Four replicated plots (dimension of 2m*3m) with six different treatments

were created at Kinsella, and four replicated plots (dimension of 1m*3m) with three different treatments were created at Larch Park (Figure 4- 1). Some treatments were amended with biochar as a fire surrogate, to examine if this C substrate might have impact on soil processes. The biochar, produced from wheat straw, had 83% moisture content and 65.6 % carbon content. 1.64 kg dry biochar was added into the soil in each plot, representing 30% of the soil total carbon as biochar carbon.

Treatments for disturbed plots at Kinsella are listed below:

- Control
- No-vegetation / With organic layer (representing grazing)
- No-vegetation / With organic layer / Biochar (simulating low intensity fire)
- No-vegetation / No organic layer / Biochar (standing for high intensity fire)
- No-vegetation / Disturbed soil (representing tillage)
- No-vegetation / Disturbed soil / Biochar (representing Larch Park with biochar addition)

The reclaimed site (Larch Park) is already disturbed; therefore the disturbed plots at Larch Park just had 3 treatments as listed below:

- Control
- No-vegetation / No organic layer / Biochar
- No-vegetation / Re-disturbed soil / Biochar

Ion exchange resin (IER)

Two resin capsules were placed inside each treatment in the disturbed plots at Kinsella and Larch Park on June 2011 to determine if nitrogen availability is affected by different treatments. Resin capsules were collected at the end of August and were transported back to the laboratory for analyses.

Soil sampling

Soil samples were taken at the end of August 2011 from 0 to 15 cm depth at five different points inside each treatment at Kinsella and Larch Park (Figure 4-2). They were mixed together to get a composite sample for reducing the variation inside each treatment and increasing the homogeneity of the soil samples. Moisture content, pH, respiration, total carbon, total nitrogen and microbial community structure (PLFA) were measured on collected samples. Soil samples were collected using a metal soil probe 2.5 cm in diameter. All sampling equipment was cleaned with 70% ethanol between samples to avoid phospholipid contamination. Samples were put into separate sterile Whirl-Pak® Sampling Bags (Nasco, USA). The samples were placed in a cooler and kept cold with ice packs until transportation back to the laboratory, then they were stored at 4 °C, except for PLFA samples, which were immediately stored at -80 °C and freeze-dried before phospholipid fatty acid extraction.

4.3.2 Laboratory analyses

PH of soil was measured by adding 0.01M calcium chloride to oven dried soil (not sieved) using a 1: 2 soil-to-solution ratio (Kalra and Maynard 1991). Moisture content of the samples was calculated based on gravimetric method by

weighing soil samples before and after oven drying at 105°C for 24 hours (Kalra and Maynard 1991).

For assessing total carbon and nitrogen (TC/TN), 1-5 g dry soil was ground (Retsch MM200 ball mill grinder). Ground samples were then encapsulated and tested for total carbon and total nitrogen by dry combustion (Costech Analytical Technologies Inc., Valencia, CA, USA) (Norris et al. 2011, Hahn and Quideau 2012).

The alkali trap method was used to measure soil microbial respiration. In this method, 50 g field fresh soil was weighed and placed in a 1L glass jar. An open scintillation vial containing 20 ml of 0.5 M sodium hydroxide (NaOH) was carefully placed into glass jar, the jar was then sealed and incubated at room temperature (25°C) for 7 days. In this method, evolved CO_2 was trapped in 0.5M sodium hydroxide (NaOH) to produce sodium carbonate (Na_2CO_3). After incubation, the scintillation vial was removed and capped immediately until titration with 0.5 M hydrochloric acid (HCl) to a clear end point (Zibilske 1994, Hopkins 2007).

Also, another set of respiration test was done on re-wet soil samples. Soil respiration was normalized by adding water to the soil samples to bring them up to moisture content of 20%. Respiration can be controlled by four different factors including temperature, moisture, types of microorganisms and types of carbon (such as soil organic matter and biochar). The effects of moisture and temperature can be controlled in the laboratory environment. By having the same moisture

content in all of soil samples and controlling temperature, the differences between respiration data will be due to the type of carbon and type of microorganisms.

Nitrogen availability of the soil was measured using ion exchange resin (IER) capsules (Dobermann et al. 1997). Resin capsules were inserted into the soil at Larch Park and Kinsella for specific time intervals, then they were collected and stored separately in plastic bags. Resin capsules were kept cold in coolers with ice packs until arrival at the laboratory where they were extracted with 2M KCl. Extracted solutions were then analyzed for ammonium and nitrate.

Phospholipid fatty acid (PLFA) analysis was performed to characterize the soil microbial community structure. PLFA samples were freeze dried before starting phospholipid fatty acid extraction. Polar lipids were extracted from the freeze-dried soil samples (2 g) with a modified Bligh and Dyer extraction (Bligh and Dyer 1959, Frostegård and Bååth 1996). The extracted solutions were filtered by pre-packed silicic acid columns (Agilent Technologies, Wilmington, DE), then they were subjected to a mild alkaline methanolysis to form fatty acid methyl esters (FAMEs). An Agilent 6890 Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE) with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column was used to separate the FAMEs. The individual fatty acids were identified by the MIDI peak identification software (MIDI, Inc., Newark, DE) (Hannam et al. 2006, Swallow et al. 2009, Hahn and Quideau 2012).

4.3.3 Statistical analyses

To test for systematic effects of disturbance, biochar addition or any interaction of both variables, analysis of variance (ANOVA) was performed. Two-way ANOVA was done on total inorganic nitrogen, respiration, total carbon and total nitrogen data. SAS software (version 9.2) was used to check the assumptions of normality and homogeneity of variance, and to perform two-way ANOVA. Factors were considered significant at an alpha of 0.05.

Total inorganic nitrogen data at Kinsella was not normally distributed among the disturbance and biochar variables, so was log transformed. Soil respiration data for field fresh samples from Larch Park were not normal for disturbance variable, thus the data were log transformed to meet the ANOVA assumptions.

Non metric multidimensional scaling (NMS) was used to examine PLFA data using PC-ORD (version 6, MjM Software Design). NMS is an iterative method that organizes the data in a reduced number of dimensions based on distances between data points. Therefore, the distances in the ordination graph represent the similarities or dissimilarities in community structure of the original data. The Sorensen (Bray–Curtis) distance measurement was used in the ordination. NMS is not assuming a linear relationship between variables, so this method can be considered more appropriate than many other ordination methods (Hannam et al. 2006, Norris et al. 2011). The main matrix was included PLFA biomarkers measured and expressed on a nmol g^{-1} basis, relativized by row and transformed using the arcsine square-root function. The second matrix contained

selected soil variables, site parameters and bio-indicators including calculated microbial indices.

4.4 Results

There were no significant effects of disturbance and biochar on total carbon and total nitrogen at Kinsella or Larch Park (data not shown here).

Biochar addition had no significant effect on total inorganic nitrogen concentrations at either Kinsella or Larch Park; however there was a significant effect of disturbance found at Kinsella ($P = 0.0001$), but not at Larch Park ($P = 0.8431$), and no significant interaction between disturbance and biochar were found at Kinsella or Larch Park (Figure 4- 3 and Figure 4- 4).

No significant effects of disturbance and biochar were found at Kinsella or Larch Park for soil basal respiration on field fresh samples (Figure 4- 5 and Figure 4- 6) and re-wet soil samples (Figure 4- 7, just Kinsella data).

The NMS ordination of the soil microbial communities made a two-dimensional ordination solution with a final stress of 11.97 after 44 iterations with 92% of the variation explained (Figure 4- 8). Microbial community structures at Larch Park and Kinsella were different. The variability of microbial community at Kinsella was higher than Larch Park based on the spread of data in the ordination graph; data are more scattered at Kinsella compared to Larch Park. Undisturbed community was dominated by fungi, gram negative bacteria, arbuscular mycorrhizal fungi (AMF) and total biomass, while the reclaimed site was dominated by actinobacteria. Also fungi to bacteria ratio, pH and respiration (for

re-wet soil sample) were higher at Kinsella compared to Larch Park, so they might be responsible for driving the ordination (Figure 4- 8).

4.5 Discussion

4.5.1 The effects of biochar on nitrogen availability and microbial activity

Several studies have indicated that adding biochar to soil caused soil fertility enhancement (Marris 2006, Steinbeiss et al. 2009). Biochar has also been shown to improve physical properties of soil by increasing specific surface area, enhancing water-holding capacity and increasing cation exchange capacity (CEC) (Anderson et al. 2011, Lehmann et al. 2011).

Biochar has the potential to absorb available nitrogen (Clough and Condron 2010), our results indicated that by adding biochar to the soil at Kinsella the level of total inorganic nitrogen declined slightly, but this decrease was not significant (Figure 4- 3). We also found a significant effect of disturbance on total inorganic nitrogen at Kinsella (Figure 4- 3) as disturbance increases nitrogen mineralization (McMillan et al. 2007), but not at Larch Park. However, the concentration of total inorganic nitrogen at "No-vegetation / Re-disturbed soil / Biochar" treatment at Larch Park was slightly higher than other treatments, but not significantally (Figure 4- 4).

Despite adding 30% of soil total carbon as biochar carbon into the soil in each treatment, we did not notice significant impacts of biochar on nitrogen availability or soil microbial activity. It might be as a result of biochar loss by wind, mineralization during microbial respiration, leaching through precipitation, or not deep enough biochar addition into the soil. Based on the literature, people

believe that biochar can remain in soil over long periods of time (Lehmann et al. 2011), but further research is required to have more information about biochar resistivity. In addition, Larch Park and Kinsella soils were classified as black chernozems which were high in nitrogen content, so biochar might not be able to increase fertility in this type of soil.

Soil basal respiration for field fresh samples at Kinsella was reduced in "No-vegetation / With organic layer / Biochar" treatment compared to the same treatment without biochar. Reduced respiration indicated that microbes were being suppressed in this treatment, but this decrease was not significant (Figure 4-5). While, the amount of respiration for field fresh samples at Larch Park was increased slightly in treatments with biochar, but this increase was also not significant (Figure 4- 6). Testing soil respiration on re-wet samples at Kinsella (Figure 4- 7) indicated that the level of respiration has been decreased gradually at disturbed treatments; it can be as a result of drastic effect of disturbance on soil microbial activity. We also found that "No-vegetation / With organic layer / Biochar" treatment had higher soil respiration than other treatments which might be due to the stimulatory impact of biochar on soil respiration (Figure 4- 7). However, these differences were not significant. Soil respiration measurement indicated a slight stimulation of microbial activities after applying biochar amendment to the soil in some treatment, but some other treatments showed respiration rates similar to the control treatment. This stimulatory effect of biochar on soil respiration might be also due to increased moisture content in the re-wet soil samples (Figure 4- 7). As a result of the increased respiration in some

treatments that received biochar we can suggest that carbon mineralization may be increased by adding biochar to the soil. But further research is needed in order to better understand the impact of biochar on soil processes.

4.5.2 The impacts of biochar addition on soil microbial community structure

One-year after applying disturbance and biochar treatments in experimental plots, PLFA data indicated non-significant impacts on soil microbial community structure, which is in agreement with the previous study (Attaeian 2010). Attaeian (2010) found that warming and defoliation treatments had limited effects on soil microbial structure in the natural grassland system. Therefore, based on stability in microbial structure, she concluded that the soil microbial community in this ecosystem might be relatively resistant to climate warming, which can be considered as a form of disturbance. This was reflected in the current research as well, where microbial communities were resilient to heavy physical disturbance at Larch Park. In addition, Larch Park was used to be an agricultural land; therefore the land-use prior to reclamation might cause a shift in microbial community.

The NMS ordination of PLFA data showed that the soil microbial community at the rebuilt site (Larch Park) was different from the natural grassland site (Kinsella), which is in agreement with the previous study on restored riparian soils of the Canadian prairie pothole region (Card and Quideau 2010). The variability of microbial community at Kinsella was higher than Larch Park because disturbance followed by land reclamation at Larch Park had significant influence on microbial community including fungi. Previous study

indicated that soil disturbance has negative effects on fungal growth as shown by reduced fungal biomarkers and increased bacterial dominance (Mummey et al. 2002). In addition, soil microbial communities of undisturbed terrestrial ecosystems have a tendency towards fungal microbial biomass dominance, which contributes to a high FBR (Mummey et al. 2002). Our finding is in agreement with these studies.

By comparing the PLFA ordination graphs of the disturbed plots and phytometer (*Festuca hallii*) in chapter 2, we found some differences in the soil microbial community composition in these areas. The ordination graph of the PLFA data under *Festuca hallii* indicated that the microbial community at Kinsella was dominated by fungi and total biomass, while Larch Park was dominated by actinobacteria and arbuscular mycorrhizal fungi (AFM) (Figure 2-14). On the other hand, the PLFA ordination graph from the disturbed plots showed that the undisturbed community was dominated by fungi, gram negative bacteria, arbuscular mycorrhizal fungi (AMF) and total biomass. But the reclaimed site was dominated by actinobacteria (Figure 4- 8).

Figures

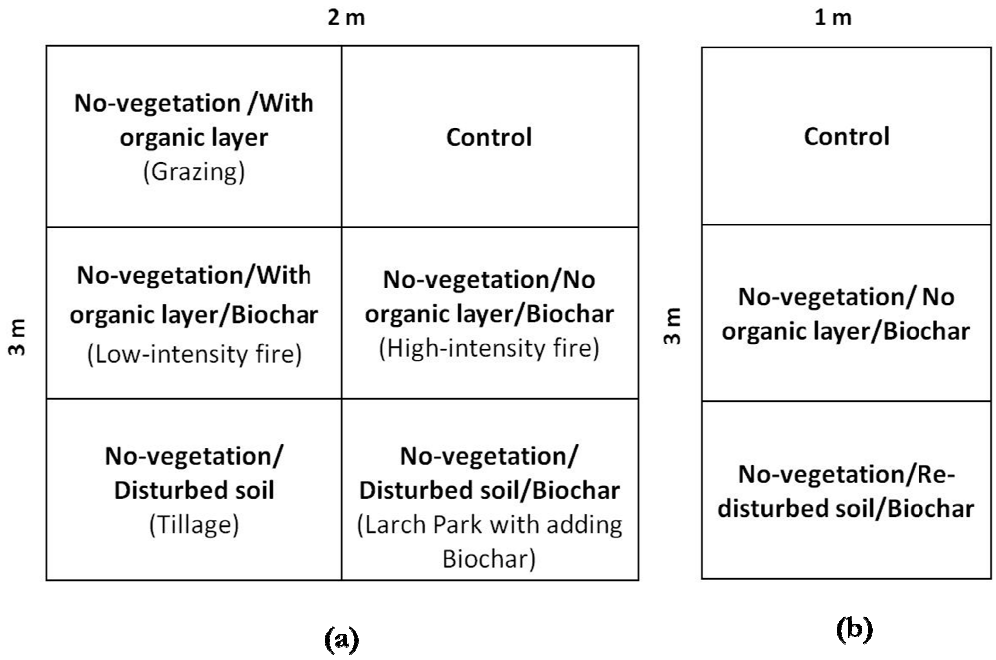

Figure 4- 1: The pattern of disturbed plots with all treatments, at a) the native grassland site and b) the reclaimed site.

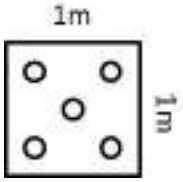

Figure 4- 2: Pattern of collecting soil samples at disturbed plots; 5 soil samples were collected from each treatment and mixed together as a composite sample.

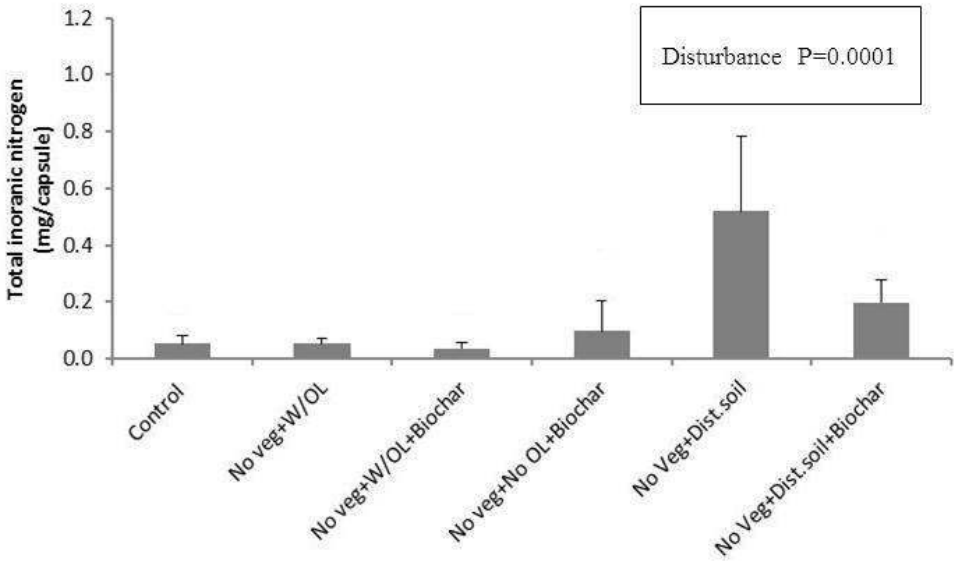

Figure 4- 3: Total inorganic nitrogen (mg/capsule), at Kinsella under disturbed plots (within 6 treatments), summer resin sampling.

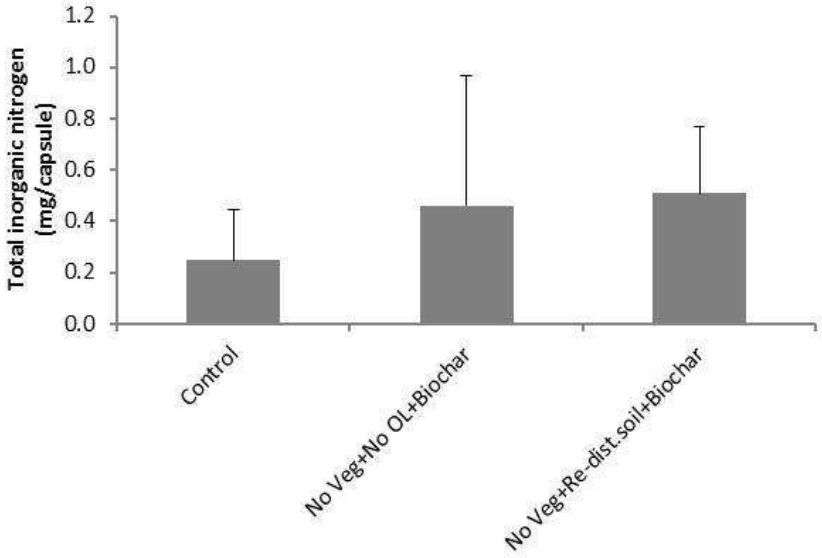

Figure 4- 4: Total inorganic nitrogen (mg/capsule), at Larch Park under disturbed plots (within 3 treatments), summer resin sampling.

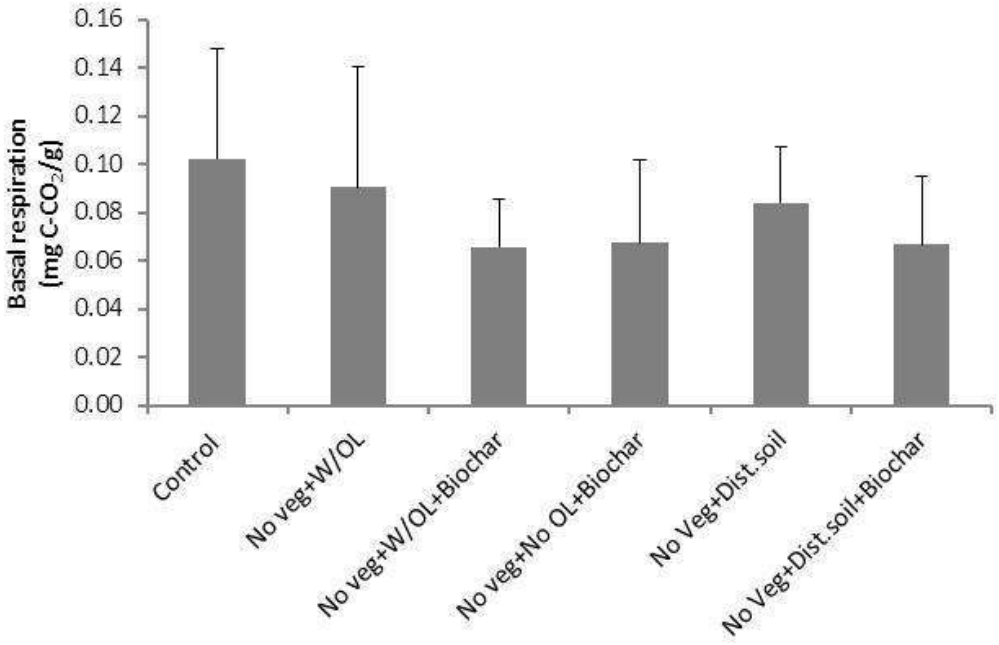

Figure 4- 5: Soil respiration (mg C-CO_2/g dry soil), at Kinsella under disturbed plots (within 6 treatments). Experiment performed on field fresh soil samples.

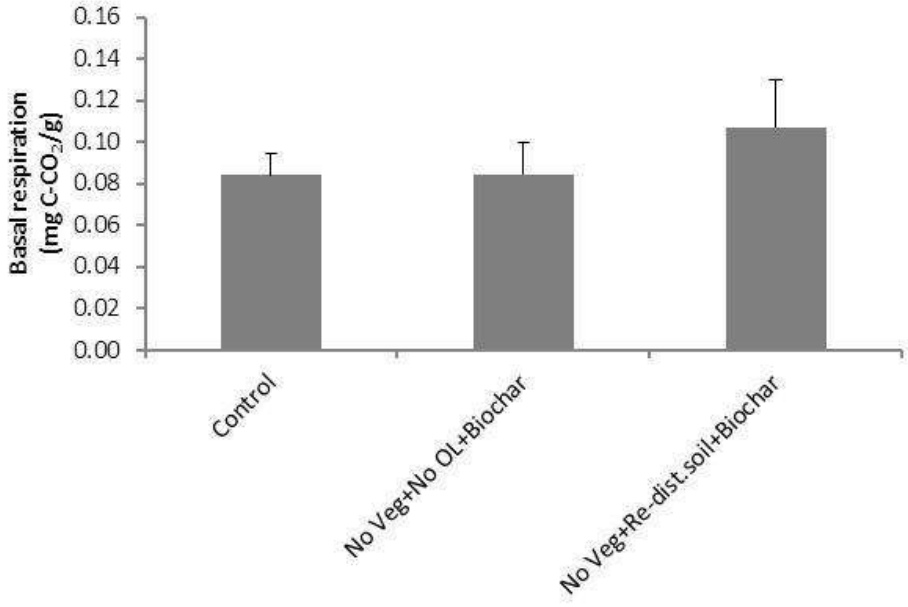

Figure 4- 6: Soil respiration (mg C-CO_2/g dry soil), at Larch Park under disturbed plots (within 3 treatments). Experiment performed on field fresh soil samples.

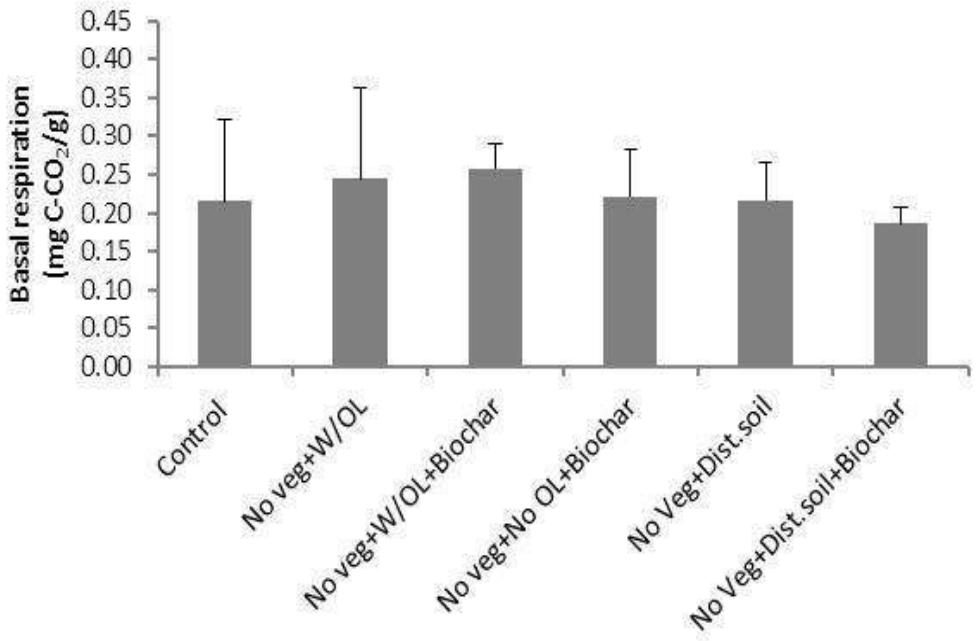

Figure 4- 7: Soil respiration (mg C-CO_2/g dry soil), at Kinsella under disturbed plots (within 6 treatments). Experiment performed on re-wet soil samples.

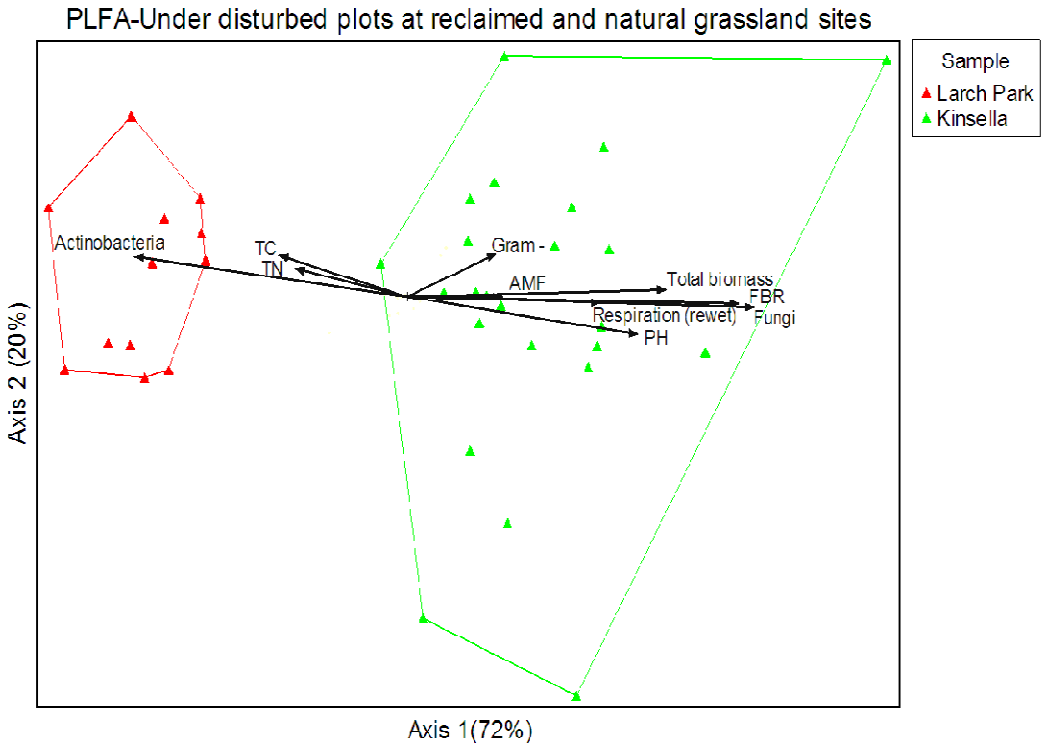

Figure 4- 8: Non-metric multidimensional scaling ordination of microbial phospholipids fatty acid (PLFA) data for Larch Park and Kinsella under disturbed plots. The proportion of variance explained by each axis is based on the correlation between distance in the ordination space and distance in the original space, and is reported after each axis heading. Data are more scattered at the native grassland site compared to the reclaimed site, so the variability of microbial community at Kinsella was higher than Larch Park. Kinsella was dominated by fungi, gram negative bacteria, arbuscular mycorrhizal fungi (AMF) and total biomass, while Larch Park was dominated by actinobacteria. Also fungi to bacteria ratio (FBR), pH and respiration (for re-wet soil sample) at Kinsella were responsible for driving the ordination.

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Chapter 5: Summary and Conclusions

5.1 Summary

Restoring native plant communities in urban development area is a novel practice and was the focus of this study. By using salvaged soil, planting native grassland communities, and adding biochar as a fire surrogate to the soil, we tried to re-establish ecosystem processes comparable to those of natural sites in as short time as possible. We expected that ecosystem function and services in the reclaimed site including carbon sequestration, plant productivity and wildlife habitat for birds, insects and amphibians to be more similar to native ecosystems and require less maintenance such as watering, fertilization, and weed control. A greenhouse study was also conducted to examine the effects of biochar and native species on soil processes.

The level of similarity of the reclaimed site (Larch Park) to the native grassland site (Kinsella), in terms of ecosystem functions and services, could determine the reclamation success. We examined ecosystem function in the reclaimed and the natural grassland sites by measuring soil nitrogen availability using resin capsules, soil microbial biomass C and N by chloroform fumigation-extraction method, microbial respiration by alkali trap method and microbial community structure with phospholipid fatty acid (PLFA) analysis.

5.2 Conclusion

We found significant differences in soil properties of the natural grassland and the reclaimed sites in many cases, indicating that the goal of re-establishing natural ecosystem function has not been achieved during this period of time.

Higher nitrogen availability, lower microbial biomass, and lower visual variability of microbial community structure in Larch Park compared to Kinsella are the result of disturbance followed by reclamation at Larch Park.

The NMS ordination of PLFA data for phytometer (*Festuca hallii*) and also disturbed plots, showed that the soil microbial community composition at the rebuilt site was different from the natural grassland site. The variability of microbial community at Kinsella was higher than Larch Park as disturbance had significant impact on microbial community composition.

As a final type of analysis, we combined the PLFA data for phytometer and disturbed plots together and examined them by non metric multidimensional scaling (NMS) ordination using PC-ORD (version 6, MjM Software Design). The NMS ordination of the soil microbial communities for phytometer and disturbed plots together made a three-dimensional ordination solution with a final stress of 8.97 after 53 iterations with 95% of the variation explained. The visual variability of microbial community at Kinsella was higher than Larch Park. Larch Park was dominated by actinobacteria, while Kinsella was dominated by fungi. Also, fungi to bacteria ratio, respiration, and total biomass were higher at Kinsella compared to Larch Park (Figure 5- 1).

In addition, Multi-Response Permutation Procedures (MRPP) analysis of the PLFA data for both phytometer and disturbed plots together performed using PC-ORD (Version 6, MjM Software Design). Groups were defined by treatments and Euclidean distance measurement was used. The MRPP results indicated a clear separation between the treatments at Larch Park and Kinsella (T= -13.339, A= 0.2, P= 0.00). Therefore, based on the good separation between the treatments we can conclude that biochar might have an impact on shifting soil microbial community structure to some extent, however it did not have a significant effect on soil chemical characteristics such as nitrogen availability.

To control the high level of available nitrogen at Larch Park, biochar amendment was applied into the soil. Biochar has the potential to affect soil fertility and possibly mitigate climate change through carbon sequestration (Woolf et al. 2010, Lehmann et al. 2011). The results indicated that biochar had some significant interaction effect on soil-plant processes. Lehmann et al.(2011) has reported that biochar can remain in soil over long periods of time. But based on our results from chapter 4, we could conclude that in field condition biochar might be blown off by wind, mineralized as a result of microbial respiration, or leached out because of precipitation. It was also possible that the amount of biochar added to the soil was not enough.

Greenhouse experiment indicated stimulatory effects of native species on microbial biomass and respiration, and decreasing impact on nitrogen availability. Also we found non-significant decreasing effect of biochar on total inorganic nitrogen and non-significant stimulatory impact on soil respiration, but further

studies are required to study the long term behavior of biochar in natural and reclaimed ecosystems.

In general, the data for this study were collected through one growing season after reclamation, but this time frame is not long enough for re-establishing natural processes and plant-soil microorganism connections. It could be possible that the soil structure has not formed yet and the soil microbial communities have not developed to the similar level of characteristics of natural ecosystems. Thus, the development of soil structure and establishment of plant-soil microorganism connections may only be achieved after specific period of time.

5.3 Reclamation Recommendation

The discrete dissimilarity between the reclaimed and the natural grassland sites shows the need for more research into the differences between soil processes in these sites, to examine what kind of management practices could be made to accelerate the reclamation and restoration projects.

Soils were rebuilt at Larch Park to have soil layers resembling the natural soil horizons present before development and to ensure that rebuilt soils are as similar to native soils as possible. The three soil horizons of chernozemic soil (Ah, Bt_1 and Bt_2) were removed and stored separately on the site. During reclamation, soil profiles were created by replacing lower soil (LS), upper soil (US), and top soil (TS) from bottom to the surface, respectively (www.gov.ab.ca/env/). Top soil had high available nitrogen and so high probability of growing weedy species. The elevated level of nitrogen availability

could be controlled by adding biochar to the soil surface. It is also suggested that a mix of marginal soil and biochar be used instead of the rich top soil on the surface layer.

Native species were planted in Larch Park in order to enhance native biodiversity and wild life habitat in the reclaimed site. Plant species have different ability to adapt to drastic changes during disturbance (McKinney 2002). We noticed that *Festuca hallii* was not ideal species for urban land reclamation. *Festuca hallii* is a late-successional, long-lived perennial bunchgrass, with a rhizomatous growth form, and it usually need three to four years for establishment (Anderson 2006). This species has been reduced as a result of over grazing (Looman 1969, 1983). *Festuca hallii* can be considered as an urban avoider species. Urban avoiders are plant species that are very sensitive to human activities and habitat disturbance, and they would include late successional plants (McKinney 2002). Therefore, it is suggested that other type of Festuca be used for reclamation practices. It was decided to use *Festuca saximontana* (Rocky Mountain fescue) for germination in greenhouse experiment; which is a proper species to use in revegetation for reclamation practices at high elevations. It grows in grasslands, meadows, open forests and sand dune of the northern plains, boreal and mountain areas and it offers good forage for livestock (Barkworth et al. 2007). This native fescue showed very well growth under the greenhouse experiment for this research.

Figures

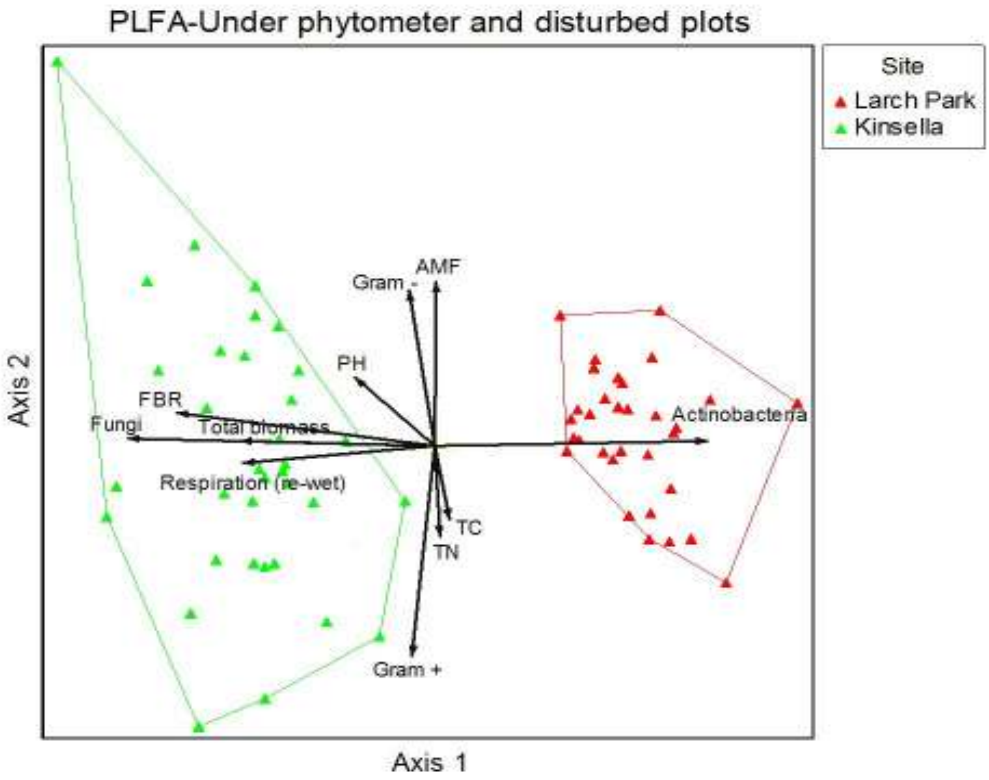

Figure 5- 1: Non-metric multidimensional scaling ordination of microbial phospholipids fatty acid (PLFA) data for both phytometer and disturbed plots at Kinsella and Larch Park. The variability of microbial community at Kinsella was higher than Larch Park. Larch Park was dominated by actinobacteria, while Kinsella was dominated by fungi. Fungi to bacteria ratio, respiration, and total biomass were higher at Kinsella compared to Larch Park.

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