七転び八起き

“Fall down seven times, stand up eight”

-Japanese proverb

University of Alberta

The ecology of boreal forest floor microbial communities in relation to environmental factors

by

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Dedication

I dedicate this work to my grandfather, Fred Swallow. You and your memorable words “The world would be much better place if everyone simply got a good night’s rest” are often on my mind.

Abstract

Soil microbial communities in boreal forests are structured by complex interactions among many factors operating simultaneously on large and small spatial scales. Of particular note in the boreal mixedwood, the microbial communities under trembling aspen and white spruce forest floors are distinct. This thesis highlights linkages that connect ecosystem characteristics observable on the landscape with microscopic processes occurring within boreal forest floors.

As indicated by phospholipid fatty acid (PLFA) and multi-substrate induced respiration analysis, prescribed burning after harvest did not alter microbial community structure or function in aspen, spruce and mixedwood stand. Instead, community structure was related to the pre-harvest overstory and local topography, with communities under aspen being different than those under similarly structured spruce and mixedwood canopies but in sites located at lower landscape positions. Microbial communities in spruce forest floor were similar regardless of the amount of moisture retained throughout the incubation, while in aspen, community structure depended on the level of moisture. Microbial community response to moisture in aspen and spruce was linked to different physical properties and subsequently, the pore habitat, inherent to the two forest floors. Microbial community structure in aspen leaf litter inoculated with forest floor bacteria is altered when ciliates are present. Ciliates moderated the growth of gram negative bacteria, potentially grazed on fungi and promoted bacteria that consumed plant auxins. However, I learned that PLFA analysis may not be a

suitable method to detect ciliates as ciliate lipid biomarkers were either absent or not correlated with ciliate abundance.

Forest floor microbial communities are shaped by the pore habitat, which in turn is generated by the litter of the plant community and predatory activity of protozoa. However, being aquatic organisms, protozoa can function only when suitable water filled pore habitat is available. On the landscape these processes depend on factors such as topography to redistribute moisture. Under these circumstances, differences between the physical properties of aspen and spruce forest floors manifest and influence the microbial communities residing within them.

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Abbreviation	Definition
DOC	dissolved organic carbon
DON	dissolved organic nitrogen
EMEND	ecosystem management emulating natural disturbance
FAME	fatty acid methyl ester
IAA	indole-3-acetic acid
MBC	microbial biomass carbon
MBN	microbial biomass nitrogen
MC	moisture content
MRPP	multi-response permutation procedure
MRT	multivariate regression tree
multi-SIR	multiple carbon-source substrate induced respiration
NMS	non-metric multidimensional scaling
PLFA	phospholipid fatty acid

Chapter 1. Introduction

1.1 The Boreal Forest and the Forest Floor

Roughly 30% of the world's boreal forest is located in Canada's boreal ecozones (Canadian Forest Service, 2011). The Canadian boreal forest covers approximately 397 million hectares, the majority of which are affected by human activity (Canadian Forest Service, 2011). This region is home to over 20,000 species of insects (Danks and Foottit, 1989) and provides breeding habitat for 250 bird species of which the majority are migrants from more southern areas (Erskine, 1977). In addition to providing habitats for wildlife, the Canadian boreal is a major component of the global carbon cycle and has traditionally behaved as a carbon sink, sequestering on average 50 million tonnes of carbon a year (Stinson et al., 2011). However, due to large scale natural and human disturbances, the future of the Canada's boreal region is unclear and could potentially begin to act as a net emitter of atmospheric carbon.

The boreal plains ecozone of Canada contains roughly 47 million hectares of forest and encompasses much of the landmass of central and northern Alberta (Power and Gillis, 2006). The majority of this forested area is composed of mixedwoods with upland regions containing large proportions of trembling aspen (*Populus tremuloides* Michx.) and white spruce (*Picea glauca* (Moench) Voss) (Rowe, 1972). Mixedwood forests containing both aspen and spruce in their overstories combine the structural elements inherent of both forests and often contain a higher diversity of floral and faunal species than pure forests alone (Macdonald et al., 2010). An area of particular importance to many researchers

working in pure and mixedwood forests is the forest floor, which occupies a relatively small portion of the forest when compared to the overstory and underlying mineral soil, yet is integral to the overall functioning of the forest ecosystem.

The forest floor is the zone where atmospheric moisture and gases interact with forest plants and the earth's surface. The product of this important environmental interface is an organic reservoir of plant and animal biomass at various stages of decomposition. The formation and composition of the forest floor arises from additions of fresh organic substrates, usually in the form of plant detritus, counteracted by losses of organic matter through decomposition. The latter part of this interaction is largely dependent upon the activity of the microbial community as forest floor litter is converted into fungal and bacterial biomass. This activity occurs in the organic layers of humus and freshly deposited plant detritus and forms the base of an intricate food web that involves multiple trophic levels of microfauna and larger mesofauna (Brussard et al., 1997).

The detrital food web of the forest floor contributes to larger regional and global biogeochemical cycles by transferring the energy and recycling the nutrients contained within the forest litter. Forest floor humus affects plant growth as it can immobilize nitrogen and other nutrients while its structure and thickness can affect seed germination (Prescott et al., 2000). Aspen and spruce forest floors have low bulk density (Redding et al., 2005) and have the potential to store large volumes of moisture derived from precipitation. The interaction of the forest floor with moisture not only affects local conditions, but also actively contributes to the

hydrology of the area in which it occurs (Devito et al., 2005). Finally, the forest floor in Canada contains vast reservoirs of carbon, estimated at roughly 7 billion tonnes, making it an important component of global carbon cycling (Goodale et al., 2002).

The boreal forest of Canada is an expansive and complex ecosystem. Changes to the forest caused by human and natural disturbance will have global implications, which at the moment remain speculative. The cumulative impact on a global scale will ultimately depend on how the smallest inhabitants of the forest (i.e.; soil microorganisms) respond to changes occurring within a few cubic centimeters. Understanding the interrelationships among these microorganisms that for the most part reside within the forest floor and how these communities are shaped by larger factors and disturbances is paramount if we are to predict how the boreal forest region of Canada will react to our uncertain climate future.

1.2 Environmental Factors and the Forest Floor

The formation of boreal soils began soon after the end of the last ice age. Soil development was conceptualized by Hans Jenny (1941) who based his "Cl,o,r,p,t" equation of soil formation on key factors. These factors include the climate of a region or "Cl"; the organisms present within the environment or "o"; the relief and topography of an area or "r"; the parent geological material or "p" and the time interval that the soil has had to develop or "t". This conceptual framework also includes the microorganisms of the forest floor (as part of the "o"

factor) and can be used as a basis to study their interaction with other soil forming factors and consequently their role in soil development.

At a distance, overstory trees, which are included within organisms under "Cl,o,r,p,t", are the most visually distinct living component of a forest. Overstory trees influence the plant understory by controlling the amount of solar radiation reaching the forest floor, competing for forest floor moisture, and shedding litter that affects the physical and chemical composition of the forest floor (Barbier et al., 2008). The combination of litter from the overstory and understory communities produces a chemical and physical environment that is unique to individual forest types (Vesterdal and Raulund-Rasmussen, 1998; Menyailo et al., 2002) which in turn, influences the activity and composition of the forest floor microbial community residing within it (Priha et al., 2001).

Topography can have a profound effect on forest ecosystems as outlined by Swanson et al. (1988). Topography affects how rain and solar radiation are intercepted by an area; it dictates how water, nutrients and other materials are translocated within a landscape by gravity; and it influences the spatial and temporal occurrence of natural disturbances. The boreal forest floor does not develop in isolation of these influences, therefore its properties, including the microbial community within it, will also be reflective of the surrounding topography. Downslope areas in boreal forests generally have more moisture, higher pH, thicker forest floors and lower C:N ratios than upslope locations (Seibert et al., 2007). Topographic variation over relatively small distances (<90 m) and very gentle slopes (2%) on the landscape can alter edaphic conditions such

as pH by the translocation of base cations to downslope positions (Giesler et al., 1998). These topographically affected conditions in turn will influence the community composition of the plant understory and ultimately the forest floor microbial community (Högberg et al., 2007). Topographic changes in the microbial community between upper slope and toe slope positions occur in grassland mineral soils (McCulley and Burke, 2004); however, it is unknown if similar topographical effects occur within the boreal forest floor.

A large proportion of the forest floor in northern Alberta has formed under white spruce (*Picea glauca* (Moench) Voss) and trembling aspen (*Populus tremuloides* Michx.). Forest floors under spruce are more acidic than under aspen (Menyailo et al., 2002; Grayston and Prescott, 2005) and are also a poorer substrate for microbes as they contain higher proportions of aromatic carbon (Hannam et al., 2004). Additionally, the physical environment also differs between aspen and spruce, with aspen forest floors being less thick but having a higher bulk density than spruce (Redding et al., 2005). Overall spruce forest floors have microbial communities that are lower in biomass and are structurally and functionally different from aspen (Hannam et al., 2006). This community distinction is quite strong and is retained even when portions of the forest floor from one forest type are transplanted into the other and left to incubate for a year under the environmental conditions generated by the corresponding overstory (Hannam et al., 2007).

In the boreal plains of Alberta, timber harvesting affects a large landscape through the harvest of millions of cubic meters a year of timber (Sustainable

Resource Development, 2010). The detrital foodweb in the forest floor continues to function even after removal of the canopy but does so in the absence of a major input of fresh litter. In this post-harvest environment, microbial biomass of the forest floor is reduced (Lindo and Visser, 2003), while the remaining organic material is enriched in aromatic carbon (Hannam et al., 2005). These changes are most likely due to loss of litter inputs from the overstory causing labile compounds to be consumed faster than they are replaced, which results in lower forest floor microbial biomass (Grady and Hart, 2006). However, the structure and function of microbial communities in aspen and spruce appears to be resilient to harvesting when measures are taken to lessen the mechanical disturbance on the forest floor (Hannam et al., 2006).

Forestry practices that conserve coarse woody debris help maintain parts of the forest ecosystem that provide the habitat for most saproxylic organisms and is indispensable for the diversity of wood-inhabiting fungi (Siitonen, 2001; Nordén et al., 2004). Forestry research sites such as EMEND have maintained coarse woody debris after harvest by redistributing slash and have implemented prescribed burns to promote early successional communities similar to those found after a fire event (Spence et al., 2002). Prescribed burning can alter the soil microbial community structure more strongly than harvesting alone (Bååth et al., 1995). However, the microbial community can quickly recover to pre-disturbance levels when fire is a frequent occurrence (D'Ascoli et al., 2005). Wildfire is common in the boreal regions of Alberta, yet it is largely unknown how the forest

floor microbial community will respond when prescribed burning is applied to harvested areas.

In humans, a doctor makes an initial diagnosis of a patient's affliction based on easily measurable symptoms that are caused by biochemical processes within the body. Treating an illness in the human body requires not only recognizing the visual symptoms but also understanding the processes causing them. Forest floors are also similar in that their properties can be associated with easily identifiable environmental factors visible on the landscape. However, as in the human body, what is observed results from many processes operating at much smaller scales. Identifying and understanding these processes only adds to the overall understanding of the forest floor and how it will respond to changes over time.

1.3 The Soil as a Habitat

Forest floor microorganisms exist and interact within the space occurring between the solid organic components of the forest floor. The pore environment within forest litter layers can occupy roughly 80-90% of the total volume (Voroney 2007) and constantly fluctuates between being an aquatic habitat when forest floor moisture is high and an air filled terrestrial habitat when moisture is low. Energy and nutrients are transferred between trophic levels within this habitat through complicated interactions among forest floor microorganisms (Elliot et al., 1980). Overall these trophic interactions depend on soil moisture dynamics and in combination form the complex detrital foodweb.

As the soil matrix dries, pore environments that were once connected become separated, causing nutrient limitations in some pore habitats as diffusion between water filled pores is inhibited or completely ceases (Stark and Firestone, 1995). Loss of moisture can also affect trophic interactions as larger aquatic soil organisms are isolated within disconnected pore habitats (Darbyshire, 1976). Over time the interaction between the pore physical environment and seasonal moisture fluctuations will affect the composition of the microbial community. Not surprisingly, microbial communities exposed to frequent wet and dry cycles are adapted to such conditions (Drenovsky et al., 2004, Frier et al., 2003; Griffiths et al., 2003; Lundquist et al., 1999), while communities not accustomed to frequent fluctuations will be less adapted and will undergo community changes when exposed to severe moisture limitations (Frier et al., 2003). The forest floor depends on the accumulation of litter within which occur recurrent changes in moisture; therefore, the microbial community found here should reflect the cumulative interactions between these factors. Spruce and aspen litters create forest floors with different physical characteristics (Redding et al., 2005), yet the impact of these differences upon the microbial communities of these forests is largely unknown.

Forest floor microbial communities and the processes they mediate are one of many integral components of soil formation. The community found within the forest floor environment results from the complex interaction of multiple factors, some likely still unknown, which operate simultaneously on both large and small spatial scales. Altering the fundamental structure of the forest pore environment,

through natural or human disturbance, could ultimately lead to a new relationship between soil moisture and soil organisms.

1.4 Soil Protozoa

Protozoa are single celled eukaryotic organisms that inhabit most aquatic environments on Earth including the water filled pores of soil (Finlay et al., 1999). They contribute to the detrital food web in the forest floor by grazing bacterial, fungal and other protozoan cells and also by ingesting metabolites produced by saprotrophic bacteria and fungi (Adl and Gupta, 2006). Their primary function in soils is summarized conceptually as the soil microbial loop, in which the nutrients and energy of saprotrophic biomass are made available to higher trophic levels of the detrital food web through protozoan grazing (Coleman, 1994). The soil microbial loop ultimately funnels the nutrients liberated by protozoan activity back to plants through root uptake. It has been demonstrated experimentally by comparing soil characteristics and plant growth in the presence and absence of protozoa. Studies using this methodology have consistently found that treatments that include protozoa have increased rates of soil nitrogen mineralisation and nitrification (Griffiths, 1986, Griffiths et al., 1999) as well as higher nitrogen contents in plant tissues (Chlarholm, 1985; Kuikman and Van Veen, 1989; Uikman et al., 1991).

The role of protozoa in the soil microbial loop includes indirect influences on plant growth in addition to the more direct nutritional effects of grazing (Bonkowski, 2004). Soil protozoa can alter the composition of soil microbial

communities by selective grazing (Rønn et al., 2002; Griffith et al., 1999). This activity can promote the growth of bacteria that produce plant auxins and leads to increased size and branching of plant root systems (Bonkowski and Brandt, 2002) as well as increased auxin content in plant tissues (Krome et al., 2010). Theoretically, increases in size and branching of the root system would produce more root contact with the soil matrix enabling plants to acquire more water and nutrients. However, enhanced plant growth in the presence of protozoa occurs even in the absence of plant auxin production, leading some to question its validity as a component of the soil microbial loop (Ekelund et al., 2009).

As a group, soil protozoa vary in size across three orders of magnitude (0.002 – 2 mm) and have traditionally been classified into groups based on their mode of mobility; the amoeba which are active on surfaces and move by extending cytoplasmic pseudopods; the flagellates which move in an erratic whip-like manner generated by large cilia; and the ciliates which are covered in fine hair-like cilia and have more direct straightforward movement. While this simple classification scheme is popular among non-specialists, it has been abandoned by protologists in favor of more comprehensive based systems which rely on shared phylogenetic and morphological traits (Adl et al., 2005). Fortunately, the majority of ciliate species are still included under similar taxonomic categories making them ideal for study by non-specialists.

Conservative estimates of soil ciliates number around 2000 species globally (Foissner, 1997) with many more species being undescribed and unstudied (Foissner et al., 2008). Their success lies in part in their ability to gain

wide access to new habitats *via* aerosol dispersal allowing them to repopulate areas after disturbance (Altenburger et al., 2010) and to colonise newly formed landscapes (Frederiksen et al., 2001). They are aquatic organisms yet can be found in nearly every terrestrial biome on earth including drier polar regions (Smith, 1996) and deserts (Bamforth, 2004, Robinson et al., 2002). As expected, their activity in terrestrial environments depends on the interaction between the soil matrix, which dictates the physical structure of the habitat, and the amount of soil moisture, which allows them to be active within the soil.

The interplay among moisture, soil structure and ciliate activity has been aptly described by Elliott and Coleman (1988). Soil ciliates occupy water films and water filled pores that are sufficient in size to support their activity and movement. Aggregate structure within the soil regulates the spatial distribution and volume of pore habitat as well as pore habitat connectivity. The interaction of these structural components with temporal fluctuations of soil moisture determines when and where soil ciliates are active. As a soil dries the water films and pores connecting suitable habitats narrow. Ciliates can adapt to narrowing pore channels, but with severely reduced mobility (Wang et al., 2005). Prolonged differences in water contents in soils with similar structural characteristics will cause dramatic differences to active ciliate populations with the drier soils having far fewer active ciliates (Darbyshire, 1976).

Soil ciliates are ideal candidates for describing the status of terrestrial ecosystems as well as monitoring the effects of human impacts given their nearly ubiquitous global presence, diverse roles in ecosystem functioning, and sensitivity

to changes in soil habitat. However, at present their use as indicators is hindered by the fact that traditional microbial enumeration techniques such as Most Probable Number (MPN) are not appropriate (Adl and Coleman, 2005), while enumeration and identification using direct count methods are time consuming and require high levels of expertise (Foissner, 1999). Molecular methods hold promise of being a potential workaround for many of these methodological hurdles; however, application of these methods to soil ciliates is still in its infancy and requires extensive fundamental groundwork (Lara and Acosta-Mercado, 2012). Currently, biomarkers based on microbial phospholipid fatty acids (PLFAs) are used to detect the presence of protozoa (Frostegård et al., 1997; Sampedro et al., 2006; Thoms et al., 2010). However, using PLFA biomarkers may not be appropriate for all protozoa as the biomarker PLFAs are often absent or inconsistent even when the presence of flagellates and amoeba has been verified using traditional MPN techniques (Rønn et al., 2002). The 20:4 PLFA biomarker may be appropriate for detecting ciliates as the original source of the biomarker (Lechevalier and Lechevalier, 1988) was based on *Paramecium*. However, this assumption has yet to be tested against more traditional enumeration methods such as direct counts.

The role of protozoa within soil ecosystems has been pondered and appreciated by protologists and soil scientists for nearly 100 years (Waksman, 1916). Hopefully over the course of the next 100 their importance in soil ecosystems will be more widely recognized and surpassed only by the expansion in knowledge about their roles within the soil.

1.5 Objectives and Outline

The primary aim of this work was to understand how the composition of microbial communities in the forest floors of aspen and white spruce stands is shaped by the action of factors operating at large and small spatial scales. Thus, this thesis addressed the following three main research objectives: 1) To understand how prescribed burning after harvesting affected microbial communities in white spruce, trembling aspen and mixedwood stands occurring at different topographical positions 2) To study how manipulating the moisture content of aspen and spruce forest floor affect the structure of their microbial communities and 3) To determine how ciliates derived from aspen forest floor affect the structure of the microbial community and certain aspects of the soil microbial loop.

The five chapters that make up this thesis include the pertinent literature summarized in this introduction followed by three chapters that each address one of the main research objectives from above, and a concluding chapter. The second chapter is an observational study based on work conducted at EMEND; my primary goals were to distinguish how the pre-harvest overstory, prescribed burning and topographic positions affected the size, structure and function of the microbial community in spruce, aspen and mixedwood forest floors. The discovery of topography as a critical driver of community structure opened up further avenues of investigation that led to the work conducted in chapter three. This chapter is a controlled experiment that manipulated the amount of moisture within the forest floor to determine the differences between pore habitats of the

aspen and spruce forest floors and how these differences affected the microbial community. A key finding from the PLFA analysis of chapter 3, namely, the potential interaction between moisture, protozoa and the microbial community in aspen forest floor, provided the basis for the final study chapter. Chapter 4 is focused on the controlled experiment that was designed to test the effects that ciliates have on the structure of microbial communities derived from aspen forest floor as well as the role of ciliates in the production of plant auxins and release of nitrogen. Finally the concluding chapter contains an overall synthesis of how the results of each chapter are related as well as specific chapter summaries, the research limitations of each chapter and potential avenues for future research.

Literature cited

Adl, S.M., Coleman, D.C. 2005. Dynamics of soil protozoa using a direct count method. Biology and Fertility of Soils 42, 168-171.

Adl, S.M., Simpson, A.G.B., Farmer, M.A., Andersen, R.A., Anderson, O.R., Barta, J.R., Bowser, S.S., Brugerolle, G., Fensome, R.A., Fredericq, S., James, T.Y., Karpov, S., Kugrens, P., Krug, J., Lane, C.E., Lewis, L.A., Lodge, J., Lynn, D.H., Mann, D.G., Mccourt, R.M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S.E., Nerad, T.A., Shearer, C.A., Smirnov, A.V., Spiegel, F.W., Taylor, M.F.J.R. 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. Journal of Eukaryotic Microbiology 52, 399-451.

Adl, S.M., Gupta, V.V.S.R. 2006. Protists in soil ecology and forest nutrient cycling. Canadian Journal of Forest Research 36, 1805-1817.

Altenburger, A., Ekelund, F., Jacobsen, C.S. 2010. Protozoa and their bacterial prey colonize sterile soil fast. Soil Biology and Biochemistry 42, 1636-1639.

Bååth, E., Frostegård, Å., Pennanen, T., Fritze, H. 1995. Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. Soil Biology and Biochemistry 27, 229-240.

Bamforth, S.S. 2004. Water film fauna of microbiotic crusts of a warm desert. Journal of Arid Environments 56, 413-423.

Barbier, S., Gosselin, F., Balandier, P. 2008. Influence of tree species on understory vegetation diversity and mechanisms involved-A critical review for temperate and boreal forests. Forest Ecology and Management 254, 1-15.

Bonkowski, M., Brandt, F. 2002. Do soil protozoa enhance plant growth by hormonal effects? Soil Biology and Biochemistry 34, 1709-1715.

Bonkowski, M. 2004. Protozoa and plant growth: The microbial loop in soil revisited. New Phytologist 162, 617-631.

Brussaard, L., Behan-Pelletier, V.M., Bignell, D.E., Brown, V.K., Didden, W., Folgarait, P., Fragoso, C., Freckman, D.W., Gupta, V.V.S.R., Hattori'S, T., Hawksworth, D.L., Klopatek, C., Lavelle, P., Malloch, D.W., Rusek, J., Söderström, B., Tiedje, J.M., Virginia, R.A. 1997. Biodiversity and ecosystem functioning in soil. Ambio 26, 563-570.

Canadian Forest Service. 2011 The State of Canada's Forests. Annual Report 2011. Natural Resources Canada, Ottawa Ontario, Canada.

Clarholm, M. 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biology and Biochemistry 17, 181-187.

Coleman, D.C. 1994. The microbial loop concept as used in terrestrial soil ecology studies. Microbial Ecology 28, 245-250.

Danks, H.V., Foottit, R.G. 1989. Insects of the boreal zone of Canada. Canadian Entomologist 121, 625-690.

D'Ascoli, R., Rutigliano, F.A., De Pascale, R.A., Gentile, A., Virzo De Danto, A. 2005. Functional diversity of the microbial community in Mediterranean maquis soils as affected by fires. International Journal of Wildland Fire 14, 355-363.

Darbyshire, J.F. 1976. Effect of water sunctions on the growth in soil of the ciliate *Colpoda steini*, and the bacterium *Azobacter Chroococcum*. Journal of Soil Science 27, 369–376.

Devito, K.J., Creed, I.F., Fraser, C.J.D. 2005. Controls on runoff from a partially harvested aspen-forested headwater catchment, Boreal Plain, Canada. Hydrological Processes 19, 3-25.

Drenovsky, R.E., Graham, K.J., Scow, K.M. 2004. Soil water content and organic matter availability are major determinants of soil microbial community composition. Microbial Ecology 48, 424-430.

Elliott, E.T., Anderson, R.V., Coleman, D.C., Cole, C.V. 1980. Habitable Pore Space and Microbial Trophic Interactions. Oikos 35, 327-335.

Elliott, E.T., Coleman, D.C. 1988. Let the soil work for us. Ecological Bulletins Swedish Natural Science Research Council 39, 23-32.

Erskine, A. J. 1977. Birds in boreal Canada: communities, densities and adaptations. Canadian Wildlife Service Report Series Number 41, Ottawa Ontario, Canada.

Finlay, B.J., Esteban, G.F., Olmo, J.L., Tyler, P.A. 1999. Global distribution of free-living microbial species. Ecography 22, 138-144.

Frederiksen, H.B., Kraglund, H.-O., Ekelund, F. 2001. Microfaunal primary succession on the volcanic island of Surtsey, Iceland. Polar Research 20, 61-73.

Frier, N., Schimel, J.P., Holden, P.A. 2003. Influence of drying-rewetting frequency on soil bacterial community structure. Microbial Ecology 45, 63-71.

Frostegård, Å., Petersen, Ø.O., Bååth, E., Nielsen, T.H. 1997. Dynamics of a microbial community associated with manure hot spots as revealed by phospholipid fatty acid analyses. Applied and Environmental Microbiology 63, 2224-2231.

Foissner, W. 1997. Global soil ciliate (Protozoa, Ciliophora) diversity: A probability-based
approach using large sample collections from Africa, Australia and Antarctica. Biodiversity and Conservation 6, 1627-1638.

Foissner, W. 1999. Soil protozoa as bioindicators: Pros and cons, methods, diversity, representative examples. Agriculture, Ecosystems and Environment 74, 95-112.

Foissner, W., Chao, A., Katz, L.A. 2008. Diversity and geographic distribution of ciliates (Protista: Ciliophora). Biodiversity and Conservation 17, 345-363.

Giesler, R., Högberg, M., Högberg, P. 1998. Soil chemistry and plants in Fennoscandian boreal forest as exemplified by a local gradient. Ecology 79, 119-137.

Goodale, C.L., Apps, M.J., Birdsey, R.A., Field, C.B., Heath, L.S., Houghton, R.A., Jenkins, J.C., Kohlmaier, G.H., Kurz, W., Liu, S., Nabuurs, G.-J., Nilsson, S., Shvidenko, A.Z. 2002. Forest carbon sinks in the Northern Hemisphere. Ecological Applications 12, 891-899.

Grady, K.C., Hart, S.C. 2006. Influence of thinning, prescribed burning, and wildfire on soil processes and properties in southwestern ponderosa pine forests: a retrospective study. Forest Ecology and Management 234, 123-135.

Grayston, S.J., Prescott, C.E. 2005. Microbial communities in forest floors under four species in coastal British Columbia. Soil Biology and Biochemistry 37, 157-1167.

Griffiths, B.S. 1986. Mineralization of nitrogen and phosphorus by mixed cultures of the ciliate protozoan *Colpoda steinii*, the nematode *Rhabditis* SP. and the bacterium *Pseudomonas fluorescens*. Soil Biology and Biochemistry 18, 637-641.

Griffiths, B.S., Bonkowski, M., Dobson, G., Caul, S. 1999. Changes in soil microbial community structure in the presence of microbial-feeding nematodes and protozoa. Pedobiologia 43, 297-304.

Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J. 2003. Physiological and community responses of established grassland bacterial populations to water stress. Applied and Environmental Microbiology 69, 6961-6968.

Hannam, K.D., Quideau, S.A., Oh, S.-W., Kishchuk, B.E., Wasylishen, R.E. 2004. Forest floor composition in aspen- and spruce-dominated stands of

the boreal mixedwood forest. Soil Science Society of America Journal 68, 1735-1743.

Hannam, K.D., Quideau, S.A., Kishchuk, B.E., Oh, S.-W., Wasylishen, R.E. 2005. Forest-floor chemical properties are altered by clear-cutting in boreal mixedwood forest stands dominated by trembling aspen and white spruce. Canadian Journal of Forest Research 35, 2457-2468.

Hannam, K.D., Quideau, S.A., Kishchuk, B.E. 2006. Forest floor microbial communities in relation to stand composition and timber harvesting in northern Alberta. Soil Biology and Biochemistry 38, 2565-2575.

Hannam, K.D., Quideau, S.A., Kishchuk, B.E. 2007. The microbial communities of aspen and spruce forest floors are resistant to changes in litter inputs and microclimate. Applied Soil Ecology 35, 635-647.

Högberg, M.N., Högberg, P., Myrold, D.D. 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150, 590-601.

Jenny, H. 1941. Factors of Soil Formation. McGraw-Hill, New York.

Krome, K., Rosenberg, K., Dickler, C., Kreuzer, K., Ludwig-Müller, J., Ullrich-Eberius, C., Scheu, S., Bonkowski, M. 2010. Soil bacteria and protozoa affect root branching via effects on the auxin and cytokinin balance in plants. Plant and Soil 328, 191-201.

Kuikman, P.J., Van Veen, J.A. 1989. The impact of protozoa on the availability of bacterial nitrogen to plants. Biology and Fertility of Soils 8, 13-18.

Lara, E., Acosta-Mercado, D. 2012. A molecular perspective on ciliates as soil bioindicators. European Journal of Soil Biology 49, 107-111.

Lechevalier, H., Lechevalier M.P., 1998. Chemotaxonomic use of lipids – an overview. In: Ratledge, C., Wilkinson, S.G. (Eds.), Microbial Lipids Volume 1. Academic Press, San Diego, 869-902.

Lindo, Z., Visser, S. 2003. Microbial biomass, nitrogen and phosphorus mineralization, and mesofauna in boreal conifer and deciduous forest floors following partial and clear-cut harvesting. Canadian Journal of Forest Research 33, 1610-1620.

Lundquist, E.J., Scow, K.M., Jackson, L.E., Uesugi, S.L., Johnson, C.R. 1999. Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/dry cycle. Soil Biology and Biochemistry 31, 1661-1675.

Macdonald, S.E., Lecomte, N., Bergeron, Y., Brais, S., Chen, H., Comeau, P., Drapeau, P., Lieffers, V., Quideau, S., Spence, J., and Work, T. 2010. Ecological implications of changing the composition of boreal mixedwood forests. A State of Knowledge Report. Sustainable Forest Management Network, Edmonton, Alberta. 48 pp.

McCulley, R.L., Burke, I.C. 2004. Microbial community composition across the great plains: landscape versus regional variability. Soil Science Society of America 68, 106-115.

Menyailo, O.V., Hungate, B.A., Zech, W., 2002. Tree species mediated soil chemical changes in a Siberian artificial afforestation experiment. Plant and Soil 242, 171-182.

Nordén, B., Ryberg, M., Götmark, F., Olausson, B. 2004. Relative importance of coarse and fine woody debris for the diversity of wood-inhabiting fungi in temperate broadleaf forests. Biological Conservation 117, 1-10.

Power, K., Gillis, M. 2006. Canada's Forest Inventory 2001. Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre Victoria.

Prescott, C.E., Maynard, D.G., Laiho, R. 2000. Humus in northern forests: Friend or foe? Forest Ecology and Management 133, 23-36.

Priha, O., Grayston, S.J., Hiukka R., Pennanen, T., Smolander A. 2001. Microbial community structure and characteristics of the organic matter in soils under Pinus sylvestris, Picea abies and Betula pendula at two forest sites. Biology and Fertility of Soils 33, 17-24.

Redding, T.E., Hannam, K.D., Quideau, S.A., Devito, K.J. 2005. Particle density of aspen, spruce, and pine forests floors in Alberta Canada. Soil Science Society of America Journal 69, 1503-1506.

Robinson, B.S., Bamforth, S.S., Dobson, P.J. 2002. Density and diversity of protozoa in some arid Australian soils. Journal of Eukaryotic Microbiology 49, 449-453.Rønn, R., Gavito, M., Larsen, J., Jakobsen, I., Frederiksen, H., Christensen, S. 2002. Response of free-living soil protozoa and microorganisms to elevated atmospheric CO 2 and presence of mycorrhiza. Soil Biology and Biochemistry 34, 923-932.

Rønn, R., McCaig, A.E., Griffiths, B.S., Prosser, J.I. 2002. Impact of protozoan grazing on bacterial community structure in soil microcosms. Applied and Environmental Microbiology 68, 6094-6105.

Rowe, J.S. 1972. Forest regions of Canada. Environment Canada, Ottawa Ontario, Canada.

Sampedro, L., Jeannotte, R., Whalen, J.K. 2006. Trophic transfer of fatty acids from gut microbiota to the earthworm *Lumbricus terrestris* L. Soil Biology and Biochemistry 38, 2188-2198.

Seibert, J., Stendahl, J., Sørensen, R., 2007. Topographical influences on soil properties in boreal forests. Geoderma 141, 139-148.

Siitonen, J., 2001. Forest management, coarse woody debris and saproxylic organisms: Fenoscandian boreal forests as an example. In: Jonsson, B.G., Kruys, N. (Eds.), Ecology of Woody Debris in Boreal Forests. Ecological Bulletins 49, 11–41.

Smith, H.G. 1996. Diversity of antarctic terrestrial protozoa. Biodiversity and Conservation 5, 1379-1394.

Spence, J.W., Volney, J.A., Sidders, D., Luchkow, S., Vinge, T., Oberle, Gilmore, D., Bielech, J.P., Wearmouth, P., Edwards, J., Bothwell, P., Shorthouse, D., Wilkinson, D., Brais, S. 2002. The EMEND Experience. In: Veeman et al. (Eds), Advances in Forest Management: From Knowledge to Practice, Proceedings of SFMN Conference, 13-15 Nov, Edmonton, Alberta. SFMN Network, Edmonton, Canada, pp. 40-44.

Stark, J.M., Firestone, M.K. 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. Applied and Environmental Microbiology 61, 218-221.

Stinson, G., Kurz, W.A., Smyth, C.E., Neilson, E.T., Dymond, C.C., Metsaranta, J.M., Boisvenue, C., Rampley, G.J., Li, Q., White, T.M., Blain, D. 2011. An inventory-based analysis of Canada's managed forest carbon dynamics, 1990 to 2008. Global Change Biology 17, 2227-2244.

Sustainable Resource Development. 2010. Fall 2010 timber volume harvested statistics. Forest Management Branch, Alberta Sustainable Resource Development.

Swanson, F.J., Kratz, T.N., Caine, N., Woodmansee, R.G. 1988. Landform effects on ecosystem patterns and processes. BioScience 38, 92–98.

Thoms, C., Gattinger, A., Jacob, M., Thomas, F.M., Gleixner, G. 2010. Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. Soil Biology and Biochemistry 42, 1558-1565.Uikman, P.J.K., Jansen, A.G., Van Veen, J.A. 1991. ^{15}N-Nitrogen mineralization from bacteria by protozoan grazing at different soil moisture regimes. Soil Biology and Biochemistry 23, 193-200.

Vesterdal, L., Raulund-Rasmussen, K. 1998. Forest floor chemistry under seven tree species along a soil fertility gradient. Canadian Journal of Forest Research 28, 1636-1647.

Voroney, R.P. 2007. The Soil Habitat. In: Paul, E.A.(Ed.), Soil Microbiology Ecology and Biochemistry third edition. Academic Press, Boston, pp. 25-49.

Waksman, S. 1916. Studies on soil protozoa. Soil Science 1, 135-152.

Wang, W., Shor, L.M., LeBoeuf, E.J., Wikswo, J.P., Kosson, D.S. 2005. Mobility of protozoa through narrow channels. Applied and Environmental Microbiology 71, 4628-4637.

Chapter 2. Microbial community structure and function: The effect of silvicultural burning and topographic variability in northern Alberta

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2.1 Introduction

Forest floors and the microbial communities that reside within them are the result of complex interactions between biotic and abiotic factors. Tree litterfall affects forest floors by influencing soil chemical properties (Menyailo et al., 2002), forest floor microbial activity (Bauhus et al., 1997), and forest floor microbial community structure (Priha et al., 2001; Grayston and Prescott, 2005). Forest floors derived from coniferous litter can have lower microbial biomass and activity than those derived from deciduous litter (Bauhus et al., 1997). Furthermore, previous work in northern Alberta has shown that microbial communities in deciduous forest floors are distinct from either coniferous or mixedwood forest floors (Hannam et al., 2006).

Harvesting in the boreal forest can cause an increase in forest floor C/N ratios while simultaneously reducing total C (Schmidt et al., 1996). In mature boreal forests, soluble organic matter leached from fresh tree litter within the forest floor can be quickly metabolized by the resident microbial community or

sorbed onto existing organic matter (Froberg et al., 2007). However, clearcut harvesting can reduce the amount of tree litterfall reaching the forest floor, although partial retention can lessen the impact (Lindo and Visser, 2003). By reducing annual litterfall, harvesting removes a source of easily degradable dissolved organic C, leaving microbial communities with only recalcitrant C compounds to fulfill their metabolic requirements as illustrated by Hannam et al. (2005) who found that clear cut forest floors in boreal mixedwoods can be more humified than uncut forest floors. Further, Grady and Hart (2006) saw that lower amounts of microbial biomass coincided with reducing annual litterfall. On the other hand, other recent studies have found that microbial community structure may be resistant to disturbance (Hassett and Zak, 2005; Hannam et al, 2006).

Post harvesting disturbances have multiple impacts on boreal forest floors. In addition to the effects of canopy removal, disturbances to the forest floor such as prescribed burning can increase soil temperature, as well as alter forest floor chemistry (Frey et al., 2003). Also, factors inherent to a site such as topography can influence soil moisture, soil carbon and soil nutrients, which in turn affect microbial activity (Florinsky et al., 2004).

Burning following harvesting can alter forest floor properties by reducing forest floor thickness, increasing pH, and increasing exchangeable cation concentrations (Scheuner et al., 2004). Prescribed burning has been reported to lower forest floor microbial biomass when compared to 40% thinned sites (Grady and Hart, 2006), or uncut sites (Chromomanska and Deluca, 2001). Both harvesting and prescribed burning may alter microbial community structure when

compared to uncut sites, but the effects seem more prominent in prescribed burns than in clear-cut sites (Bååth et al., 1995). However, recovery of soil microbial community functional diversity to pre-disturbance levels can occur as quickly as three months following fire (D'Ascoli et al., 2005).

Soil microbial communities can also be impacted by slight to moderate changes in topography. In alpine tundra soils, Fisk et al. (1998) found that wetter low lying areas supported more active microbial communities. In addition, McCulley and Burke (2004) observed structural differences between uplands and lowlands in microbial communities found in grassland soils. In boreal forest soils, wetter topographic locations have thicker forest floors, higher pH and lower C:N ratios (Seibert et al., 2007). Extensive work in Fennoscandian boreal forests by Högberg et al., (2007) has found that changing edaphic conditions along a topographic gradient coincide with differently structured forest floor microbial communities.

The Ecosystem Management Emulating Natural Disturbance (EMEND) site in northwestern Alberta was established to determine how forestry practices could best be adapted to maintain ecosystem sustainability within mixedwood boreal forests (Spence et al., 2002) and consists of replicated blocks of variable retention harvesting at an operational scale. As fire is the main disturbance agent in boreal forests (Stocks et al., 2003), it was reintroduced across parts of the harvested areas at EMEND through the use of prescribed burning in unharvested sites and as silvicultural burning in post-harvested sites. Prescribed burning has been shown to control populations of tree damaging insects (McCullough et al., 1998) as well

as promote tree seedling growth in northern climates (Ballard, 2000). While a large amount of literature worldwide exists on the effects of prescribed burning on soil and forest floor microbial communities (Bååth et al., 1995, Jurgen and Saano, 1999), very little is known about these relationships within the forest floor of Canadian boreal mixedwoods.

In this study we examined how environmental factors, including silvicultural burning, stand type, forest floor nutrients and topographic position, influenced microbial community size, structure and function at EMEND. Our original research questions were directed at studying stand type and silvicultural treatment, specifically: 1) What is the effect of slash burning on microbial community structure and function as compared to slash alone; 2) how does stand type influence the structure and function of boreal forest floor microbial communities; and (3) how is the structure and function of forest floor microbial communities influenced by landscape level topographic changes?

2.2 Materials and Methods

2.2.1 Study Site

The EMEND research installation is located within the boreal mixedwood plains of northwestern Alberta (56° 46’ 13” N, 118° 22’ 28” W). It encompasses approximately 1000 ha of boreal mixedwood forest, comprising sites that are deciduous-dominated, consisting of primarily trembling aspen (*Populus tremuloides);* coniferous-dominated, consisting primarily of white spruce (*Picea glauca*); and mixedwood sites of coniferous and deciduous species. Elevation

changes from 880 to 677 m in a south to north direction over a distance of roughly 8 kilometers. A comprehensive description of the harvesting treatments at the EMEND site can be found in McDonald and Fenniak (2007). In brief, harvesting occurred within 20 m wide strips from an adjacent 5 m wide machine corridor. Machine traffic was restricted to the 5 m machine corridor to minimize the impact upon the forest floor within the 20 m harvesting corridor. Generally, soils within EMEND are fine textured, Orthic and Dark Gray Luvisols derived from glacio-lacustrine parent materials with an average forest floor thickness of 12 cm, much of which is moderately humified. On average, all forest floor layers throughout the EMEND installation have a bulk density of between 0.07-0.08 g cm^{-3}, with the deeper, highly humified portions of the forest floor having an acidic pH of 4.95 compared to a pH of 5.30 found in the moderate to less humified portions of the forest floor (Kishchuk, 2004).

Climatic records from the Peace River weather station (56° 13.800' N, 117° 27.000' W) show that between 1971 and 2000, the mean annual temperature for the area was 1.2°C, while mean annual precipitation was 402.3 mm (Environment Canada, 2002). In 2005 and 2006, the area received one third of the annual precipitation during June and July, while only five percent of the annual precipitation occurred in August.

2.2.2 Sampling

Sampling occurred in ten-hectare coniferous, deciduous and mixedwood sites of the EMEND installation during August, 2005 and July, 2006. Each site used in

this study was harvested (bole only) to 10% retention of the standing volume in the winter of 1998 with the remaining slash evenly distributed on the forest floor surface. It was then divided in two, with half of the site being burned in October 2003. Specific data for the prescribed burn treatments does not exist, however all aspen prescribed burns were considered to be low intensity while the mixedwood and coniferous prescribed burns were low to moderate intensity (Jason Edwards, personal communication). In all, 18 five-hectare sites were studied (3 replicates by 3 stand types by 2 silvicultural treatments). An elevation gradient of roughly 71 m exists with our sites; however, this was not one of the original criteria for the establishment of silvicultural treatments (Table 2-1).

Within each site, forest floor samples were collected over two field seasons along pre-delineated tree mensuration transects at three randomly selected positions. However, two conditions were applied to the random locations: 1) that sampling occurred off the machine corridor to ensure soils were not compacted, and 2) that visibly charred areas were sampled in the burn treatment to capture the effect of fire. Sampling spots were located two meters from the transect line to reduce the chance of human disturbance. In August 2005, three forest floor samples were collected for analysis of nutrients, microbial biomass and PLFA analysis along the tree measurement transects. A 15 x15 cm frame was used to delineate the sampling area, and forest floor material was carefully collected to the interface with mineral soil. A subsample of the homogenized forest floor samples was removed and placed in a separate sterile plastic bag for phospholipid fatty acid (PLFA) analysis. All sampling equipment was washed with 70%

ethanol between samples to minimize phospholipid contamination. Samples were kept chilled in coolers until they were brought back to the laboratory where they were stored at 4°C, except for PLFA samples, which stored at -80°C and freeze dried before PLFA extraction. During the 2006 field season, the same sites were sampled in the same way as in 2005, but samples were sieved to 4 mm and composited by site on the day of collection. These composite samples were kept in coolers until being transported back to the lab and stored at 4°C to await multiple carbon-source substrate-induced respiration (multi-SIR) analyses.

2.2.3 Laboratory Analysis

Moisture content (MC) of the forest floor samples was determined gravimetrically by weighing samples before and after drying at 65°C for 48 hrs. Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined using the chloroform-fumigation extraction method as described in Hannam et al. (2004). Briefly, 10 g dry weight equivalent of forest floor was placed in a 100ml beaker, fumigated with chloroform for 24 hrs, extracted in 100 ml of 0.5M K_2SO_4 and vacuum filtered with Whatman P2 fine porosity filter paper. Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) concentrations in the extracts were determined using a Shimadzu TOC-VTN instrument (Mandel Scientific Company Inc., ON, Canada). MBC and MBN were estimated by calculating the difference in DOC and DON concentrations between fumigated and unfumigated samples. To allow these data to be more comparable with fumigation extraction literature from other boreal sites and

because no conversion factors currently exist for boreal ecosystems, no correction factors were used when calculating MBC and MBN. Concentrations of ammonium and nitrate were measured colorimetrically on the unfumigated extracts using the nitroprusside-salicylate method for ammonium (Mulvaney, 1996) and the cadmium reduction method for nitrate (Mulvaney, 1996) on a Technicon Auto Analyzer II (Technicon Industrial Systems, Tarrytown, New York).

2.2.4 PLFA Analysis

Microbial community structure was characterized using PLFA analysis of samples collected in 2005. A detailed description of the PLFA methodology can be found in Hannam et al. (2006). In brief, polar lipids were extracted from freeze-dried forest floor samples using a modified Bligh and Dyer (1959) extraction process. Extracts were purified on pre-packed silicic acid columns (Agilent Technologies, Wilmington, DE) before being subjected to mild alkaline methanolysis to form fatty acid methyl esters (FAMEs). The FAMEs were separated using an Agilent 6890 Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column. The MIDI peak identification software (MIDI, Inc., Newark, DE) was used to identify individual fatty acids.

The nomenclature for PLFAs follows the standard format of X:YωZ, where X is the number of carbon atoms in the molecule, Y is the number of double bonds and Z records the location of the first double bond from the aliphatic end of

the molecule represented by ω. *Cis* or *trans* configuration is represented by the suffixes "c" or "t" respectively. Branching occurring at the second or third carbon from the aliphatic end of the molecule is designated by the prefix 'i' or 'a', respectively. The existence of a cyclopropyl group occurring along the carbon chain is indicated by the prefix 'cyc'. The prefix '10Me' indicates a methyl group located on the 10^{th} carbon from the carboxyl end of the molecule.

2.2.5 Multi-Substrate Induced Respiration (Multi-SIR) Analysis

Microbial community function was determined on samples collected in June 2006 using a multi-SIR analysis (Hannam et al., 2006), following the addition of a diverse range of carbon substrates in separate laboratory incubations. Theoretically, each forest floor community will have a respiration profile based on its response to all of the substrates used. A total of 17 different carbon substrates were used, including: D-glucosamine, L-arginine, L-glutamine, L-histidine, DL-lysine, urocanic acid, D-glucose, D-mannose, L-ascorbic acid, D-gluconic acid, DL-malic acid, pantothenic acid, (-)-quinic acid, DL-tartaric acid fumaric acid, Na-citrate and malonic acid. Field-moist composite forest floor samples were incubated in the dark at room temperature for three weeks prior to being analyzed to ensure the most readily available carbon was consumed prior to the multi-SIR incubation. Samples were removed from the incubation chamber 24 hrs prior to substrate additions and transferred into multi-SIR incubation flasks. Carbon substrate solutions were prepared to concentrations listed in Degens et al. (2000) on the day of the incubation by using 500 ml of distilled

water with pH adjusted to between 6-7 using concentrated HCL or NaOH. Substrate solution was applied to each vial with a 1:1 ratio of solution to soil. Vials were capped with a rubber seals and incubated at 25ºC for one hour after solutions were added. Headspace CO_2 was measured after incubation using an HP 5890 series II gas chromatograph with a one meter Poropak Q colomn and a HP 3396 series II integrator, with helium being used as the carrier gas.

2.2.6 Statistical Analysis

Silvicultural effects were separated into two levels, which were unburned and burned while effects of stand type which were separated into three levels: coniferous, deciduous and mixedwood. A two-way ANOVA for a completely randomized design was performed on MBC, MBN, NH_4^+, NO_3^-, MC, DOC and DON data using PROC MIXED (version 9.1, SAS institute Inc.). Mean separation was tested using the LSMEANS statement. All data conformed to the assumptions of ANOVA, except for NO_3^- data which had to be log transformed to ensure homogeneity of variance. Factors were deemed significant at an α of 0.05.

Multivariate regression trees (MRT) were constructed to compare the response among sites of the microbial community structure (PLFA) to categorical environmental data (De'ath, 2002). In brief, MRT create dichotomies, where sites that share similar species composition in relation to environmental factors are clustered together. It is a hierarchical method which aims to minimize the dissimilarity of sites contained within a cluster by repeatedly splitting the data based on environmental variables. The MRT used the same mol% PLFA data as

the ordination (see below). A Bray-Curtis dissimilarity measure was used to determine dissimilarity. Categorical environmental variables used in the analysis included stand type, topographic position and silvicultural treatment. The MRT were computed using the R package (version 2.6.2, R Development Core Team) and the mvpart library (Therneau & Atkinson, 2005).

Microbial community structure (PLFA) and microbial community function (multi-SIR) were also characterized with a non-metric multidimensional scaling (NMS) ordination using PC-ORD (version 4, MjM Software Design). Only PLFA associated with soil microorganisms were used in the ordination. Bacterial PLFA included from Frostegård and Bååth (1995) were 15:0, a15:0, i15:0, i16:0, 16:1ω9c, 17:0, a17:0, i17:0, cyc17:0 18:1ω7c and cyc19:0. Additional bacterial PLFA included 14:0, 16:1ω7c, 18:0 (Myers et al. 2001), 18:1ω5c, 19:0 (Hasset and Zak 2005), 16:1 2OH and 17:0 3OH (Hamman et al. 2007). Fungal PLFA included 18:2ω6c (Frostegård and Bååth, 1996), 18:1ω9c, 20:1ω9c (Myers et al. 2001), 16:1ω5c, 18:3ω6c (Hamman et al. 2007). The PLFA used for actinomycetes included 10Me19:0 (O'Donnell et al. 1982), 10Me16:0 and 10Me 18:0 (Myers et al. 2001). The PLFA 20:4ω6c was included for protists (Myers et al. 2001). All measured PLFA were expressed on a mol% basis and were arcsine squareroot transformed due to its suitability for proportional data (McCune and Grace 2002). The CO_2 respiration response of a forest floor sample to an individual substrate was defined as a proportion of its total response to all substrates (Degens et al. 2000). The SIR data had to be log transformed prior to

being ordinated to ensure a stable ordination solution. A Sørensen (Bray-Curtis) dissimilarity measurement was used in both ordinations.

The presence of grouping patterns linked to silvicultural treatment, stand type and topographic position were tested for significance in the NMS analyses using a multi-response permutation procedure (MRPP). In addition, PLFA data were grouped by how each site clustered in the MRT and these MRT groups were also tested in ordination space using MRPP. The MRPP is a non-parametric test, which generates two values that were tested for significance. The T value indicates separation between groups, with a larger T value indicating a stronger separation, while the A value indicates within-group homogeneity compared to random expectation (McCune and Grace, 2002). Indicator species analyses were performed using the grouping variables shown by MRPP to have a significant effect on forest floor microbial community patterns (Hannam et al., 2006) as well as MRT clusters (Jacobs et al., 2007). A detailed explanation of indicator species analysis can be found in McCune and Grace (2002). Briefly, indicator species analysis generates an indicator value based on the abundance and frequency of a particular PLFA in a given treatment combination. Larger values represent a stronger relationship between the occurrences of a particular PLFA with a specific treatment combination. The statistical significance of the indicator value is tested against a randomized Monte Carlo test.

2.3 Results

2.3.1 Forest Floor Chemistry and Microbial Biomass

Burning had a significant effect on MBC (p=0.001) and MBN (p=0.007) with significantly lower concentrations in all burned sites than in unburned sites (Table 2-2). Stand type had no apparent effect on either measure of microbial biomass (MBC and MBN). Silvicultural treatments had no significant effect on extractable NH_4^+ however, deciduous sites had significantly more NH_4^+ than mixedwood sites (p=0.006). A single unburned conifer outlier was removed from the extractable NO_3^- data after which, no differences in extractable NO_3^- concentrations were found among sites. However, burned sites had significantly higher extractable NO_3^- concentrations than unburned sites (p=0.01). Forest floor moisture, DOC and DON were not significantly affected by silvicultural treatment or stand type. No interactions between silvicultural treatment and stand type were observed.

2.3.2 Microbial Community Structure and Function

Multiple cross validations consistently produced MRT ending in three groups which explained 71% of the variation in PLFA data (Figure 1). In all, 1000 cross validations produced MRT that branched into trees containing three groups 651 times, one group 224 times, and five groups 135 times. The first branching of the MRT was caused by topography, and explained 36% of the variation. Group 1 contained all sites with higher elevations (greater than 740 meters) and included both unburned and burned coniferous, deciduous and mixedwood sites. The second branching of the MRT contained sites with lower elevations (less than 740 meters) and explained 36% of the variation. This split was related to stand type,

with burned and unburned coniferous and mixedwood sites grouping separately under group 2 from burned and unburned deciduous sites found under group 3. Silvicultural treatment appeared to have no effect on how sites clustered in the MRT.

The NMS ordination produced a two-dimensional ordination solution with a final stress of 7.06 was achieved after 96 iterations with 64% and 33% of the variation being explained by axis one and axis two, respectively (Figure 2). As seen in Figure 2, strong grouping patterns for community structure were related to topographic position rather than stand type.

The MRPP analysis found that forest floor microbial communities of all stand types in sites with higher elevations grouped differently than sites with lower elevations (P=3.98*10^{-5}, A=0.16; Table 2-3). However, larger A values and lower P values were found when sites were grouped in relation to their MRT cluster (Table 2-3). All coniferous, deciduous and mixedwood sites found at higher elevations were clustered under group 1 of the MRT (Figure 2). Microbial community structure of the group 1 sites was significantly different from sites clustered under group 2, which contained the coniferous and mixedwood sites at lower elevations (P=1.5*10^{-4}, A=0.19) and group 3, which contained only deciduous sites at lower elevations (P=4.5*10^{-3}, A=0.30; Table 2-3). Sites grouping under groups 2 and 3 also had significantly different grouping patterns (P=3.8*10^{-3}, A=0.29). Stand related differences were present but not as strong as those linked to the MRT groups or topographic positions (Table 2-3). The coniferous and mixedwood sites grouped together (P=0.73, A=-0.03) while the

deciduous sites were separated from the coniferous (*P*=0.04, A=0.08) and mixedwood sites (*P*=0.01, A=0.11; Table 2-3). Finally, no distinct grouping patterns could be seen between the two silvicultural treatments (*P*=0.44, A=-0.002).

The strongest indicator species values were associated with topographic position and the MRT group rather than with stand type. In all, three PLFAs were associated with MRT groups and two PLFAs were associated with elevation (Table 2-4). Two actinomycetal PLFA's 10Me19:0 and 10Me18:0, were indicators for group 3 of the MRT. However, the indicator value for 10Me18:0 was much weaker than the indicator value for 10Me19:0. The fungal PLFA 20:1ω9c was a strong indicator for group 1 as well as the upper elevation class. In contrast, the fungal PLFA 18:3ω6c was weakly associated with upper sites and moderately associated with lower sites.

The NMS ordination of the SIR data produced a 3-dimensional ordination with a final solution stress of 10.53 after 145 iterations (not shown). The MRPP found stand related grouping patterns (*P*=0.048 A=0.032), however unlike the PLFA ordination, only mixedwood sites had any significant separation from deciduous sites (*P*=0.039 A=0.046)(Table 2-3). In addition, the function of forest floor microbial communities in mixedwood sites was similar to coniferous sites, while coniferous sites were similar to deciduous sites. No effect of elevation or silvicultural treatment was observed in terms of microbial function.

2.4 Discussion

2.4.1. Effects of Silvicultural Treatments

Prescribed burning and wildfire have both been observed to cause a reduction in microbial biomass when compared to undisturbed native and harvested forests (Peitikäinin and Fritze, 1993; Prietro-Fernández et al., 1998). Initially, burning reduces microbial biomass by directly heating the soil (Certini, 2005), however, this effect would not explain why we observed lower amounts of microbial biomass in burned sites than unburned sites (Table 2-2), since they were sampled two years after burning had occurred. Fernández et al. (1996) observed that the C content in soils that were burned naturally or in the laboratory was significantly lower than unburned soils. In addition, they found that the remaining organic matter found in the burned soils was more humified and consequently a poor substrate for microbial growth, as it contained proportionally more humin and had a higher Klason lignin content than unburned soil organic matter. Modification of organic matter by burning can influence forest floor microbial communities for extended periods of time as Pietikäinin et al. (2000) found following a 6-month incubation, where humus samples which had been heated and re-inoculated with active soil bacteria had lower amounts of microbial biomass than unheated humus samples. The final amount of microbial biomass was further dependant upon the temperature at which the humus was initially subjected to; temperatures ranging between 100ºC to 230ºC reduced biomass by roughly 34% to 70%, respectively (Pietikäinin et al., 2000). Organic matter in our burned sites may have been a

lower quality microbial substrate, which would explain why it supported less microbial biomass than the unburned sites.

Heating soils can result in nitrogen losses through volatilization or transformation of organic nitrogen into more recalcitrant forms (Knicker et al., 1996). Large amounts of organic N can also be converted into ammonium through thermal reduction and the resulting pulse of ammonium can last up to one or two years after the fire (Certini, 2005). Chromanska and Deluca (2001) hypothesized that higher nitrate levels found in burned sites could be the result of heat-intolerant nitrifying bacteria recolonizing the burned site and converting the thermally generated ammonium into nitrate. Our results appear to agree with this hypothesis since nitrate was higher in burned sites, while the amount of ammonium was not different between burned and unburned sites (Table 2).

In contrast with what was observed in our study (Table 2-2), some authors have reported elevated DOC (Palese et al., 2004) following burning. However, Prieto-Fernandez et al. (1998) found the average concentration of DOC to be lower in wildfire sites than control sites 13 years after the fire took place. Pietikäinin and Fritze (1993) observed DOC to increase initially after prescribed burning and then fall to similar levels as unburned sites. The conflicting findings may be caused by unique combinations of environmental factors such as soil moisture, fire severity and humus composition existing at each site at the time of burning (Gonzáles-Pérez et al., 2004).

Microbial community structure in our study did not appear to be influenced by burning (Figure 2 and Table 3). This may have been a result of sampling depth, as

the charred organic matter was homogenized with the underlying forest floor, as we attempted to measure the effect of the slash burn treatment on the entire forest floor. However, in boreal forest soils, Bååth et al. (1995) demonstrated that, one year after prescribed burning, the microbial communities of clear-cut sites, and clear-cut and burned sites were structurally similar, but that both were different from unharvested sites. Archaeal diversity in soils has also been found to respond similarly to clear-cutting and clear-cutting followed by burning (Jurgen and Saano, 1999). A trend similar to that observed by Bååth et al. (1995) may have occurred at our sites; however, undisturbed forest sites were not included since the initial aim of this study was to determine the differences between slash and slash burning following harvesting. Furthermore, previous work at EMEND by Hannam et al. (2006) had failed to uncover any influence of harvesting on forest floor microbial communities. Recently, Hamman et al. (2007) found that sites subjected to low and high severity wildfires had microbial community structures that were different from neighboring unburned sites. These structural differences included lower concentrations of the fungal PLFAs 18:2ω6c and 18:3ω6c found in the fire sites. We did not see a reduction in fungal biomarkers and this contrast between our findings and theirs could be linked to time since fire and fire severity. Our sites were mildly impacted by prescribed fire and had two growing seasons to recover before being sampled while the sites in Hamman et al. (2007) were sampled 14 months after wildfire.

2.4.2. Effects of Stand Type and Topographic Position

Forest floor litter composition has been shown to influence the chemical composition and concentration of DOC (Smolander et al., 2005). Our study did not observe differences in DOC concentrations or most of the other soil chemistry indices. However, deciduous sites had higher NH_4^+ than mixedwood sites. In addition, our SIR analysis also showed functional differences in the forest floor microbial communities between deciduous and mixedwood sites. The trend in NH_4^+ concentrations has been observed previously in past work at EMEND (Jerabkova et al., 2006) and warrants further inquiry.

In addition to forest floor chemistry, forest floor litter composition can also influence microbial community structure (Saetre and Bååth, 2000). Forest floors from coniferous and deciduous sites tend to have differently structured microbial communities (Merilä et al., 2002). At EMEND, Hannam et al. (2004 and 2006) found that the chemical composition of coniferous and deciduous forest floors was different and that pre-harvest stand type, rather than disturbance by harvesting, had the strongest influence on microbial community structure. As with the results found in our study from both PLFA and SIR analyses, Hannam et al. (2006) found that the microbial communities of conifers and mixedwoods were structurally similar but different from deciduous communities. The separation of mixedwood from deciduous communities could be caused by the influence conifers have on PLFA patterns and concentrations (Saetre, 1999).

In our study, the strongest separation among PLFA profiles was represented by their MRT grouping, which represents a combination of stand type and topographic location better than either of these factors alone (Table 3). On the

other hand, the lack of separation between sites at higher and lower elevations for the SIR ordination may be due to a combination of higher regional precipitation at the time of sampling. The standard deviation of mean elevation for each site in Table 1 shows that sites found above 740 meters have greater elevation changes than sites found below 740 meters. In addition, according to Environment Canada weather data, the Peace River region received six times more precipitation in July 2006, when the SIR samples were collected, than in August 2005, when the PLFA samples were collected (Environment Canada, 2002). Forest floor moisture may not have had an influence on the upper sites when samples were collected in July 2006, but most likely was a factor in August 2005 since the upper bench topography at these sites would cause them to be better drained and drier. Precipitation may also explain why Hannam et al. (2006) did not observe any topographical grouping patterns in their ordinations. Regional precipitation during June 2003, when they collected samples, was four times higher than in August 2005 (Environment Canada, 2002).

Topography appeared to affect deciduous microbial communities more so than coniferous and mixedwood. As shown in Figure 2, the microbial communities of coniferous, deciduous and mixedwood sites at higher elevations were grouped together while at lower elevations, the coniferous and mixedwood communities were similar to each other and both differed from the deciduous communities. The large separation seen in the ordination along axis one between deciduous sites located at lower and higher elevations could be an indication that the structure of deciduous forest floor microbial communities is strongly

influenced by seasonal moisture trends, more so than coniferous and mixedwood communities. In addition, the grouping of sites from all stand types under MRT group 1 suggests that seasonal moisture trends affected sites at higher elevations by causing a homogenization of forest floor microbial community structure regardless of the overstory composition.

The indicator species analysis revealed that the actinomycetal PLFA 10Me19:0 was a very strong indicator of MRT group 3 and nearly absent in MRT groups 1 and 2. In Western Canada, Jayasinghe and Parkinson (2008) found that actinomycetes prefer moister and organically rich deciduous soils to coniferous soils. Group 3 of the MRT contained only lower elevation deciduous sites, which would be presumably moister than the upper deciduous sites and have richer organic matter than the coniferous and mixedwood sites at either upper or lower elevation. The strong association of the fungal indicator PLFA 20:1ω9c with MRT group 1 may be the result of a combination of biological and environmental factors. Zak and Kling (2006) studied tundra ecosystems and found that fungal biomarkers were highest in drier ecosites. Additionally, the presence of actinomycetes can hinder fungal growth (Jayasinghe and Parkinson, 2008). The upper sites were most likely a better habitat for fungi since these sites were better drained with reduced competition for metabolic resources by actinomycetes.

A common trend is beginning to emerge among many of the communities studied independently at EMEND. The same community separation patterns observed in our study, namely that mixedwood and coniferous communities are similar to each other but different from deciduous communities, have also been

observed in understory plant community structure (Macdonald and Fennaik, 2007), and spider community structure (Pinzon, unpublished observations). Hence, the mechanisms responsible for the separation of deciduous communities from mixedwood and coniferous communities do not appear limited or confined to one particular community within the boreal forest ecosystem. The findings at EMEND illustrate the need for future work in boreal forest ecosystems that incorporates more than one ecological level. Furthermore, our results clearly indicate that topographic position can supersede the influence of overstory composition on forest floor microbial communities and must be taken into account when working within these ecosystems.

Tables and Figures

Table 2-1. Elevation ranges for the sites studied at EMEND.

Stand Type	Treatment	Elevation Class	Mean Elevation(m)
Deciduous	Slash/burn	Upper	768(2.88)
Deciduous	Slash only	Upper	773(1.83)
Deciduous	Slash/burn	Upper	809(7.02)
Deciduous	Slash only	Upper	791(4.51)
Coniferous	Slash/burn	Upper	824(3.12)
Coniferous	Slash only	Upper	824(3.92)
Mixedwood	Slash/burn	Upper	753(2.71)
Mixedwood	Slash only	Upper	748(2.51)
Coniferous	Slash/burn	Lower	715(2.64)
Coniferous	Slash only	Lower	711(2.42)
Coniferous	Slash/burn	Lower	712(0.777)
Coniferous	Slash only	Lower	712(0.827)
Mixedwood	Slash/burn	Lower	699(0.369)
Mixedwood	Slash only	Lower	700(0.147)
Mixedwood	Slash/burn	Lower	703(1.26)
Mixedwood	Slash only	Lower	701(1.10)
Deciduous	Slash/burn	Lower	695(1.42)
Deciduous	Slash only	Lower	697(1.42)

Values are mean elevation with standard deviation in parentheses.

Table 2-2. Microbial biomass and selected chemical characteristics of forest floors at EMEND sampled in 2005.

	Silvicultural Treatment		Stand Type		
	burned (n=9)	unburned (n=9)	coniferous (n=6)	deciduous (n=6)	mixedwood (n=6)
MBC (μg-C g-soil^{-1})	1337 (285)**b**	2606 (532)**a**	1942 (839)	2032 (929)	2000 (607)
MBN (μg-N g-soil^{-1})	295 (33)**b**	401 (65)**a**	337 (87)	377 (92)	329 (30)
NO_3 (μg-NO_3 g-soil^{-1})	18 (7)**a**	6 (8)**b**	17 (8)	13 (12)	7 (8)
NH_4 (μg-NH_4 g-soil^{-1})	25 (8)	32(13)	30 (13)**ab**	36 (10)**a**	20 (2.3)**b**
DOC (μg-DOC g-soil^{-1})	1531 (473)	1165 (352)	1506 (294)	1100 (308)	1439 (617)
DON (μg-DON g-soil^{-1})	55 (8)	60 (13)	58 (14)	56 (13)	59 (6)
Moisture content (%)	161 (52)	170 (38)	181 (42)	142 (39)	175 (50)

Note: Values are means with standard deviations in parentheses. Lower case letters represent statistical significance at p = 0.05 for a given chemical property within treatment factors. An outlier for NO_3^- concentration values resulted in n=5 for coniferous (stand type) and n=8 for unburned (silvicultural treatment) for the NO_3^- ANOVA.

Table 2-3. Multi-response permutation procedure results for microbial community structure (PLFA) and function (SIR).

		PLFA			SIR		
	Comparison	T	A	*p*	T	A	*p*
MRT group	1 vs. 2 vs. 3	-8.007	0.303	$1.7*10^{-6}$			
	1 vs. 2	-6.964	0.195	$1.5*10^{-4}$			
	1 vs. 3	-4.966	0.300	$4.4*10^{-3}$			
	2 vs. 3	-5.095	0.290	$3.0*10^{-3}$			
Topographic Position	upper vs. lower	-7.111	0.164	$3.9*10^{-5}$	-1.294	0.016	0.10
Stand Type	conifer vs. deciduous vs. mixedwood	-2.405	0.081	0.022	-1.789	0.032	0.04
	deciduous vs. conifer	-2.085	0.081	0.040	-0.645	0.012	0.24
	deciduous vs. mixedwood	-2.769	0.110	0.015	-1.997	0.046	0.03
	conifer vs. mixedwood	0.702	-0.026	0.734	-0.873	0.015	0.17
Silvicultural Treatment	burned vs. unburned	0.660	$-1.5*10^{-3}$	0.44	-.0385	$4.8*10^{-4}$	0.44

Table 2-4. PLFA indicator species associated with MRT group and elevation class.

			Indicator Value			Monte Carlo	Indicator Value			Monte Carlo
PLFA	Origin	Mean	1	2	3	$p<0.05$	Mean	Upper	Lower	$p<0.05$
10Me18:0	Actinomycetes	35.4(1.35)	29	30	41	$6.8*10^{-3}$				
10Me19:0	Actinomycetes	29.6(13.9)	1	3	80	0.016				
20:1ω9c	Fungi	38.4(10.8)	85	6	0	$2*10^{-4}$	41.5(8.87)	88	4	$4*10^{-4}$
18:3ω6c	Fungi						53.1(3.42)	33	62	$3.8*10^{-3}$

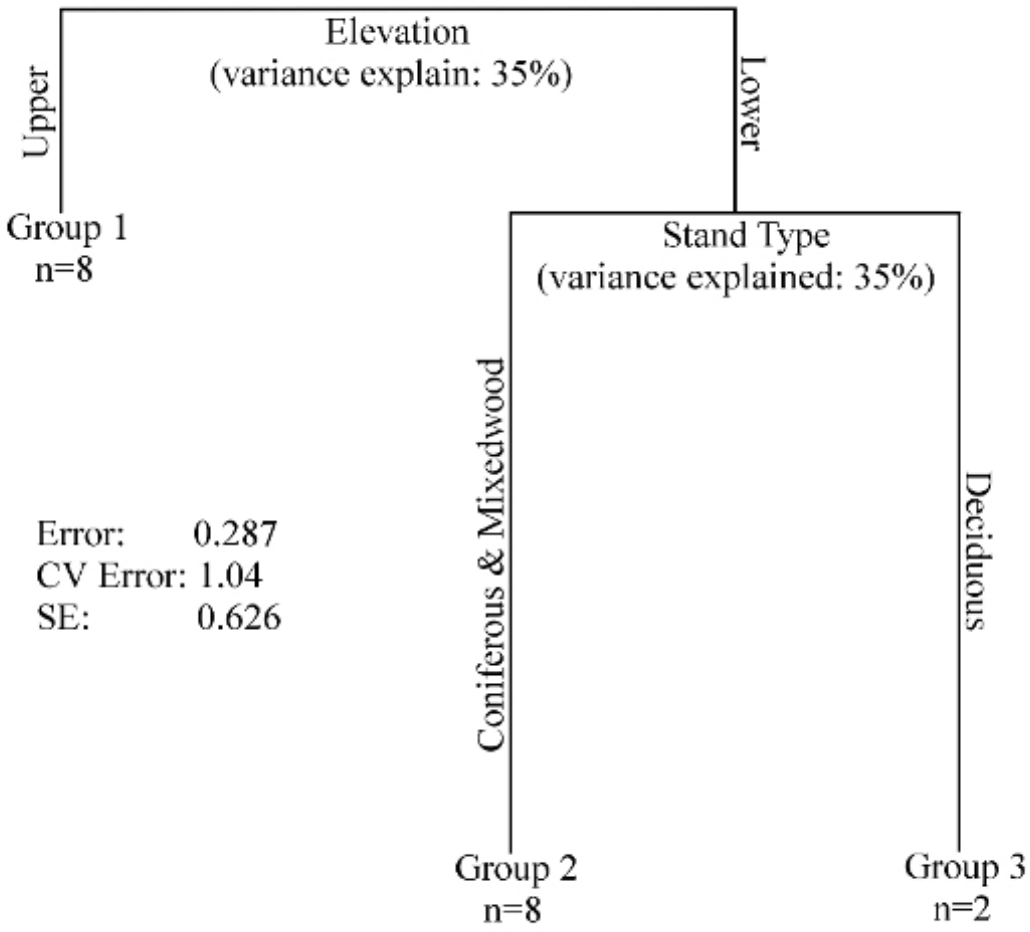

Figure 2-1. Multivariate regression tree of mol% PLFA data from harvested sites at EMEND in northern Alberta. Groups are combinations of topographic position (upper >740 meters, lower < 740meters), stand type and silvicultural treatment.

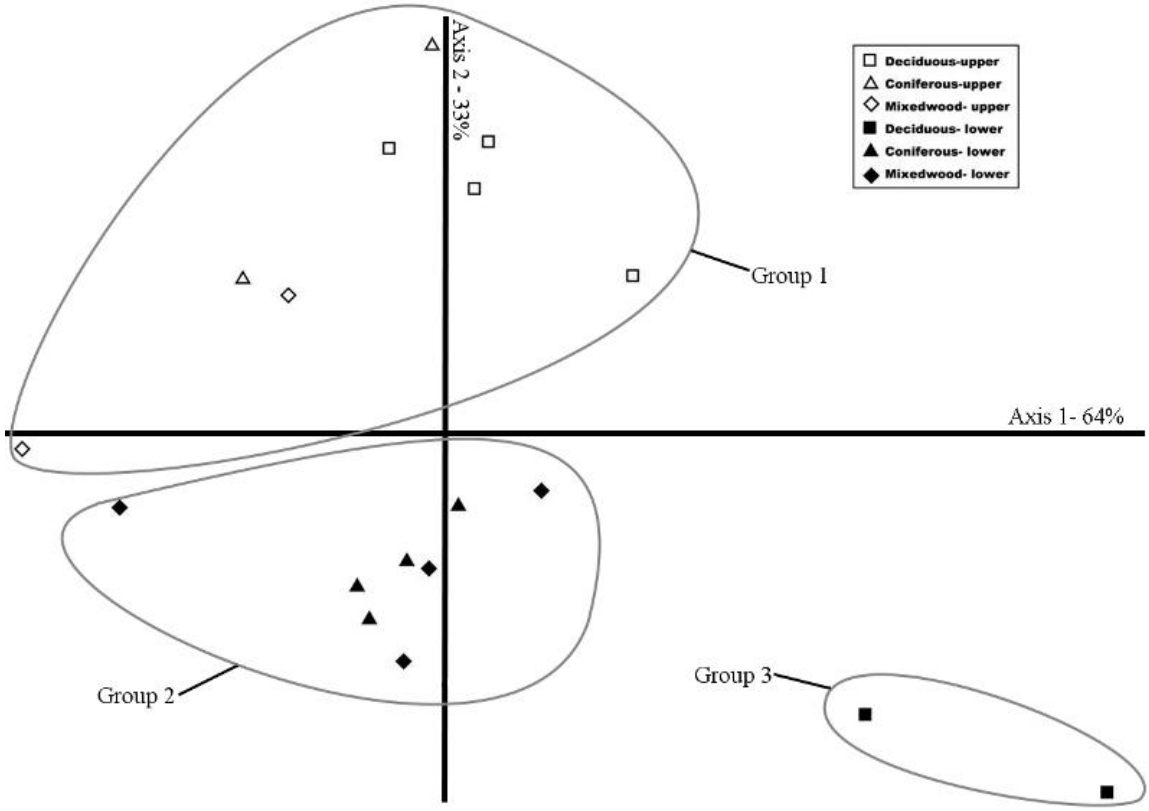

Figure 2-2. NMS ordination of forest floor PLFA profiles from harvested sites at EMEND in northern Alberta, delineated by stand type and topographic position (upper >740 meters, lower < 740meters). Grouping based upon MRT analysis is highlighted with circles.

Literature Cited

Bååth, E., Frostegård, Å., Pennanen, T., Fritze, H. 1995. Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. Soil Biology and Biochemistry 27, 229-240.

Ballard, T.M. 2000. Impacts of forest management on northern forest soils. Forest Ecology and Management 133, 37-42.

Bauhus, J., Paré D., Côté, L. 1997. Effects of tree species stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. Soil Biology and Biochemistry 30, 1077-1089.

Bligh, E.G., Dyer, W.J. 1959. A rapid method for the total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911-917.

Certini, G. 2005. Effects of fire on properties of forest soils: a review. Oecologia 143, 1-10.

Choromanska U., DeLuca, T.H. 2001. Prescribed fire alters the impact of wildfire on soil biochemical properties in a ponderosa pine forest. Soil Science Society of America Journal 65, 232-238.

De'ath, G. 2002. Multivariate regression trees: a new technique for modeling species-environment relationships. Ecology 83, 1105-1117.

D'Ascoli, R., Rutigliano, F.A., De Pascale, R.A., Gentile, A., Virzo De Danto, A. 2005. Functional diversity of the microbial community in Mediterranean

maquis soils as affected by fires. International Journal of Wildland Fire 14, 355-363.

Degens, B.P., Schipper, L.A., Sparling, G.P., Vojvodic-Vukovic, M. 2000. Decreases in organic C reserved in soils can reduce the catabolic diversity of soil microbial communities. Soil Biology and Biochemistry 32, 189-196.

Environment Canada. 2002. Climate data online. Environment Canada. Gatineau, Quebec. (on line) http://www.climate.weatheroffice.ec.gc.ca/climateData/canada_e.html (13 December, 2007)

Florinsky, I.V., McMahon, S., Burton, D.L. 2004. Topographic control of soil microbial activity: a case study of denitrifies. Geoderma 119, 33-53.

Fernández I., Cabaneiro A., Carballas T. 1996. Organic matter changes immediately after a wildfire in an Atlantic forest soil and comparison with laboratory soil heating. Soil Biology and Biochemistry 29, 1-11.

Fisk, M.C., Schmidt, S.K., Seastedt, T.R. 1998. Topographic patterns of above- and belowground production and nitrogen cycling in alpine tundra. Ecology 79, 2253-2266.

Frey, B.R., Lieffers, V.J., Munson, A.D., Blenis, P.V. 2003. The influence of partial harvesting and forest floor disturbance on nutrient availability and understory vegetation in boreal mixedwoods. Canadian Journal of Forest Research 33, 1180-1118.

Fröberg, M., Kleja, D.B., Hagedorn, F. 2007. The contribution of fresh litter to dissolved organic carbon leached form a coniferous forest floor. European Journal of Soil Science 58, 108-114.

Frostegård, Å. Bååth, E. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59–65.

Grady, K.C., Hart, S.C. 2006. Influence of thinning, prescribed burning, and wildfire on soil processes and properties in southwestern ponderosa pine forests: a retrospective study. Forest Ecology and Management 234, 123-135.

González-Pérez J.A., González-Vila F.J., Gonzalo A., Knicker H. 2004. The effect of fire on soil organic matter-a review. Environment International 30, 855-870.

Grayston, S.J., Prescott, C.E. 2005. Microbial communities in forest floors under four species in coastal British Columbia. Soil Biology and Biochemistry 37, 157-1167.

Hamman S.T., Burke, I.C., Stromberger, M.E. 2007. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biology and Biochemistry 39, 1703-1711.

Hannam, K.D., Quideau, S.A., Oh S.-W., Kishchuk, B.E., Wasylishen, R.E. 2004. Forest floor composition in aspen- and spruce-dominated stands of the boreal mixedwood forest. Soil Science Society of America Journal 68, 1735-1743.

Hannam, K.D., Quideau, S.A., Kishchuk, B.E., Oh S.-W., Wasylishen, R.E. 2005. Forest-floor chemical properties are altered by clear-cutting in boreal mixedwood forests stands dominated by trembling aspen and white spruce. Canadian Journal of Forest Research 35, 2457-2468.

Hannam, K.D., Quideau S.A., Kishchuk B.E. 2006. Forest floor microbial communities in relation to stand composition and timber harvesting in northern Alberta. Soil Biology and Biochemistry 38, 2565-2575.

Hassett, J.E., Zak, D.R. 2005. Aspen harvest intensity decreases microbial biomass, extracellular enzyme activity and soil nitrogen cycling. Soil Science Society of America Journal 69, 227-235.

Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., Maldonado-Ramirez, S., Lynch, S.T., Nelson, E.B. 2000. Methods for assessing the composition and diversity of soil microbial communities. Applied Soil Ecology 15, 25-36.

Högberg, M.N., Högberg, P., Myrold, D.D. 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150, 590-601.

Jacobs, J.M., Spence, J.R., Langor D.W. 2007. Influence of boreal forest succession and dead wood qualities on saproxylic beetles. Agricultural and Forest Entomology 9, 3–16.

Jayasinghe, B.A.T.D., Parkinson, D. 2008. Actinomycetes as antagonists of litter decomposer fungi. Applied Soil Ecology 38, 109-118.

Jerabkova, L., Prescott, C.E., Kishchuk, B.E. 2006. Nitrogen availability in soil and forest floor of contrasting types of boreal mixedwood forests. Canadian Journal of Forest Research 36, 112-122.

Jurgen, G., Saano, A. 1999. Diversity of soil Archaea in boreal forest before, and after clear-cutting and prescribed burning. FEMS microbiology Ecology 29, 205-213.

Kishchuk, B. 2004. Soils of the Ecosystem Management Emulating Natural Disturbance (EMEND) experimental area, northerwestern Alberta. Nat. Resour. Can., Can. For. Serv., North. For. Cent., Edmonton, Alberta. Inf. Rep. NOR-X-397.

Knicker, H., Almendros, G., González-Vila, F.J., Martin, F., Lüdemann, H.-D. 1996. ^{13}C and ^{15}N-NMR spectroscopic examination of the transformation of organic nitrogen in plant biomass during thermal treatment. Soil Biology and Biochemistry 28, 1053-1060.

Leckie, S.E. 2005. Methods of microbial community profiling and their application to forest soils. Forest Ecology and Management 220, 88-106.

Lindo, Z., Visser, S. 2003. Microbial biomass, nitrogen and phosphorus mineralization, and mesofauna in boreal conifer and deciduous forest floors following partial and clear-cut harvesting. Canadian Journal of Forest Research 33, 1610-1620.

Macdonald, S.E., Fenniak, T.E. 2007. Understory plant communities of boreal mixedwood forests in western Canada: Natural patterns and response to

variable-retention harvesting. Forest Ecology and Management 242, 34-48.

McCulley, R.L., Burke, I.C. 2004. Microbial community composition across the great plains: landscape versus regional variability. Soil Science Society of America 68, 106-115.

McCune B., and Grace J. B. 2002. Analysis of ecological communities. MjM Software Design, Oregon, 284 pp.

Menyailo, O.V., Hungate, B.A., Zech, W. 2002. Tree species mediated soil chemical changes in a Siberian artificial afforestation experiment. Plant and Soil 242, 171-182.

Merilä, P., Strömmer, R., Fritze, H. 2002. Soil microbial activity and community structure along a primary succession transect on the land-uplift in western Finland. Soil Biology and Biochemistry 34, 1674-1654.

McCullough, D.G., Werner, R.A., Neuman, D. 1998. Fire and insects in northern and boreal forest ecosystems in North America. Annual Review of Entomology 43, 107-127.

Mulvaney, R.L. 1996. Nitrogen—inorganic forms. In: Sparks, D.L., (Ed.), Methods of Soil Analysis Part 3-Chemical Methods. Soil Science Society of America. Madison, pp. 1123–1184.

Myers, R.T., Zak, D.R., White, D.C., Peacock, A. 2001. Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Science Society of America Journal 65, 359-367.

O'Donnell, A.G., Goodfellow, M., Minnikin, D.E. 1982. Lipids in the classification of *Nocardiodes*; reclassification of *Arthobacter simplex* (Jensen) Lochhead in the genus *Nocardiodes* (Prauser) emend. O'Donnell et al. as *Nocardioides simplex* comb nov. Archives of Microbiology 133, 323-329.

Palese, A.M., Giovannini, G., Lucchesi, S., Dumontet, S., Perucci, P. 2004. Effect of fire on soil C, N and microbial biomass. Agronomie 24, 47-53.

Pietikäinen, J., Fritze, H. 1993. Microbial biomass activity in the humus layer following burning: short-term effects of two different fires. Canadian Journal of Forest Research 23, 1275-1285.

Pietikäinen, J., Hiukka, R., Fritze, H. 2000. Does short-term heating of forest humus change its properties as a substrate for microbes? Soil Biology and Biochemistry 32, 277-288.

Prieto-Fernández, A., Acea, M.J., Carballa, T. 1998. Soil microbial and extractable C and N after wildfire. Biology and Fertility of Soils 27, 132-142.

Priha, O., Grayston, S.J., Hiukka R., Pennanen, T., Smolander A. 2001. Microbial community structure and characteristics of the organic matter in soils under *Pinus sylvestric*, *Picea abies* and *Betula pendula* at two forest sites. Biology and Fertility of Soils 33, 17-24.

Saetre, P. 1999. Spatial patterns of ground vegetation, soil microbial biomass and activity in a mixed spruce-birch stand. Ecography 22, 183-192.

Saetre, P., Bååth, E. 2000. Spatial variation and patterns of soil microbial community structre in a mixed spruce-birch stand. Soil Biology and Biochemistry 32, 909-917.

Scheuner, E.T., Makeschin, F., Wells E.D., Carter P.Q. 2004. Short-term impacts of harvesting and burning disturbances on physical and chemical characteristics of forest soils in western Newfoundland Canada. European Journal of Forest Research 123, 321-330.

Schmidt, M.G., Macdonald, S.E., Rothwell, R.L. 1996. Impacts of harvesting and mechanical site preparation on soil chemical properties of mixed-wood boreal forest sites in Alberta. Canadian Journal of Soil Science, 76, 531-540.

Seibert, J., Stendahl, J., Sørensen, R. 2007. Topographical influences on soil properties in boreal forests. Geoderma 141, 139-148.

Smolander, A., loponen, J., Suominen, K., Kitunen, V. 2005. Organic matter characteristics and C and N transformation in the humus layer under two tree species, *Betula pendula* and *Picea abies*. Soil Biology and Biochemistry 37, 1309-1318.

Spence, J., Volney, W.J.A., Sidders, D., Luchkow, S., Vinge, T., Oberle, F., Gilmore, D., Bielech, J.P., Wearmouth P., Edwards J., Bothwell P., Shorthouse D., Wilkinson, D., Brais, S. 2002. The EMEND Experience. In: T Veeman et al. (Eds.), Advances in Forest Management: From Knowledge to Practice, Proceedings of SFMN Conference, 13-15 Nov, Edmonton, Alberta. SFMN Network, 40-44.

Stocks, B.J., Mason, J. A., Todd, J.B., Bosch, E.M., Wotton, B.M., Amiro, B.D., Flannigan, M.D., Hirsh, K.G., Logan, K.A., Martell, D.L., Skinner, W.R. 2003. Large forest fires in Canada, 1959-1997. Journal of Geophysical Research 108, FFR 5-1-FFR 5-12.

Therneau, T.M., Atkinson, B. 2005. Recursive Partitioning. R package version 3.1-23. ported 6.x original at http://mayoresearch.mayo.edu/mayo/research/biostat/splusfunctions.cfm (5 September, 2008).

Zak, D.R., Kling G.W. 2006. Microbial community composition and function across an arctic tundra landscape. Ecology 87, 1659-1670.

Chapter 3. Moisture effects on microbial communities in boreal forest floors are stand-dependent

A version of this chapter has been submitted for publication to Applied Soil Ecology.

3.1. Introduction

The organic horizons, or forest floors, of forest soils are often much thinner than the underlying mineral soil horizons. Nevertheless, forest floors are at the forefront of a multitude of biogeochemical cycles and interactions between the biotic and abiotic components of forest ecosystems. Landscape scale factors including forest stand composition (Grayston and Prescott, 2005) along with human (Bååth et al., 1995) and natural disturbances such as fire (D'Ascoli et al., 2005) all play a role in determining the structure and function of the microbial community in the forest floor. Likewise, the community structure of forest floor microbes is the primary driver behind biogenic greenhouse gas release (Schmiel & Gullege, 1998) and nitrogen mineralization (Fraterrigo et al., 2006).

Larger scale factors such as stand composition can have quantifiable effects on forest floor chemical and physical properties (Vesterdal and Raulund-Rasmussen, 1998). However, describing large scale factors alone does not sufficiently capture the micro-environmental gradients present in forest floors. For example, physical attributes such as bulk density dictate the quantity and availability of moisture within the forest floor. Microbial communities exhibit adaptive resilience to wet-dry cycles when such conditions are present *in situ*

(Frier et al., 2003, Griffiths et al., 2003; Lundquist et al., 1999). Conversely, moisture fluctuations can alter soil microbial community structure (Drenovsky et al., 2004), particularly for microbial communities from stable moisture environments (Frier et al., 2003).

The boreal landscape of Northern Alberta is predominantly composed of white spruce (*Picea glauca* (Moench) Voss) and trembling aspen (*Populus tremuloides* Michx.) stands. Research in the area has consistently shown that aspen and spruce forest floors have structurally and functionally distinct microbial communities (Hannam et al., 2006; Swallow et al., 2009). Aspen and spruce communities are able to retain their distinctiveness over time even when reciprocally transplanted into the other forest floor environment (Hannam et al., 2007). Past work in this region (Hannam et al., 2006, 2007) has attributed microbial community differences to chemical properties such as pH. However, other factors influencing community composition have been less studied; in particular, aspen and spruce forest floors provide different physical habitats for microorganisms as aspen forest floors can have higher bulk density but be thinner than spruce forest floors (Redding et al., 2005).

In this study we investigated how the forest floor microbial communities under either aspen or spruce responded when exposed to different physical microenvironments generated by a gradient of soil moisture. Microbial community structural diversity was characterized using phospholipid fatty acid (PLFA) analysis, which has successfully been used to fingerprint soil microbes across a broad range of ecosystems and is often times more sensitive at detecting

community changes than other physiological and molecular methods (Ramsey et al., 2006). We described the functional diversity of the microbial communities using the MicroResp^{TM} system developed by Campbell et al. (2003). This system utilizes the whole soil approach of the original multi-SIR developed by Degens and Harris (1997), but increases the speed and efficiency at which samples can be processed and has been shown to be more sensitive to changes of community function than other physiological based methods (Campbell et al., 2003).

3.2. Materials and Methods

3.2.1 Sample Collection and Experimental Design

Material for the laboratory incubations was collected during the summer of 2008 from representative aspen and white spruce sites located within the boreal mixedwood plains of northwestern Alberta near Fort McMurray, Alberta. The mature aspen site had an overstory of trembling aspen interspersed with the occasional white spruce in the understory, while the mature spruce site was dominated almost entirely by white spruce. Collection of the forest floor material occurred along a 10 m transect at 1 m intervals with material being collected from a square area 15 cm by 15 cm until the underlying mineral horizon was reached. Fresh leaf litter was removed prior to the collection of forest floor material and all forest floor material was kept cool during transport to the lab. The forest floor collected at 1 m intervals was sieved through to 4 mm. After sieving, the material from all 10 collection points was composited by thoroughly mixing to yield a

homogeneous material for the incubation. Afterwards, the material was air dried and stored for one month prior to the start of the incubation.

Each sample used in the incubation consisted of 12 g dry weight of forest floor material. Pressure plates were used to adjust the water content of each sample after saturation with distilled water. The highest water content was set at field capacity (-15 kPa), while the wilting point (-1.5 MPa) was used as the lowest water content. According to the Young-Laplace equation, which assumes all pores in a material are cylindrical tubes, the field capacity treatment would correspond to water being retained in all pores ≤ 20 μm in diameter, while water would be held in pores ≤ 0.2 μm in diameter for the wilting point treatment (Standing and Killham, 2007). Organic soil horizons are physically complex environments, which may not conform to the simple assumptions of the Young-Laplace equation. However, the use of water potential ensures that the moisture environment available to forest floor microorganisms is comparable between materials with different water holding properties. Indeed, this technique has been used extensively to generate different water filled microbial habitats in mineral soils (Heijnen and van Veen, 1991; Ruamps et al., 2011). Originally, an intermediate pressure of -300 kPa was included; however, preliminary tests showed that, due to the physical properties of the forest floor materials, this pressure potential had nearly identical moisture content to the materials at the wilting point. Therefore, the moisture content of samples subjected to the intermediate treatment was adjusted to 60% of the field capacity by weight. Samples were incubated at 30°C in 130 ml test tubes for 45 days. Water contents

were maintained by weighing the samples daily and using distilled water to compensate for moisture loss. After the incubation was complete, each sample was thoroughly homogenized and then subsampled for PLFA and MicroResp™ analyses.

3.2.2 Analytical Methods

Structural diversity of the viable microbial communities was determined by the identification and quantification of microbial Phospholipid Fatty Acids (PLFAs). The protocol used has been thoroughly described in Hannam et al. (2006). In short, polar lipids were extracted from freeze-dried samples using a modified Bligh and Dyer (1959) process. The extracted lipids were purified on pre-packed silicic acid columns (Agilent Technologies, Wilmington, DE) and then converted to fatty acid methyl esters (FAMEs). MIDI peak identification software (MIDI, Inc., Newark, DE) was used for peak identification and quantification of FAMEs that were separated using a Agilent 6890 Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column. The PLFAs were described with a standard format of X:YωZ, with "X" indicating the total number of carbons, "Y" the number of unsaturations and "Z" the location of the first unsaturation in the molecule from the aliphatic end indicated by "ω". The suffixes "c" and "t" specify that the molecule is in the cis or trans configuration, respectively. Saturated branching from the aliphatic end is denoted as an "i" if it occurs on the second carbon or an "a" if it occurs on the third carbon. Other

prefixes such as “cyc” and “10Me” are used to indicate the presence of a cyclopropyl group and a methyl group found on the 10^{th} carbon from the carboxyl end, respectively.

The functional composition of the microbial communities was characterized using the MicroResp™ system (Campbell et al., 2003). This system measures microbial respiration with the use of a 96 well carbon dioxide detection microplate with each well containing cresol red indicator (12.5 μg ml^{-1}), potassium chloride (150 nM) and sodium bicarbonate (2.5 mM) in a 1% agar solution. Soil samples and 15 carbon substrates plus distilled water were placed in a 1.2 ml deepwell plate and connected to the inverted detection microplate by a MicroResp™ silicon gasket. A total of two samples were analyzed on one deep-well plate with each substrate in triplicate. Each of the 96 deep-wells contained 0.06 g dry weight of forest floor. Basal respiration was determined using water only while the carbon substrates included L-alanine, citric acid, L-arginine, galactose, amniobutyric acid, malic acid, oxalic acid, dihydroxybenzoic acid, arabinose, glutamic acid, mannose, glucose, L-lysine, N-acetylglucosamine, and trehalose. The carbon substrates were delivered at a content of 30 mg g^{-1} soil in 25 μl aliquots. Small clamps were used to ensure that there was no gas leakage between the deepwell and the detection plates. The assembled plates were incubated at 30°C over a period of 6 hours. Microbial respiration was determined by the color change of the indicator dye caused by the absorption of CO_2 released by microbial respiration.

3.2.3 Statistical Design and Analysis

The experiment was designed as a randomized 2 by 3 factorial design with each treatment combination being replicated 10 times. Due to sample loss, the final replication for spruce samples (FC, 60%FC and WP) was 8, 10, and 8, while for aspen samples (FC, 60%FC and WP) was 9, 8, and 8. Factors were deemed significant at α of 0.05. Sample locations within the incubation chamber were assigned randomly. Analyses only included PLFAs that have been shown to be microbial in origin. These included the following biomarkers for bacteria: 15:0, a15:0, i15:0, i16:0, 16:1ω9c, 17:0, a17:0, i17:0, cyc17:0 18:1ω7c and cyc19:0, 14:0, 16:1ω7c, 18:0 (Myers et al., 2001), 18:1ω5c (Hasset and Zak, 2005), 16:1 2OH and 17:0 3OH (Hamman et al., 2007); for fungi: 18:2ω6c (Frostegård and Bååth, 1996), 18:1ω9, 20:1ω5 (Myers et al., 2001), 16:1ω5c and 18:3ω6c (Hamman et al., 2007); and for actinomycetes: 10Me19:0 (O'Donnell et al., 1982), 10Me16:0 and 10Me 18:0 (Myers et al., 2001). Finally, the PLFA 20:4 was included for protozoa (Myers et al., 2001). Total concentrations for gram positive bacteria (including all saturated PLFAs; e.g. 15:0), gram negative bacteria (all unsaturated bacterial PLFAs; e.g. 16:1ω7c), fungi, and total PLFAs as well as the gram positive: gram negative and fungi:bacteria ratios were analysed using two way ANOVA or one way in the case of a significant interaction effect with PROC MIXED (version 9.1, SAS institute Inc.). Mean separation between stand types and among moisture treatments was tested using the LSMEANS statement. The PLFA data for protozoa and actinomycetes were non-normal and were analysed using a 2-way non-parametric ANOVA (Scheirer-Ray-Hare extension of the

Kruskal-Wallis test). Outliers were identified and removed using the fourth-spread method (Devore, 2002) due to the conservative nature of the non-parametric tests used. Finally, a Kruskal-Wallis non-parametric ANOVA was used to conduct post-hoc tests to compare among moisture treatments within the aspen and spruce samples separately.

The structural and functional diversity of the microbial communities was analyzed using a non-metric multidimensional scaling (NMS) ordination with PC-ORD (version 5, MjM Software Design). Prior to the NMS analysis, all PLFAs were expressed on a mol% basis and were arcsine square-root transformed to be analyzed as proportional data (McCune and Grace, 2002). In terms of the results from the MicroResp™ analyses, the sample response to each substrate was calculated proportionally to the total response of all substrates (Degens et al., 2000). Both NMS ordinations used a Sørensen (Bray–Curtis) dissimilarity measurement. Sample groupings in the NMS ordination space associated with both moisture treatment and stand composition were tested for significance with a multi-response permutation procedure (MRPP). A MRPP is a non-parametric procedure that tests the separation between groups by generating a "T" value, which has a corresponding p value to indicate significance. The MRPP also calculates an "A" value, which determines within group homogeneity and is an indication of effect size (McCune and Grace, 2002).

3.3. Results

3.3.1 Moisture Retention

The amount of moisture retained at a specific retention pressure was related to the origin of the litter material. On average, the gravimetric water content for the spruce material was 123% for field capacity (FC), 118% for 60% FC and 111% for the wilting point (WP). Overall, aspen material held less gravimetric moisture at FC (110%), 60% FC (94%) and WP (74%) than spruce. Variation between sample moisture content was low with the coefficient of variation for samples within each moisture treatment being less than 1%.

3.3.2 Microbial Community Diversity

The ordination of the PLFA data produced a two dimensional solution (final stress = 8.44 after 97 iterations) with a distinct separation occurring between the aspen and spruce samples (Figure 3-1). An MRPP analysis of the ordination data did not show any difference in groupings by moisture treatment within the spruce samples but a clear difference was found among all moisture treatments within the aspen samples (Table 3-1). Even though the ordination of the multi-SIR data was weaker (three dimensional solution with a final stress of 17 after 500 iterations), it showed the same relationship as the PLFA analysis among the moisture treatments, i.e., no differences within the spruce but significant differences within the aspen samples. Further groupings within the aspen samples were associated with the presence or absence of the protozoa PLFA biomarker (20:4ω6c) as aspen samples lacking this PLFA clustered more closely regardless of moisture treatment (MRPP results $p<0.05$, $T= -14.1$, $A = 0.19$, Figure 3-1).–The joint plots of two actinonomycete PLFA concentrations

(10Me16:0 and 10Me18:0) showed a strong positive relationship (r^2 >0.50) towards the drier aspen samples. Additionally, the actinomycete PLFA, 10Me19:0 and the gram positive PLFA, i16:0, also scaled in the direction of the drier aspen samples, but had a weaker association.

3.3.3 Group Specific Analyses

The ANOVA analysis demonstrated significant interactions between moisture treatment and stand composition for concentrations of both gram positive and total PLFAs (Table 3-2). Conducting a one-way ANOVA on moisture treatments for the aspen samples showed that the gram positive PLFA concentrations increased in the treatments with less moisture; specifically, the FC samples had significantly lower gram positive PLFA concentrations than the 60%FC and WP treatments. On the other hand, moisture had no influence on the gram positive PLFA concentrations in the spruce samples, as these stayed relatively constant across all moisture treatments. The significant interaction between stand and moisture for the total PLFAs further indicated that moisture effects on total PLFA concentrations were dependant on the origin of the forest floor material. A one-way ANOVA of total PLFAs in the aspen samples revealed that the FC treatment harbored significantly lower total PLFA concentrations than the WP treatment, but neither one was significantly different from the 60%FC. Total PLFA concentrations in the spruce samples were not influenced by moisture. Furthermore, the significant interaction for total PLFA concentrations was not driven entirely by the effect of gram positive PLFAs, as it was still

present in the 2-way ANOVA results even after the gram positive PLFAs were removed from the calculation of the total PLFAs (data not shown).

There were no treatment effects on the concentrations of gram negative PLFAs. Fungal PLFA concentrations were significantly higher in the spruce treatment, but there was no apparent effect of moisture (Table 3-2). The ratios of gram positive to gram negative bacteria were significantly higher in the WP treatment than in the other moisture treatments. This trend was also observed in the ratio of fungi to bacteria; however the WP treatment was only higher than the FC treatment.

Protozoa PLFA concentrations were significantly affected by moisture, as was revealed through a non-parametric ANOVA (Scheirer-Ray-Hare extension of the Kruskal-Wallis test) analysis (Table 3-3). Post-hoc comparisons (conducted with a Kruskal-Wallis one way ANOVA) within each stand type showed that protozoan PLFA concentrations differed only in the aspen samples, with the FC and 60%FC treatments having similar PLFA concentrations but both differing significantly from the WP treatment ($P<0.016$ after Bonferroni correction; Figure 3-2). The spreading of the data was much larger for the aspen WP treatment than for all other treatments and occurred at lower concentrations than the aspen FC and 60%FC treatments (Figure 3-2). Additionally, the aspen WP treatment had a median of 0 $nmol \cdot g\text{-}soil^{-1}$ since many samples lacked any of the protozoan PLFA biomarker.

Actinomycete PLFA concentrations were significantly affected by the stand treatment with significant moisture differences only becoming apparent in

post-hoc comparisons (Table 3-3). Overall, the medians for actinomycete PLFA concentrations in the spruce moisture treatments were lower than in the aspen moisture treatments, while all treatments, excluding the aspen 60%FC, had relatively small dispersion in the data (Figure 3-2). As evidenced by post-hoc tests, the WP, 60%FC and FC moisture treatments for the aspen samples all contained significantly different concentrations of actinomycete PLFAs. Concentrations of actinomycete PLFAs in aspen further increased as moisture decreased; the WP samples showed the highest concentrations with a median value of 75.5 nmol·g-soil^{-1}, while the medians of the 60%FC and FC treatments were 63.6 and 54.9 nmol·g-soil^{-1}, respectively.

3.4. Discussion

3.4.1 Drivers of Microbial Community Diversity

The chemical environment of the litter generated by the dominant trees and understory plants has often been offered as the primary explanation for the influence that plant communities have on the structure of forest floor microbial communities (Priha et al., 2001). In this study, the origin of the plant litter was a strong determinant of the structure and function of the microbial community. The air-drying of the litter most likely had a strong effect upon the microbial communities. Nonetheless, the response of the microbial communities to the rewetting of the litter and subsequent drying down on the pressure plates was dependent on the litter origin. In spruce, moisture differences exhibited no effect on either microbial community structure or total PLFA concentrations, which has

also been observed in Norway spruce forest floors (Wilkinson and Anderson, 2001), and attributed to a drought-adapted microbial community. This adaptation may be linked to the physical structure of the litter material. During a precipitation event, the upper layers of coniferous needle litter drain primarily in a vertical direction (Sato et al., 2004). This action would limit the amount of moisture retained in the litter during a given event, as gravitational flow would predominate. In contrast, moisture in the upper layers of deciduous leaf litter will primarily flow in a horizontal direction prior to draining laterally (Sato et al., 2004), due to the plate-like structure formed by deciduous leaves falling on top of one another. This allows deciduous litter to retain more of the moisture that it intercepts during a precipitation event because air-filled spaces could be filled with horizontally flowing water. In a similar fashion, the forest floor materials used in this study exhibited distinct physical properties. Aspen litter would then wet more evenly and as it dries, produce a gradient of water-filled habitats, allowing for species better adapted to different conditions to flourish.

3.4.2 Response of Protozoa to Moisture and Forest Floor Composition

The PLFA biomarker literature in regards to protozoa is rather lacking and poorly researched. However, 20:4 has been linked to ciliated paramecium and doesn't appear to be abundant in the flagellate species that have been studied (Lechevalier and Lechevalier, 1988). Different responses seen in the concentration of this PLFA to moisture between aspen and spruce (Figure 3-2) may be linked to the differences in the habitable pore space provided by the

litters. Habitat structure limits protozoan predation on bacteria as water content decreases, because water films connecting larger pores diminish and isolate larger water filled pores (Vargas and Hattori, 1986). When this occurs, protozoa cannot migrate into areas that have active bacteria populations but lack protozoa. The spruce litter pore habitat was primarily composed of very large and small pores. The maximum water filled pore neck diameter of the FC treatment would have been roughly 20 µm and would become restrictive to ciliate activity with minimal moisture loss, as a 12% loss in moisture (the difference between FC and WP) would result in a pore neck diameter of approximately 0.2 µm. Flagellate and amoeba activity is inhibited once maximum water filled pore necks diameters are < 3µm (Uikman et al., 1991), which would have occurred in the spruce litter at a very high moisture content**.** Optimal moisture content, along with other biotic and abiotic signals, must exist in order for protozoa to begin to excyst (Adl and Gupta, 2006). It is likely that the water contents used in this study may not have been high enough to produce conditions triggering substantial ciliate excystment and activity in the spruce litter. On the other hand, the pore habitat of the aspen litter didn't appear to limit ciliate activity as severely as the spruce. The 60% FC moisture treatment in the aspen samples contained 16% less moisture than the FC treatment while the WP treatment contained 36% less moisture. This indicates that pore habitat in the aspen litter was more variable and habitat exclusion would have occurred after a higher percentage of moisture was lost from the litter. Ciliate adaptation to the pore habitats of aspen and spruce may also account for the lack of protozoa PLFA in the aspen WP treatment. All samples were saturated

overnight on pressure plates before the drying pressure was applied. If a large proportion of ciliates in the aspen litter became active during this time they may not have had sufficient time to encyst during the rapid drying caused when the WP pressure was applied. The ciliates in spruce may have required more time under wet conditions to become active and therefore fewer cells would have perished when the WP pressure treatment was applied.

3.4.3 Response of Bacteria and Fungi

Elevated concentrations of actinomycete PLFAs in the aspen compared to the spruce forest floors have also been observed in previous studies comparing aspen and spruce forest floors in Northern Alberta (Hannam et al., 2006), and in other studies comparing coniferous and deciduous litters in Canada (Iverson and Sowden, 1959). Authors working within Northern Alberta have consistently reported that aspen litter has a higher pH than spruce litter (Lindo and Visser, 2003; Hannam et al., 2006). The higher pH of aspen litter may be responsible for the higher amounts of actinomycete PLFAs since studies in mineral soils (El-Tarabily et al., 1996) and forest floors (Bååth et al., 1995) have both shown that actinomycetes respond favorably to conditions with a more neutral pH.

Some strains of actinomycetes have been established to tolerate levels of moisture far lower than used in this study (Williams et al., 1972; Zvyagintsev et al., 2007). Past studies have demonstrated that certain strains of actinomycetes prefer to occupy the outer aggregate regions of mineral soils (Hattori et al., 1976) and grow best in air-filled pores with high relative humidity (Williams et al.,

1972). Outer aggregate surfaces with negligible amounts of moisture can also be areas that are protected from protozoan predation (Vargas and Hattori, 1986) as protozoan activity in these spaces tends to diminish when soil moisture reaches a threshold level below which protozoa are forced to occupy disconnected water filled zones. The ability of actinomycetes to grow under conditions of lower moisture may allow them to occupy habitat that is inaccessible to other organisms, which could explain why the highest actinomycete PLFA concentrations occurred in the WP treatment in the aspen samples. It is also of note that one biomarker for actinomycetes (10Me19:0) was only observed in the aspen samples that did not contain the protozoan biomarker PLFA.

Recent work in pine forests by Landesman and Dighton (2010) also observed a lack of response of gram negative and fungal PLFAs to low moisture. Dix and Frankland (1987) found that the growth of litter decomposing fungi from a variety of forest habitats was only affected at moisture levels much lower than those used in this study, which may be why fungal PLFA concentrations were not affected even by the lowest moisture treatment. It is possible that most of the gram-negative bacteria of the aspen and spruce forest floors colonized the smaller water filled pores where moisture limitations were less drastic. Preferential colonization of aggregate interiors in mineral soils by gram-negative bacteria (Hattori et al., 1976) and more recently, α-protoebacteria (Mummey et al., 2006) has been observed. However, the literature in this area is sparse and to the knowledge of the authors, nonexistent for the organic horizons of forest soils.

Differences between the concentrations of fungal PLFAs in coniferous and deciduous forest floors have often been attributed to the chemical composition of coniferous litter and the association of ectomycorrhizal fungi with coniferous trees (Zechmeister-Boltenstern et al., 2010). However, the role that the physical environment of the forest floor has upon fungal growth is largely unknown. In mineral soils, the spread of fungal hyphae has been found to occur primarily in environments that are composed of smaller and evenly distributed pore space, while growth in larger pores tends to occur along the pore walls rather than across air filled voids (Harris et al., 2003). The spruce litter in this study contained a larger proportion of smaller pores than the aspen litter and, if evenly distributed, would have provided a habitat more suitable for the spread of hyphae.

It is clear that, in addition to supplying different levels of carbon and other nutrients, forest floors generated under spruce and aspen provide distinct physical environments. These environments have a large influence upon the composition of the microbial communities residing in these forest floors. However, the interactions between microorganisms such as bacteria and protozoa in relation to the forest floor physical environment are poorly understood. Considering these relationships and how they are affected by their respective physical environment would broaden our overall understanding of the many biogeochemical cycles which are driven primarily by these groups of microorganisms.

Tables and Figures

Table 3-1. MRPP results of groupings from the NMS ordination of PLFA and multi-SIR data after Bonferroni corrected alpha of 0.0083.

Variable	Stand	Moisture	T	A	p<0.0083
PLFA	Spruce	FC vs 60%FC	-0.05	0.00	No
		FC vs WP	-2.46	0.05	No
		60%FC vs WP	-3.28	0.06	No
	Aspen	FC vs 60%FC	-4.37	0.09	Yes
		FC vs WP	-7.66	0.26	Yes
		60%FC vs WP	-4.78	0.17	Yes
Multi-SIR	Spruce	FC vs 60%FC	0.01	0.00	No
		FC vs WP	0.35	0.00	No
		60%FC vs WP	0.12	0.00	No
	Aspen	FC vs 60%FC	-5.42	0.06	Yes
		FC vs WP	-4.49	0.05	Yes
		60%FC vs WP	-3.71	0.04	Yes

Table 3-2. Results from the ANOVA analysis for parametric PLFA indices with standard errors in parentheses. Significant interactions between stand and moisture treatments are denoted by † and resulted in a one-way ANOVA being conducted to test for moisture differences within each stand. Statistical differences between stands are indicated by *($p<0.05$) while differences after Bonferroni correction ($p<0.016$) among moisture treatments are denoted by different letters.

Stand	Gram positive†			Gram negative			Gram positive:Gram negative		
	FC	60%FC	WP	FC	60%FC	WP	FC	60%FC	WP
	----------nmol g^{-1}·soil----------			----------nmol g^{-1}·soil----------					
Spruce	239.7(9.8)	234.4(10.1)	244.9(12.8)	184.2(6.8)	186.8(7.6)	176.9(8.6)	1.30(0.03)^{b}	1.25(0.03)^{b}	1.38(0.04)^{a}
Aspen	197.5(7.4)^{b}	237.9(8.6)^{a}	268.3(11.2)^{a}	165.4(7.9)	186.9(7.3)	193.0(7.2)	1.20(0.03)^{b}	1.28(0.04)^{b}	1.39(0.02)^{a}
	Fungi			Fungi:Bacteria			Total†		
	FC	60%FC	WP	FC	60%FC	WP	FC	60%FC	WP
	----------nmol g^{-1}·soil----------						----------nmol g^{-1}·soil----------		
Spruce	110.3(3.2)*	105.9(4.3)*	99.4(3.8)*	0.26(0.01)*^{a}	0.25(0.01)*^{a}	0.24(0.01)*^{b}	562.8(19.8)	556.2(21.9)	547.6(25.5)
Aspen	77.6(3.2)	83.9(2.9)	83.3(2.9)	0.21(0.01)^{a}	0.20(0.004)^{a}	0.18(0.004)^{b}	506.5(19.2)^{a}	574.6(19.0)^{ab}	622.2(24.4)^{a}

Table 3-3. Results of non-parametric 2-way ANOVA (Scheirer-Ray-Hare extension of the Kruskal-Wallis test) on concentrations of PLFA biomarkers for protozoa and actinomycetes.

Variable	Source of Variation	χ^2	df	$p<0.05$
Protozoa	Moisture	8.24	2	Yes
	Stand	4.12	1	Yes
	Moisture x stand	2.54	2	No
Actinomycetes	Moisture	0.51	2	No
	Stand	42.26	1	Yes
	Moisture x stand	0.51	2	No

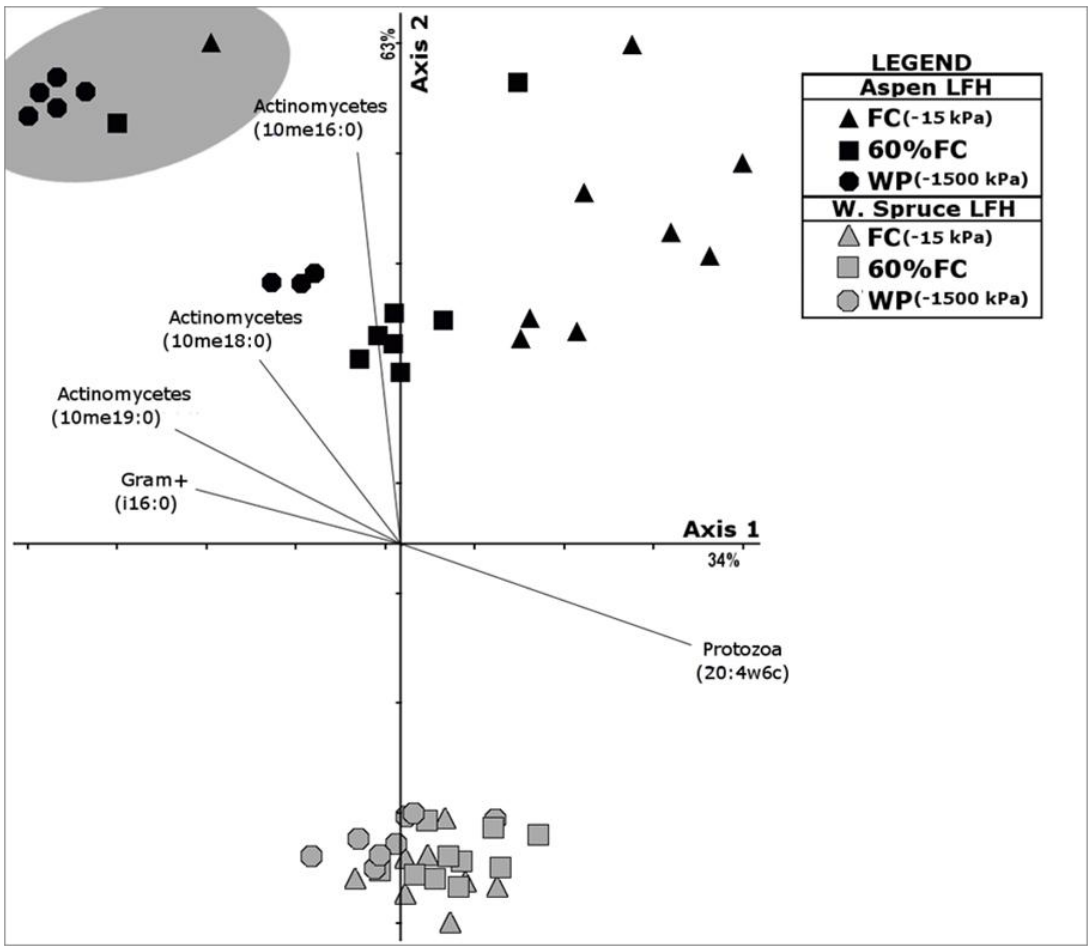

Figure 3-1. Two-dimensional NMS ordination displaying the structural composition of the microbial community. Aspen samples lacking the protozoa biomarker are highlighted within the grey circle.

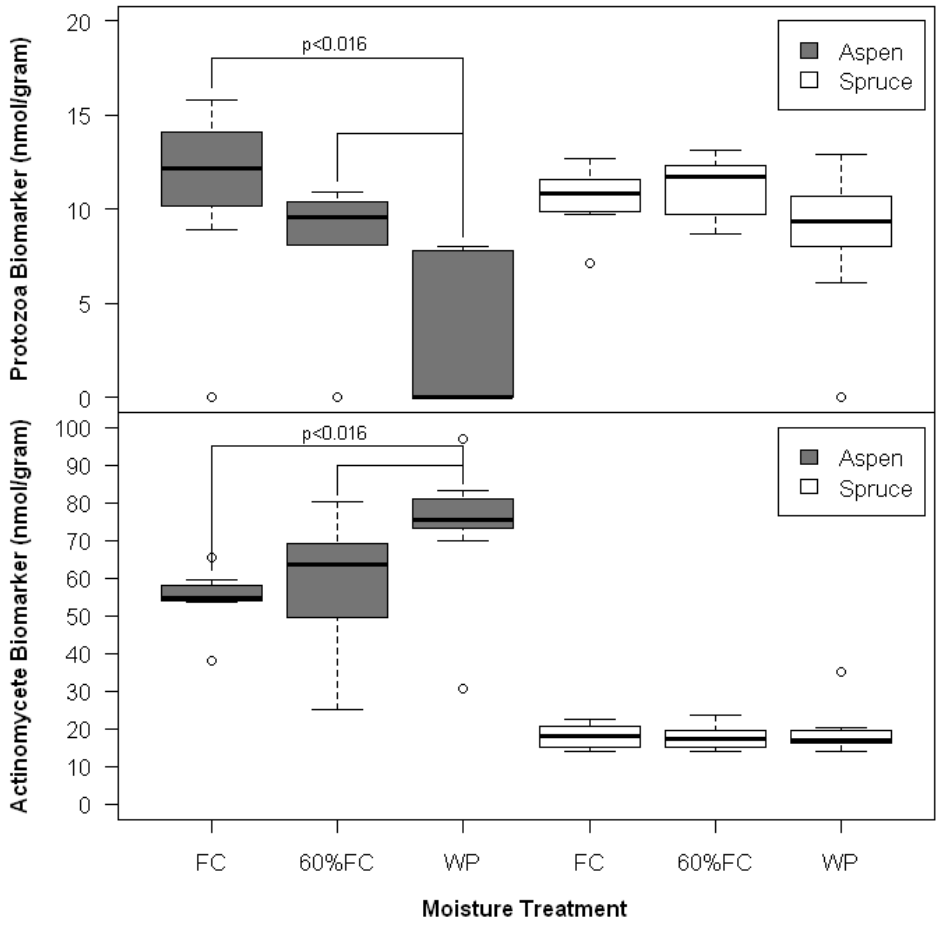

Figure 3-2. Boxplot of PLFA biomarkers concentrations for protozoa and actinomycetes. Median values are indicated by the horizontal bar within the box. The first and third quartiles are denoted by the lower and upper box limits respectively, and the 5th and 95th percentiles by the bars at the end of the vertical bars projecting from the box. Outliers are identified by open circles. Significant moisture treatment differences after Bonferroni correction found by the post-hoc Wilcoxon tests within aspen and spruce are indicated by adjoining lines.

Literature Cited

Adl, S.M., Gupta, V.V.S.R. 2006. Protists in soil ecology and forest nutrient cycling. Canadian Journal of Forest Research 36, 1805-1817.

Bååth, E., Frostegård, Å., Pennanen, T., Fritze, H. 1995. Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. Soil Biology and Biochemistry 27, 229-240.

Bligh, E.G., Dyer, W.J. 1959. A rapid method for the total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911-917.

Campbell, C.D., Champman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M. 2003. A rapid microtitier plate method to measure carbon dioxide evolved from carbon substrate ammentments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied Environmental Microbiology 69, 3593-3599.

D'Ascoli, R., Rutigliano, F.A., De Pascale, R.A., Gentile, A., Virzo De Danto, A. 2005. Functional diversity of the microbial community in Mediterranean maquis soils as affected by fires. International Journal of Wildland Fire 14, 355-363.

Degens, B.P., Harris, J.A. 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biology and Biochemistry 29, 1309-1320.

Degens, B.P., Schipper, L.A., Sparling, G.P., Vojvodic-Vukovic, M. 2000. Decreases in organic C reserved in soils can reduce the catabolic diversity of soil microbial communities. Soil Biology and Biochemistry 32, 189-196.

Devore, J.L. 2000. Probability and statistics for engineering and the sciences, Fifth ed. Pacific Grove, Califronia.

Dix, N.J., Frankland, J.C. 1987. Tolerance of litter-decomposing agarics to water stress in relation to habitat. Transactions of the British Mycological Society 88,127-129.

Drenovsky, R.E., Graham, K.J., Scow, K.M. 2004. Soil water content and organic matter availability are major determinants of soil microbial community composition. Microbial Ecology 48, 424-430.

El-Tarabily, K.A., Hardy, G.E. St.J., Sivasithamparam, K., Kurtböke, I.D. 1996. Microbiological difference between limed and unlimed soils and their relationship with cavity spot disease of carrots (Dausus carota L.) caused by Pythium coloratum in Western Austrailia. Plant and Soil 183, 279-290.

Fraterrigo, J.M., Balser, T.C., Turner, M.G. 2006. Microbial community variation and its relationship with nitrogen mineralisation in historically altered forests. Ecology 87, 570-579.

Frier, N., Schimel, J.P., Holden, P.A. 2003. Influence of drying-rewetting frequency on soil bacterial community structure. Microbial Ecology 45, 63-71.

Frostegård, Å., Bååth, E. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59-65.

Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J. 2003. Physiological and community responses of established grassland bacterial populations to water stress. Applied and Environmental Microbiology 69, 6961-6968.

Grayston, S.J., Prescott, C.E. 2005. Microbial communities in forest floors under four tree species in coastal British Columbia. Soil Biology and Biochemistry 37, 1157-1167.

Hamman, S.T., Burke, I.C., Stromberger, M.E. 2007. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biology and Biochemistry 39, 1703-1711.

Hannam, K.D., Quideau S.A., Kishchuk, B.E. 2006. Forest floor microbial communities in relation to stand composition and timber harvesting in northern Alberta. Soil Biology and Biochemistry 38, 2565-2575.

Hannam, K.D., Quideau, S.A., Kishchuk, B.E. 2007. The microbial communities of aspen and spruce forest floors are resistant to changes in litter inputs and microclimates. Applied Soil Ecology 35, 635-347.

Harris, K., Young, I.M., Gilligan, C.A., Otten, W., Ritz, K. 2003. Effect of bulk density on the spatial organization of the fungus Rhizoctonia solani in soil. FEMS Microbiology Ecology 44, 45-56.

Hassett, J.E., Zak, D.R. 2005. Aspen harvest intensity decreases microbial biomass, extracellular enzyme activity and soil nitrogen cycling. Soil Science Society of America Journal 69, 227-235.

Hattori, T., Hattori, R., McLaren A.D. 1976. The physical environment in soil microbiology: an attempt to extend principles of microbiology to soil microorganisms. Critical Reviews in Microbiology 4, 423-461.

Heijnen, C.E., van Veen, J.A. 1991. A determination of protective microhabitats for bacteria introduced into soil. FEMS Microbiology Ecology 85, 73-80.

Ivarson, K.C., Sowden, F.J. 1959. Decomposition of forest litters I. Production of ammonia and nitrate nitrogen, changes in microbial population and rate of decomposition. Plant and Soil 11, 237-248.

Landesman, W.J., Dighton, J. 2010. Response of soil microbial communities and the production of plant-available nitrogen to a two-year rainfall manipulation in the New Jersey Pinelands. Soil Biology and Biochemistry 42, 1751-1758.

Lechevalier, H., Lechevalier, M.P. 1988. Chemotaxonomic use of lipids- an overview. In: Ratledge, C., Wilkinson S.G., (Eds.), Microbial Lipids Volume 1. Academic Press., London, pp.869-902.

Lindo, Z., Visser, S. 2003. Microbial biomass, nitrogen and phosphorus mineralization, and mesofauna in boreal conifer and deciduous forest floors following partial and clear-cut harvesting. Canadian Journal Forest Research 33, 1610-1620.

Lundquist, E.J., Scow, K.M., Jackson, L.E., Uesugi, S.L., Johnson, C.R. 1999. Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/dry cycle. Soil Biology and Biochemistry 31, 1661-1675.

McCune, B., Grace, J.B., 2002. Analysis of ecological communities. Oregon.

Myers, R.T., Zak, D.R., White, D.C, Peacock, A. 2001. Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Science Society of America Journal 65, 359-367.

Mummey, D., Holben, W., Six, J., Stahl, P. 2006. Spatial stratification of soil bacterial populations in aggregates of diverse soils. Microbial Ecology 51, 404-411.

O'Donnell, A.G., Goodfellow, M., Minnikin, D.E. 1982. Lipids in the classification of *Nocardiodes*; reclassification of *Arthobacter simplex* (Jensen) Lochhead in the genus *Nocardiodes* (Prauser) emend. O'Donnell et al. as *Nocardioides simplex* comb nov. Archives of Microbiology 133, 323-329.

Priha, O., Grayston, S.J., Hiukka, R., Pennanen, T., Smolander, A. 2001. Microbial community structure and characteristics of the organic matter in soils under Pinus sylvestris, Picea abies and Betula pendula at two forest sites. Biology and Fertility of Soils 33, 17-24.

Ramsey, P.W., Rillig, M.C., Feris, K.P., Holben, W.E., Gannon, J.E. 2006. Choice of methods for soil microbial community analysis: PLFA

maximizes power compared to CLPP and PCR-based approaches. Pedobiologia 50, 275-280.

Ruamps, L.S., Nunan, N., Chenu, C. 2011. Microbial biogeography at the soil pore scale. Soil Biology and Biochemistry 43, 280-286.

Redding, T.E., Hannam, K.D., Quideau, S.A., Devito, K.J. 2005. Particle density of aspen, spruce, and pine forests floors in Alberta Canada. Soil Science Society of America Journal 69, 1503-1506.

Sato, Y., Kumagi, T., Kume, A., Otsuki, K., Ogawa, S. 2004. Experimental analysis of moisture dynamics of litter layers- the effects of rainfall conditions and leaf shapes. Hydrological Processes 18, 3007-3018.

Schimel, J.P., Gulledge, J. 1998. Microbial community structure and global trace gases. Global Change Biology 4, 745-758.

Standing, D., Killham, K. 2007. The soil environment. In: van Elsas, J.D., Jansson, K., Trevors, J. (Eds.), Modern Soil Microbiology 2nd Edition. CRC press., Boca Raton, pp. 1-22.

Swallow, M., Quideau, S.A., MacKenzie, M.D., Kishchuk, B.E. 2009. Microbial community structure and function: the effect of silvicultural burning and topographic variability in northern Alberta. Soil Biology and Biochemistry 41, 770-777.

Uikman, P.J.K., Jansen, A.G., van Veen, J.A. 1991. 15N-mineralization from bacteria by protozoa grazing at different soil moisture regimes. Soil Biology and Biochemistry 23, 193-200.

Vargas, R., Hattori, T. 1986. Protozoan predation of bacterial cells in soil aggregates. FEMS Microbiology Letters 38, 233-242.

Vesterdal, L., Raulund-Rasmussen, K. 1998. Forest floor chemistry under seven tree species along a soil fertility gradient. Canadian Journal of Forest Research 28, 1636-1647.

Wilkinson, S.C., Anderson, J.M. 2001. Spatial patterns of soil microbial communities in Norway spruce (Picea abies) plantation. Microbial Ecology 42, 248-255.

Williams, S.T., Shameemullah, M., Watson, E.T., Mayfield, C.I. 1972. Studies on the ecology of actinomycetes in soil-vi. The influence of moisture tension on growth and survival. Soil Biology and Biochemistry 4, 215-225.

Zechmeister-Boltenstern, S., Michel, K., Pfeffer, M. 2011. Soil microbial community structure in European forests in relation to forest type and atmospheric deposition. Plant and Soil 343, 37-50.

Zvyagintsev, D.G., Zenova, G.M., Doroshenko, E.A., Gryadunova, A.A., Gracheva T.A., Sudnitsyn, I.I. 2007. Actinomycete growth in conditions of low moisture. Biology Bulletin 34, 242-247.

Chapter 4. Auxin production in soils may not be linked to the activity of soil ciliates

4.1 Introduction

In forest ecosystems, the abundant protozoan community runs the gamut of soil ecological niches; they are grazers of bacteria, fungi and other protozoa; they are saprotrophs involved in the primary and secondary stages of litter decomposition; they are detrivores, consuming the products of litter decomposition (Adl, 2006). In particular, protozoa such as soil ciliates are nearly ubiquitously distributed worldwide (Foissner, 2006), have high species diversity (Foissner et al., 2008) and are able to quickly colonize soils *via* aerosol dispersal (Altenburger et al., 2010). When active, ciliates have a disproportionally large spatial influence relative to their size due to their high mobility within the soil matrix (Adl, 2007). Nonetheless, the role of ciliates and other protozoa in soil biogeochemical processes is often overlooked or only briefly touched upon in studies of forest ecosystems.

As early as the onset of the 20th century, soil protozoa have been recognized as important drivers of the soil nitrogen cycle (Waksman, 1916). Studies from the later part of the century showed that protozoans stimulate nitrogen mineralization in soils (Griffiths, 1986), promote nitrification (Griffiths et al., 1999), and that their activities lead to higher nitrogen contents in plant tissues (Chlarholm, 1985; Kuikman Van Veen, 1989; Uikman et al., 1991). The culmination of research conducted throughout the 20th century led to the conceptualization of the "soil microbial loop". In brief, soil protozoa participate in

the “soil microbial loop” by increasing nutrient availability in the soil environment because their nutritional needs are less than the bacteria they consume (Coleman, 1994).

Protozoa can influence the composition of microbial communities through selective grazing of bacteria (Rønn et al., 2002b; Griffith et al., 1999), which can have far-reaching ecological effects. In particular, protozoa-induced compositional changes to the soil microbial community can increase the activity of auxin producing bacteria, an important plant growth hormone (Bonkowski and Brandt, 2002). Plants seedlings growing under in the presence of protozoa produce root systems that are able to access soil moisture and nutrients from a larger volume as they are larger with more lateral branching (Bonkowski and Brandt, 2002), have longer fine roots and more root tips (Bonkowski et al., 2001). These changes to plant root architecture have led to the expansion of the “soil microbial loop” concept to include non-nutritional influences in addition to grazing-related nutritional effects (Bonkowski, 2004).

Given their diverse roles in ecosystem function, protozoa communities are excellent candidates for describing the status of soil ecosystems as well as for monitoring effects of human impact on soil health. Soil ciliates, in particular, are ideal as they are nearly ubiquitously distributed (Foissner, 2006). However, at present their use as indicators is hindered by the fact that most species are not described, and their enumeration and identification is time consuming and requires a high level of expertise (Foissner, 1999a). Molecular methods appear to be a potential workaround for many of the methodological hurdles; however,

applying these methods to soil ciliates is still in its infancy and requires extensive fundamental groundwork (Lara and Acosta-Mercado, 2012). One currently available option to detect the presence of soil ciliates is the use of microbial phospholipid fatty acids (PLFAs). Currently, the PLFA 20:4 is a general biomarker for protozoa commonly used in the literature (Frostegård et al., 1997; Sampedro et al, 2006; Thoms et al., 2010). However, this biomarker may not be appropriate for all protozoa as it is often absent or gives inconsistent results, even when the presence of flagellates and amoeba has been verified using traditional MPN techniques (Rønn et al., 2002a). The biomarker may be useful for studying ciliates as indicated by work linking it to *Paramecium* (Lechevalier and Lechevalier, 1988). However, this assumption has yet to be tested against more traditional enumeration methods such as direct counts.

In this study, we inoculated defaunated aspen leaf litter with ciliates and other microorganisms isolated from an aspen forest floor in order to test how ciliates participate in specific aspects of the soil microbial loop. In particular we wanted to test i) if ciliate activity changes the structural composition of the microbial community and promotes auxin-producing bacteria and ii) if ciliates enhance the release of nitrogen from leaf litter. Additionally, we assessed the effectiveness of PLFA analysis as a tool to detect and potentially quantify ciliates by measuring and analyzing microbial phospholipids linked to protozoa against direct counts of ciliates.

4.2 Materials and Methods

4.2.1 Experimental Design

The experiment was laid out as a 2-factor completely randomized design (2 treatments and 3 time periods) with three replications per treatment for a total of 18 experimental units. The treatments applied to samples included two different combinations of microbial inoculants so that half of the samples were inoculated with only forest floor bacteria ('bacteria-only' treatment) while the other half were inoculated with forest floor bacteria and ciliates ('bacteria+ciliate' treatment). After the inoculations were applied, the samples were randomly assigned to three different time or incubation periods (week-1, week-2 and week-3) at which point they were destructively sampled for analysis. A replicated set of samples was also taken immediately after inoculation to establish the effects of the fumigation treatment.

4.2.2 Mesocosm Preparation

The leaf litter used in the study was collected in August of 2009 from trembling aspen (*Populus tremuloides* Michx.) stems grown at the University of Alberta's Ellerslie Research Farm, Edmonton, Alberta, Canada. The aspen trees were from open pollinated local Edmonton stock planted in 2004 and subject to climactic conditions of the Edmonton region. Upon collection all leaves were air-dried and stored. For this experiment, the leaf litter was coarsely ground, weighed and soaked in distilled water for two hours and then allowed to drain until water ceased to pool underneath the material. Protozoa were removed by fumigating the drained litter with chloroform in two 48-hour cycles (Bonkowski et al., 2001).

After fumigation the litter was rinsed with sterilized distilled water in a sterile laminar flow hood, drained and weighed. The moisture content at field capacity, calculated by using the initial dry weight of the litter and the final wet weight, was estimated to average 312%.

Reusable filter holders (Nalgene reusable filter holders with receiver, Thermo Fisher Scientific, Waltham) were used as the incubation mesocosms. The mesocosms were autoclaved at 120°C for 20 minutes prior to addition of the leaf litter. Further, the mesocosms were kept sterile from atmospheric contamination by plugging all points of access found on the lid and on the receiver of the mesocosms with silicon plugs or sterilised cotton fiber. Each mesocosm contained 16 g (3.88 g dry weight) of the wet leaf litter placed evenly in the upper chamber on top of a 2.5 cm sand bed supported by a layer of glass wool. All mesocosms were kept within a sterile airtight acrylic glove box with inlet and outlet valves during the entire duration of the study. Gas exchange within the glove box was maintained with compressed building air passed through a 0.25 µm air filter. Sample moisture was conserved by adding water as needed to keep weights constant in relation to bi-weekly monitoring of mesocosm weights. Sterilized water and the implements used for maintaining water content were kept within the glove box.

4.2.3 Inoculant Preparation

Microbial inoculants used to create the bacteria-only and bacteria+ciliate treatments were prepared from the forest floor of a mature site of trembling aspen

collected from the Fort.McMurray region in the summer of 2010. Fresh surface litter was removed on site before the underlying decomposition layers (F and H horizons based on the Canadian system of soil classification) of the forest floor were sampled. The forest floor sample were kept cold during transport back to the laboratory, where it was hand sorted to remove coarse woody fragments and roots, and was thoroughly homogenized after being spread to a depth of 10-15 cm and air dried. The material was rewetted to field capacity using a soak and drain method (Puustjarvi, 1973) one month prior to the preparation of the inoculations. After draining, the forest floor was weighed and transferred to a large plastic tub, spread to a depth of 3 cm (to prevent anaerobic conditions) and covered. The moisture content was maintained by daily weighing to monitor moisture loss, and rewetting as necessary.

The bacteria-only and bacteria+ciliate treatments were created by using a combination of three distinct inoculants which are described below. Mesocosms receiving the bacteria+ciliate treatment had all three inoculations applied to them, while mesocosms receiving the bacteria-only treatment received only the two bacteria inoculants. The inoculant preparation and rationale are described below.

The ciliate inoculant was made by creating a 1:20 solution (by weight) of moistened forest floor with 10% standard soil solution (SSS) plus wheat grass (Adl et al., 2008) and incubating for two days at 25°C. The culture viability as a source of soil protozoa was ensured by inspecting it with an inverted phase contrast microscope at 160X. A sample of this protozoa culture, taken from the water column to avoid contamination by amoebae, was then added to a solution of

10 % SSS plus wheat grass. This solution was incubated for five days to ensure that all flagellates were consumed to extinction by the ciliates. This was verified by inspecting the solution for flagellates with a hemocytometer under a phase contrast microscope at 400X before the solution was used as an inoculant for the bacteria+ciliate treatment.

Bacteria inoculant #1 was prepared from forest floor bacteria mixing distilled water to the moistened aspen forest floor (3:1 water:forest floor). The solution was stirred for 20 minutes and then left to settle, after which the supernatant was filtered twice through a 3 µm nitrocellulose membrane (MF-Millipore mixed cellulose ester membrane filter, EMD Millipore, Billerica) to exclude protozoa. The solution was incubated overnight at room temperature and then inspected under the microscope to ensure that no protozoan contamination had occurred.

Following the protocol recommended by Jentschke et al. (1995), bacteria inoculant #2 was added to the experimental design to reduce any potential differences in bacterial diversity arising from the culture conditions of bacterial inoculant #1 and the ciliate inoculant. This inoculant was prepared by filtering a portion of the ciliate inoculant in the same manner as bacteria inoculant #1. The filtrate was then incubated overnight and checked for protozoan contamination.

4.2.4 Auxin Analysis

The detection of the plant auxin Indole-3-acetic acid (IAA) was conducted as described by Frankenberger and Brunner (1983). At the time of sampling, two

sub-samples were taken from each mesocosm and amended with a 0.2 M phosphate buffer. One of the sub-samples was amended with 0.05 M L-tryptophan, while distilled water was added to the second one to be used as a control, as it was found that the leaf litter had a high background level of IAA. After amendment, all sub-samples were incubated in the dark at 35°C for a period of 24 hours. The sub-samples were filtered and acidified with H_3PO_4 to a pH of 2.6 and then extracted by partitioning them into 3 aliquots of ethyl acetate at 20 minute intervals. The ethyl acetate fraction was collected and dried with N_2 and reconstituted in methanol.

The reconstituted sample extracts were run on a Dionex GPM-1 gradient pump through a C_{18} partisil 10 ODS-3 column with a mobile phase of 60:40 methanol/deionised water and 0.01M l-heptanesulfonic acid acidified to pH 2.5. Analysis for IAA was done using a Dionex VDM-2 detector set to 254 nm. Peak identification was achieved by using several standards of known products of tryptophan metabolism in soil (Martens and Frankenberger, 1993). The standards used were IAA, indole-3-acetamide, indole-3-pyruvic acid, indole-3-ethanol, indole-3-acetaldehyde, anthranilic acid, and serotonin acquired from Sigma Aldrich, Canada. Curve fitting of the chromatograms for peak quantification was done using Fityk software version 1.2.0 (Wojdyr, 2010). Finally, IAA production potential was calculated by subtracting the subsample amended with distilled water from the subsample amended with L-trpytophan.

4.2.5 Ciliate Abundance

Counts to estimate ciliate abundance were done with an inverted phase contrast microscope using the modified direct count methodology from Adl et al. (2006). A subsample of each mesocosm was placed into a 6-well microtiter plate and moistened with 2 ml of distilled water. Ciliate abundance was estimated in all six wells were estimated using a transect method (Krebs, 1989). All mesocosms were inspected under a phase contrast microscope for flagellates by using a portion of the solution and a hemocytometer.

4.2.6 Microbial Community Analysis

Microbial communities were analyzed using microbial phospholipid fatty acids. A detailed methodology can be found in Hannam et al. (2006). In brief, lipids were extracted using a modified Blight and Dyer (1959) process, purified on pre-packed silicic acid columns (Agilent Technologies, Wilmington, DE) and finally converted to fatty acid methyl esters (FAMEs). The FAMEs were separated using an Agilent 6890 Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE) with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column and identified using MIDI peak identification software (MIDI, Inc., Newark, DE).

The PLFAs are described using a X:YωZ format where X represents the number of carbons, "Y" the number of unsaturated bonds between carbons and "Z" the location of the first unsaturated bond from the aliphatic end (indicated by ω) of the PLFA. Additional nomenclature includes chain branching from the aliphatic end denoted by "i", the occurrence of a cyclopropyl group denoted by

"cyc" and "10Me" indicating the presence of a methyl group located on the 10^{th} carbon from the carboxyl end. The recovery efficiency of the entire process was achieved by adding a known quantity of the PLFA 19:0 to all samples prior to the extraction. Quantification of the PLFAs was done by adding a known quantity of the FAME 10:0 to samples prior to separation and identification on the gas chromatograph.

4.2.7 Leachate Analysis

Litter leachates were determined by adding 100 ml of distilled water to the mesocosms on the day they were sampled. The volume of leachate recovered from each mesocosm was recorded after draining for approximately 10 minutes. The collected leachate samples were stored frozen and prepared for analysis for dissolved carbon (<0.2 μm) and nitrogen in accordance to McGill et al. (1986). Dissolved carbon and nitrogen were measured using a Shimadzu TOC-V CSH/CSN Model (Shimadzu Corporation, Analytical & Measuring Instrument Division, Kyoto, Japan).

4.2.8 Statistical Analysis

Treatment effects for ciliate abundance, IAA analysis, leachate analysis and PLFA indicator groups were tested using a one or two-way ANOVA with the PROC MIXED statement for mixed models (version 9.1, SAS institute Inc). Following significant main effects, pairwise comparison of treatments was completed using the LSMEANS statement. All data were met the assumptions of

an ANOVA based on Levene's test for homogeneity of variance and Shapiro-Wilk test for normality. Quantification of PLFA indicator groups were on a mole percentage of the total PLFA concentration in a sample (expressed as nmol·per g leaf litter). Indicator PLFAs for bacteria were based on Myers et al. (2001) and included 15:0, 17:0 and 18:0 for gram positive bacteria and cyc17:0, cyc19:0, 16:1ω7c for gram negative bacteria. The indicator for microbial growth phase was determined by taking the ratio of cyc17:0/16:1ω7c (Knivett and Cullen, 1965). Actinomycete PLFAs included 10Me18:0 (Myers et al., 2001) and 10Me19:0 (O'Donnell et al., 1982). The PLFAs used for fungi were 18:2ω6c (Frostegård and Bååth, 1996) and 18:3ω6c (Hamman et al., 2007).

Microbial community analysis using the PLFA data was conducted with PC-ORD (version 5, MjM Software Design). The analysis used a non-metric multidimensional scaling (NMS) ordination with a Sorenson (Bray-Curtis) dissimilarity measurement. All PLFAs used in the ordination were expressed on a mole percentage of the total PLFAs, and were arcsine square-root transformed. A total of 21 PLFAs occurred in three or more samples and were included in the ordination and included 10:0 2OH, 12:0, 12:0 2OH, 14:0, 15:1ω6c, 16:0N alc, 16:0 2OH, a17:1ω9c, 17:1ω8c, 20:1ω9c, 19:1ω6c in addition to PLFA biomarkers. Effects of inoculation and time on patterns of microbial community composition were tested using a multi-response permutation procedure (MRPP). The MRPP test is a non-parametric procedure that tests grouping patterns by generating a “T” value and a corresponding test for significance. Another value

listed as “A” determines within group homogeneity and indicates the size of the effect (McCune and Grace, 2002).

4.3 Results

4.3.1 Experimental Manipulation and Ciliates

Leaf litter samples analysed immediately after inoculation contained a high proportion of the fungal PLFA 18:2ω6c (Table 4-1). In contrast, the two bacteria inoculants used to generate the treatments did not contain 18:2ω6c; the concentration of 18:2ω6c in the ciliate inoculation was negligible when compared to leaf litter. Taken together this indicates that the fungi most likely originated from the leaf litter, having survived the fumigation treatment. Gram positive bacteria and actinomycetes also originated from the leaf litter as all of the PLFA biomarkers, except 18:0, were absent in the three treatment inoculants. On the other hand, it is likely that gram negative bacteria were introduced by the three treatment inoculants as the PLFA cyc17:0 and cyc19:0 were absent in the leaf litter.

Double fumigation of the leaf litter was apparently successful in killing all protozoa as none were observed in any of the bacteria-only samples throughout the entirety of the incubation (Figure 4-1). Bacteria-only samples were found to be free of ciliates and other protozoa. All bacteria+ciliate samples had quantifiable ciliate populations and appeared to be free of flagellate and amoeba contamination. There were 3 distinct ciliate morphospecies (two colpodid and one non-colpodid) identified in the samples based on size, movement and shape.

Ciliate abundances were not distinguished among these morphospecies, however, and overall abundances fluctuated with time (Figure 4-1). A strong drop in abundances occurred between week-1 and week-2 ($p=0.05$) followed by a weak increase between week-2 and week-3 ($p=0.16$). Interestingly, the protozoan biomarker PLFA 20:4 was not detected in any of the samples throughout the study.

4.3.2 Microbial Community Response

An initial ordination showed that the differences in microbial community structure between leaf litter sampled immediately after inoculation and samples taken in later weeks were so strong that other patterns were undetectable. Consequently, the NMS ordination focused on samples taken in week-1, week-2 and week-3 (Figure 4-2). The final NMS ordination of PLFA data resulted in a two dimensional solution with a final stress of 10.4 after 41 iterations. As seen in Figure 4-2, axis-1 accounted for 53% of the variation in the data while 38% was accounted for by axis-2. Community patterns along axis-1 were associated with the PLFA cyc19:0 (r^2 0.90), cyc17:0 (r^2 0.74), 14:0 (r^2 0.56), 18:3ω6c (r^2 0.54), and 17:0 (r^2 0.52). Patterns along axis-2 were most associated with 10me19:0 (r^2 0.42) and 10me18:0 (r^2 0.38).

The multi-response permutation procedure (MRPP) suggests that throughout the entire incubation, the bacteria-only and bacteria+ciliate samples possessed distinct community structures (Table 4-2). The patterns of community structure seen along axis-1 in bacteria-only and bacteria+ciliate samples coincided

with an increase of incubation time. Community structure in bacteria-only samples was different between week-1 and week-2 but became similar between week-2 and week-3. In the bacteria+ciliate samples, each week exhibited distinctive grouping patterns. Bi-plots of measured quantitative variables showed that the ratio of 16:1ω7c/ cyc17:0 had a strong correlation with axis 1 (r = 0.69) and projected through ordination space in the same direction as samples that had been incubated for longer periods of time.

The indicator of microbial growth phase (cyc17:0/16:1ω7c) increased over time in bacteria-only and bacteria+ciliate samples, but displayed strong differences between the two inoculants at every time interval (Figure 4-3). This indicator increased gradually in bacteria+ciliate samples, as a strong difference only occurred when comparing between week-1 and week-3; it remained consistently lower than in bacteria-only samples. In contrast, in bacteria-only samples, the indicator showed a strong initial increase between week-1 and week-2 and only marginally increased afterwards, showing a very strong difference between week-1 and week-3 ($p=0.005$) but no apparent difference between week-2 and week-3.

Gram negative PLFAs from week-1, week-2 and week-3 were dominated by the cyclopropyl PLFAs cyc17:0 and cyc19:0. On average, in bacteria-only samples, 60% of the mole percent was derived from cyc17:0 and 28% was derived from cyc19:0. In bacteria+ciliate samples, cyc17:0 accounted for 49% on average of the total mole percent while cyc19:0 accounted for 38%. The mole percent of gram negative PLFAs responded very strongly to the inoculants and

time when cyclopropyl PLFA were included; however, the response of non-cyclo gram negative PLFA was negligible (Figure 4-3). The difference of total gram negative PLFA between bacteria-only and bacteria+ciliate samples was strong and consistently lower in bacteria+ciliate samples (Figure 4-3). Additionally, mole percentages showed a very strong increase over time in samples of both treatments.

The behavior of fungal and gram positive PLFAs depended on the strong interaction effect ($p=0.03$ and $p=0.05$, respectively) between inoculant and time (Figure 4-3). In bacteria-only samples, both groups decreased in mole percentage over time as the proportion of gram negative bacteria increased. Interestingly, the behavior of fungal and gram positive PLFAs in bacteria+ciliate samples appeared to be unrelated to changes in gram negative PLFAs. In particular, mole percent of fungal PLFAs in the bacteria+ciliate samples moderately increased between week-1 and week-2 ($p=0.1$) and returned to a lower mole percent in week-3. In comparison, fungal PLFAs in bacteria-only samples decreased strongly between week-1 and week-2 and continued to decrease. However, the decrease after week-2 was negligible and only apparent when comparing the very strong difference between week-1 and week-3. Gram positive mole percentages in bacteria-only samples showed strong weekly differences and decreased with time. On the other hand, gram positive mole percentages in bacteria+ciliate samples showed a strong drop between week-1 and week-2, but returned to an intermediate mole percent in week-3.

Correlations between different microbial groups depended on the presence or absence of ciliates (Table 4-3). In the bacteria-only samples, the rise in the proportion of gram negative bacteria occurred at the expense of all other microbial groups. In particular, gram positive bacteria and fungi were strongly affected as each group had a negative relationship with gram negative bacteria ($r = -0.85$). Actinomycetes, on the other hand, were less affected and shared only a moderate negative relationship with gram negative bacteria ($r = -0.33$). In bacteria+ciliate samples, mole percentages of other microbial groups were less related to the proportion of gram negative bacteria. Interestingly, the relationship of fungi to gram positive bacteria and actinomycetes went from being positive in the absence of ciliates, to strongly negative when ciliates were present.

4.3.3 Auxin Production Potential and Litter Leachate

The bacteria+ciliate inoculant did not result in a stimulation of IAA generation potential compared to the bacteria-only inoculant (Figure 4-4.). Potential generation was similar in all weeks between the two treatments except in week-1 where the bacteria inoculant had moderately higher IAA generation than the bacteria+ciliate inoculant ($p=0.15$). In both inoculants, IAA generation decreased strongly between week-2 and week-3, with week-3 averages being close to or below background IAA. The generation of IAA in both inoculants coincided with a strong positive relationship with fungal PLFA and weak negative relationships with all other PLFA groups (Table 4-4). Finally, ciliate abundances had a negligible relationship with IAA production (Table 4-4).

In contrast, the presence of ciliates stimulated the generation potential of anthranilic acid as it was totally absent in all but one bacteria-only sample (Figure 4-4). Interestingly, anthranilic acid generation shared a strong negative relationship with ciliate counts, even though the presence of ciliates was generally required for its occurrence (Table 3). Potential anthranilic acid generation fluctuated strongly between time periods by increasing from week-1 to week-2 and then decreasing in week-3 back to levels comparable to week-1 (Figure 4). Anthranilic acid generation also coincided with an increase in the proportion of fungal PLFAs as they shared a strong positive relationship (Table 3). All other microbial groups had either negligible or negative relationships, which was similar to the relationship of these groups with fungal PLFAs in the bacteria+ciliate treatment.

Sample variability in the amount of total dissolved nitrogen was high in all time periods for the bacteria-only samples; however, in week-3 bacteria-only samples nitrogen increased moderatly in comparison to week-2 and was also elevated compared to bacteria+ciliate samples in the same time period (Figure 4-4). There was negligible change in leachate C/N of bacteria+ciliate samples throughout the incubation. However, in bacteria-only samples, leachate C/N decreased markedly between week-2 and week-3. There were no strong differences between bacteria-only and bacteria+ciliate leachate C/N, however, except in week-3, when bacteria-only samples showed a strong drop in C/N (coinciding with an increase in dissolved N).

4.4 Discussion

4.4.1 Microbial Community Response

Recently, Kuppartd (2010) successfully used longer chained PLFAs (20 carbon or higher) to track the grazing behavior of a single species of ciliates on bacteria in wastewater. However, in our study, the biomarker 20:4 was absent in the bacteria+ciliate samples, in spite of the presence of viable ciliate populations. Additionally, in re-analysis of these samples for longer eukaryotic PLFAs (>20 carbon), I found no increases in the total response of longer chained PLFAs in bacteria-ciliate samples compared to bacteria-only samples (data not shown). For a biomarker to be useful, it should be relatively common across species and environmental conditions. The results of our study indicate that more research is required if PLFAs are to be used as common biomarkers for ciliates and that any conclusions based on the use of 20:4 should be drawn with caution.

Stationary growth, which typically occurs once free resources in the local environment have been consumed and incorporated into the microbial biomass, can be identified by the predominance of the cyclopropyl PLFA, cyc17:0, over its straight chained precursor, 16:1ω7 (Knivett and Cullen, 1965). In my NMS ordination (Fig. 4-2) axis-1 was highly correlated with this ratio and the cyclopropyl PLFAs cyc17:0 and cyc19:0. This suggests that progression of samples along axis-1 coincided with a change over time in the metabolic growth phase of gram negative bacteria.

Gram negative biomarkers, namely the cyclopropyl and monosaturated PLFAs, are generally more sensitive to the effects of chloroform fumigation

(Zelles et al., 1997). In my experiment, it appears that fumigation treatment greatly reduced the gram negative portion of the microbial community as indicated by the lack of cyclopropyl PLFAs in leaf litter analyzed immediately after the fumigation treatment. Overall, the rapid rise of cyclopropyl in the weeks following inoculation indicates that the gram negative community was able to re-establish itself.

On the whole, ciliate grazing appeared to moderate rather than inhibit overall growth of gram negative bacteria as the mole percentage of gram negative PLFAs, while being lower than in the bacteria-only samples, continued to rise in the bacteria+ciliate samples as the incubation progressed. Analysis of the growth phase indicator suggests that in bacteria-only samples, the lack of protozoa resulted in more gram negative bacterial entering a stationary growth phase. In contrast, ciliate predation in bacteria+ciliate samples appeared to reduce the proportion of stationary growth bacteria. When studying the feeding behavior of two ciliates species, Gruber et al. (2009) demonstrated that *E. coli* populations entering a stationary growth phase were preferentially consumed by ciliates over cells that were actively growing. It is likely that this feeding preference also occurred throughout the incubation since, in addition to having higher cyc17:0/16:1ω7 ratios, bacteria-only samples had consistently higher proportions of cyclopropyl gram-negative PLFAs in every time period. Also, non-cyclopropyl gram-negative PLFAs appeared to be unaffected by the presence of ciliates as there was no relationship between these PLFAs and ciliate counts.

Direct effects of ciliates appeared to be strongest for fungi, actinomycetes and total gram positive bacteria as signatures of these groups were highly correlated with ciliate counts. At least one of the ciliate species recovered from the aspen litter in my study may have been mycophagous as fungal PLFA mole percentages were negatively related to ciliate counts. While an uncommon feeding behavior in protozoa (Adl and Gupta, 2006), mycophagous colpodids have been recovered from soil that has been dried and rewetted (Foissner, 1999b). Additionally, the cultured protozoa inoculation used in this study contained fungal PLFAs and could have provided the relevant fungi needed for the mycophagous ciliates to grow during the incubation. Microbial communities in aspen litter often have a large fungal component (Hasset and Zak, 2005) and could be a prime habitat for the presence of mycophagous ciliates. Evidence for ciliate mycophagy is limited (Petz et al., 1986; Foissner, 1999b) but if such species are present and common in aspen litter they would be an integral component of the microbial ecology of these ecosystems.

Actinomycetes inhabiting aspen forest floors can be antagonistic to the growth of many types of saprotrophic fungi common to aspen leaf litter (Jayasinghe and Parkinson, 2008). However, the rapid growth of gram negative bacteria in the absence of ciliates appeared to overshadow any potential interactions among these groups. Detection of the interaction between actinomycetes and fungi was only possible when ciliate grazing moderated the growth of gram negative bacteria. The potential for mycophagous behavior by ciliates coupled with the fungal inhibition by actinomycetes may have accounted

for the strong, albeit opposite, relationship of actinomycetes with fungi and ciliate counts.

4.4.2 Auxin Generation Potential and Litter Leachate

The production of IAA was not enhanced by the presence of protozoa, which is in agreement with Ekelund et al. (2009) who used a mixed culture of flagellates and observed no increase in the proportion of IAA producing bacteria in the presence of protozoa. On the other hand, Krome et al. (2009) used a single species of amoebae and saw beneficial growth in plants prior to a measureable increase in the concentration of available nutrients. We propose that IAA-generating bacteria in soil could benefit from the presence and activity of amoeba but not of ciliates or flagellates. Additionally, soil ciliates appear to stimulate bacteria that can catabolise L-tryptophan as a source of energy and nitrogen as indicated by the production of anthranilic acid (Martens and Frankenberger, 1993). This suggests that, in addition to directly contributing to soil N by grazing bacterial biomass, soil ciliates also indirectly influence soil N by increasing the catabolism of phytohormone precursors by soil bacteria.

Studies targeting the interactions of protozoa with plants and bacteria generally show that the addition of protozoa increases nitrogen content in plant tissues (Alphei et al., 1996; Kuikman et al., 1990; Bonkowski et al., 2000) and soil leachates (Bonkowski et al., 2000). The apparent discrepancy between our study and the literature may be a result of the leaf litter used in the incubation. Most studies isolating the effects of protozoa have used mineral soils (Alphei et

al., 1996; Kuikman et al., 1990) or grass litter (Bonkowski et al., 2000) as substrates. Aspen leaf litter is known to produce leachates with high concentrations of phenolic compounds and dissolved sugars but low nitrogen levels (Natalia et al., 2008) and has been shown to cause nitrogen immobilization in the field (Kochy and Wilson, 1997). In such a nitrogen-poor environment, any available nitrogen released by ciliate grazing may have been quickly taken up by other bacteria and immobilized. In fact, ciliate grazing may have promoted nitrogen immobilization by redistributing it faster than diffusion alone and could account for bacteria-only samples in week-3 having higher leachate nitrogen and lower C/N than samples containing ciliates..

4.4.3 Concluding Remarks

The soil microbial loop is a powerful conceptual tool that facilitates an understanding of the numerous interactions that soil protozoa have with other organisms in the soil ecosystem. We observed ciliates altering the microbial community over time, selectively feeding on cells in stationary growth, and potentially consuming fungi. However, it is clear that some of the roles attributed to protozoa, namely, the promotion of auxin producing bacteria, should not be applied broadly and perhaps should be limited to the activity of soil amoeba. While soil ciliates isolated in this study may not be involved in auxin production, they were responsible for indirectly affecting the catabolism of auxin precursors which could influence the availability of these precursors to organisms that produce IAA. We found that microbial PLFA are useful in monitoring the results

of ciliate activity on the microbial community, but do not appear to be an effective way to measure the presence or abundance of soil ciliates.

Tables and Figures

Table 4-1 Indicator PLFA from inoculants and leaf litter sampled immediately after inoculation. Leaf litter values are mean mol percent (n = 3) with standard error in parentheses

	Indicator PLFA										
	Gram +			Gram -		Actinomycetes			Fungi		
	15:0	17:0	18:0	cyc17:0	cyc19:0	16:1ω7c	10me18:0	10me19:0	18:2ω6c	18:3ω6c	Total (nmol·g)
Leaf litter bacteria-only	0.94(0.01)	8.60(0.07)	19.9(0.54)	0	0	0.33(0.01)	0.78(0.03)	0.75(0.06)	65.9(0.56)	2.78(0.05)	5095(430)
Leaf litter bacteria+ciliate	0.93(0.01)	8.64(0.02)	20.4(0.06)	0	0	0.50(0.08)	0.81(0.02)	0.72(0.01)	65.1(0.03)	2.94(0.03)	5221(323)
Bacteria inoculant #1	0	0	0	36.6	0	63.4	0	0	0	0	0.76
Bacteria inoculant #2	0	0	0	36.9	14.7	14.4	0	0	0	34.1	7.03
Ciliate inoculant	0	0	11.5	17.9	17.9	24.5	0	0	7.94	20.3	0.34

Table 4-2. MRPP results for microbial PLFAs. Highlighted are comparisons of time (week) within a treatment and comparisons of treatments within a specific time period.

Group	Comparison	T	A	P
bacteria+ciliate	week 1 vs. 2	-2.64	0.32	0.024
	week 2 vs. 3	-1.83	0.23	0.047
bacteria-only	week 1 vs. 2	-2.32	0.19	0.026
	week 2 vs. 3	-0.49	0.03	0.303
week 1	bacteria-only	-2.26	0.22	0.028
week 2	vs.	-2.05	0.21	0.035
week 3	bacteria+ciliate	-2.93	0.24	0.021

Table 4-3. Pearson correlation coefficients of microbial groups present in bacteria+ciliates and bacteria-only samples. Correlations of note are bolded with associated p-values indicated in parentheses.

	Gram -		Gram +		Actinomycetes		Fungi		Ciliate Count
	Inoculant								
	bacteria-only	bacteria+ciliate	bacteria-only	bacteria+ciliate	bacteria-only	bacteria+ciliate	bacteria-only	bacteria+ciliate	bacteria+ciliate
Gram -	/	/	**-0.85(<0.01)**	-0.29(0.44)	-0.33(0.40)	-0.07(0.86)	**-0.85(<0.01)**	-0.32(0.40)	-0.34(0.37)
Gram +	**-0.85(<0.01)**	-0.29(0.44)	/	/	0.32(0.40)	**0.94(<0.01)**	0.47(0.21)	**-0.77(0.01)**	**0.634(0.07)**
Actino	-0.33(0.40)	-0.07(0.86)	0.32(0.40)	**0.94(<0.01)**	/	/	0.16(0.67)	**-0.81(0.01)**	**0.606(0.08)**
Fungi	**-0.85(<0.01)**	-0.32(0.40)	0.47(0.21)	**-0.77(0.01)**	0.16(0.67)	**-0.81(0.01)**	/	/	**-0.451(0.22)**

Table 4-4. Pearson correlation coefficients of microbial groups and potential production of IAA and anthranilic acid in bacteria+ciliates and bacteria-only samples. Correlations of note are bolded with associated p-values indicated in parentheses.

	IAA Generated		Anthranilic acid	
	Inoculant			
	bacteria-only	bacteria+ciliate	bacteria-only	bacteria+ciliate
Gram -	-0.25(0.55)	-0.25(0.55)	-	-0.03(0.94)
Gram +	-0.26(0.54)	-0.26(0.54)	-	**-0.60(0.11)**
Actino	-0.23(0.56	-0.24(0.56)	-	**-0.62(0.10)**
Fungi	0.47(0.23)	0.48(0.23)	-	**0.59(0.12)**
Ciliate Counts	-	-0.18(0.67)	-	**-0.64(0.06)**

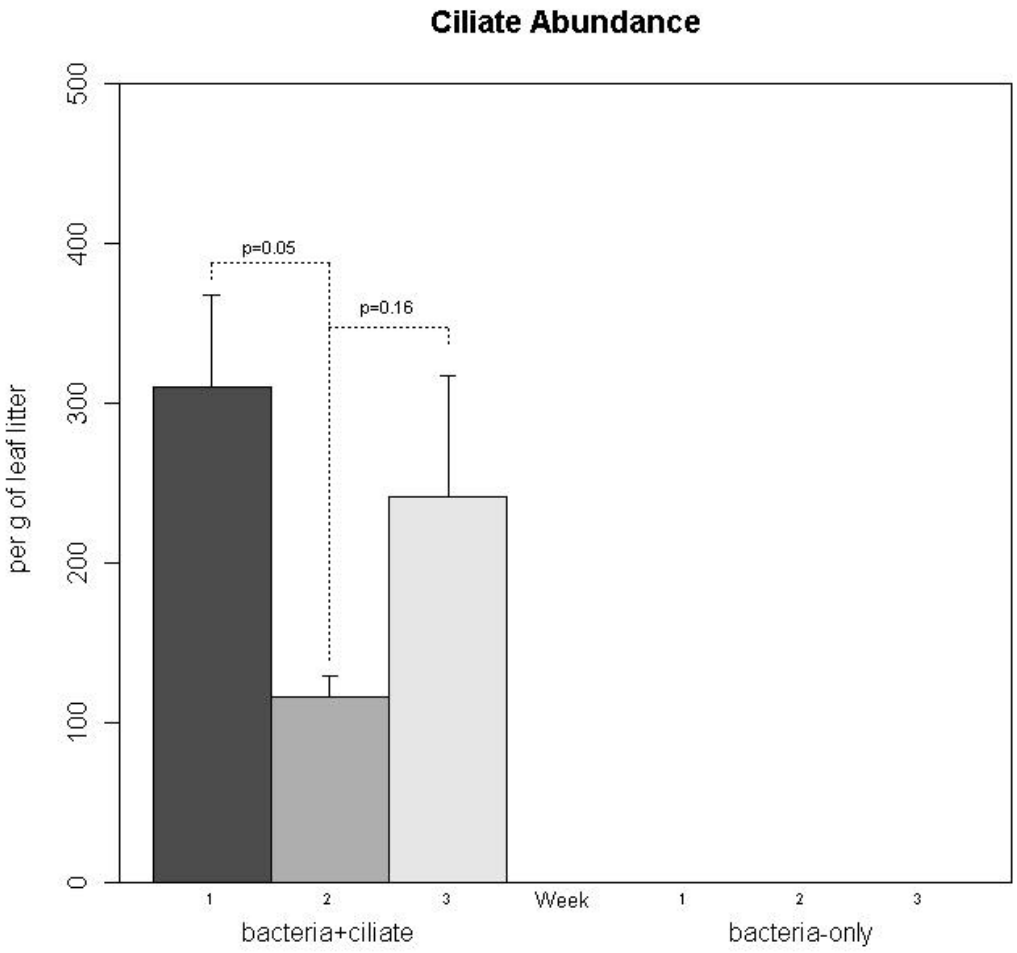

Figure 4-1. Mean values of ciliate abundance in bacteria+ciliate and bacteria-only samples measured over time. Error bars represent one standard error from the mean. Differences among incubation times within a treatment are expressed with adjoining dotted lines and associated p-values centered above.

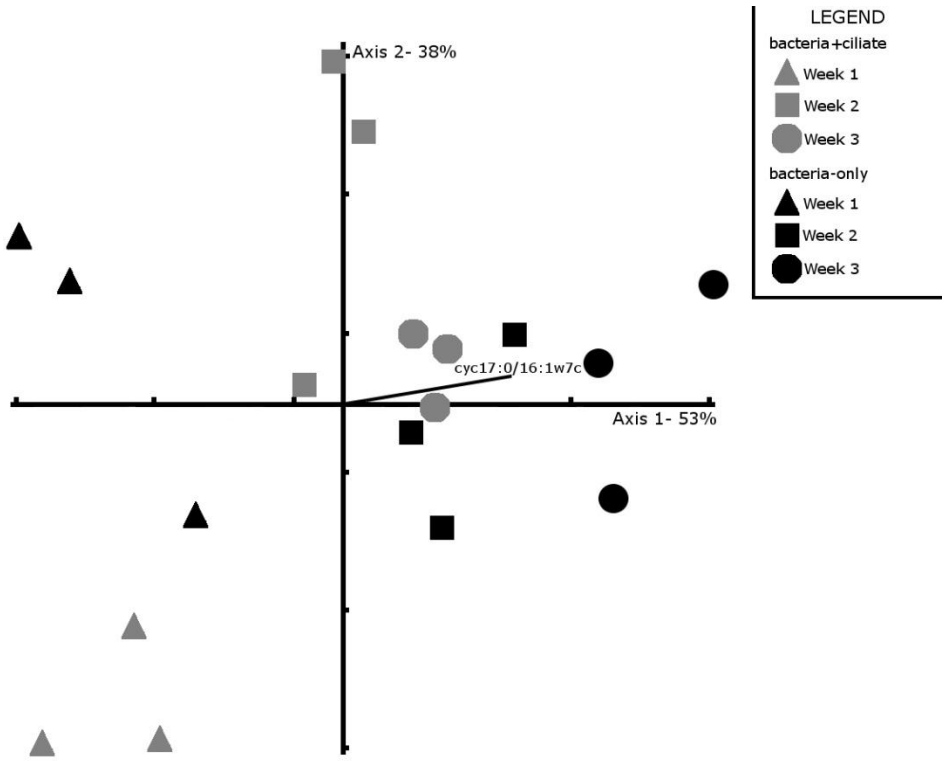

Figure 4-2. Graphical plot of the 2-dimensional NMS ordination for microbial PLFAs

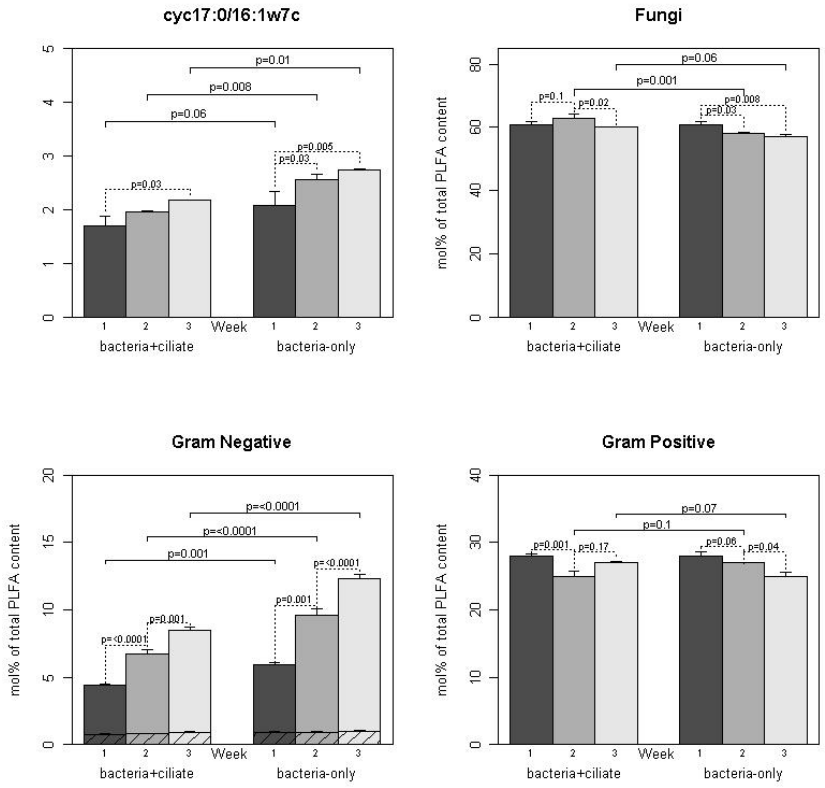

Figure 4-3. Mean values of relevant microbial community indices derived from mole percent of total PLFA data in bacteria+ciliate and bacteria-only samples measured over time. Error bars represent one standard error from the mean. The statistical difference between treatments within an incubation time week is indicated by an adjoining solid line and associated p-value centered above. Differences among incubation times within a treatment are expressed with adjoining dotted lines and associated p-values centered above. Hash marked columns in the gram negative panel represent concentrations of non-cyclopropane gram negative PLFAs.

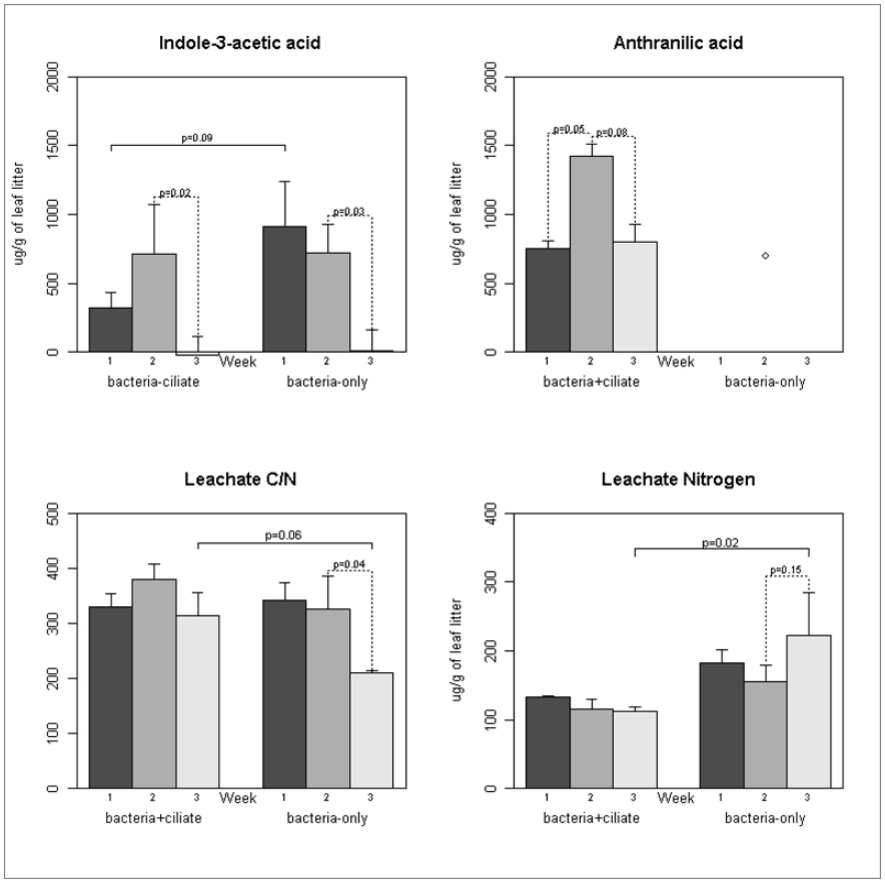

Figure 4-4. Mean values of potential production of Indole-3-acetic acid and anthranilic acid, leachate C/N and total leachate nitrogen in bacteria+ciliate and bacteria-only samples measured over time. Error bars represent one standard error from the mean. The statistical difference between treatments within an incubation time week is indicated by an adjoining solid line and associated p-value centered above. Differences among incubation times within a treatment are expressed with adjoining dotted lines and associated p-values centered above.

Literature Cited

Adl, S.M., Coleman, D.C., Read, F. 2006. Slow recovery of soil biodiversity in sandy loam soils of Georgia after 25 years of no-tillage management. Agriculture, Ecosystems and Environment 114, 323-334.

Adl, S.M., Gupta, V.V.S.R. 2006. Protists in soil ecology and forest nutrient cycling. Canadian Journal of Forest Research 36, 1805-1817.

Adl, S.M. 2007. Motility and migration rate of protozoa in soil columns. Soil Biology and Biochemistry 39, 700-703.

Adl, S. M., Acosta-Mercado, D., Lynn, D.H. 2008. Protozoa. In: Carter, M.R., Gregorich, E.G. (Eds.), Soil Sampling and Methods of Analysis Second Edition. CRC Press, Boca Raton, pp.455-469.

Alphei, J., Bonkowski, M., Scheu, S. 1996. Protozoa, nematoda and lumbricidae in the rhizosphere of Hordelymus europaeus (Poaceae): Faunal interactions, response of microorganisms and effects on plant growth. Oecologia 106, 111-126.

Altenburger, A., Ekelund, F., Jacobsen, C.S. 2010. Protozoa and their bacterial prey colonize sterile soil fast. Soil Biology and Biochemistry 42, 1636-1639.

Bonkowski, M., Griffiths, B., Scrimgeour, C. 2000. Substrate heterogeneity and microfauna in soil organic 'hotspots' as determinants of nitrogen capture and growth of ryegrass. Applied Soil Ecology 14, 37-53.

Bonkowski, M., Jentschke, G., Scheu, S. 2001. Contrasting effects of microbial partners in the rhizosphere: Interactions between Norway Spruce seedlings

(*Picea abies* Karst.), mycorrhiza (*Paxillus involutus* (Batsch) Fr.) and naked amoebae (protozoa). Applied Soil Ecology 18, 193-204.

Bonkowski, M., Brandt, F. 2002. Do soil protozoa enhance plant growth by hormonal effects? Soil Biology and Biochemistry 34, 1709-1715.

Bonkowski, M. 2004. Protozoa and plant growth: The microbial loop in soil revisited. New Phytologist 162, 617-631.

Clarholm, M. 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biology and Biochemistry 17, 181-187.

Coleman, D.C. 1994. The microbial loop concept as used in terrestrial soil ecology studies. Microbial Ecology 28, 245-250.

Ekelund, F., Saj, S., Vestergård, M., Bertaux, J., Mikola, J. 2009. The "soil microbial loop" is not always needed to explain protozoan stimulation of plants. Soil Biology and Biochemistry 41, 2336-2342.

Foissner, W. 1999a. Soil protozoa as bioindicators: Pros and cons, methods, diversity, representative examples. Agriculture, Ecosystems and Environment 74, 95-112.

Foissner, W. 1999b. Description of two new, mycophagous soil ciliates (Ciliophora, Colpodea): Fungiphrya strobli n. g., n. sp. and Grossglockneria ovata n. sp. Journal of Eukaryotic Microbiology 46, 34-42.

Foissner, W. 2006. Biogeography and dispersal of micro-organisms: A review emphasizing protists. Acta Protozoologica 45, 111-136.

Foissner, W., Chao, A., Katz, L.A. 2008. Diversity and geographic distribution of ciliates (Protista: Ciliophora). Biodiversity and Conservation 17, 345-363.

Frankenberger, W., Brunner, W. 1982. Method of detection of auxin-indole-acetic acid in soils by high performance liquid chromatography. Soil Science Soceity of America Journal 47, 237-241.

Frostegård, Å., Bååth, E. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59-65.

Frostegård, Å., Petersen, Ø.O., Bååth, E., Nielsen, T.H. 1997. Dynamics of a microbial community associated with manure hot spots as revealed by phospholipid fatty acid analyses. Applied and Environmental Microbiology 63, 2224-2231.

Griffiths, B.S. 1986. Mineralization of nitrogen and phosphorus by mixed cultures of the ciliate protozoan Colpoda steinii, the nematode Rhabditis SP. and the bacterium Pseudomonas fluorescens. Soil Biology and Biochemistry 18, 637-641.

Griffiths, B.S., Bonkowski, M., Dobson, G., Caul, S. 1999. Changes in soil microbial community structure in the presence of microbial-feeding nematodes and protozoa. Pedobiologia 43, 297-304.

Gruber, D.F., Tuorto, S., Taghon, G.L. 2009. Growth phase and elemental stoichiometry of bacterial prey influences ciliate grazing selectivity. Journal of Eukaryotic Microbiology 56, 466-471.

Hamman, S.T., Burke, I.C., Stromberger, M.E. 2007. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biology and Biochemistry 39, 1703-1711.

Hannam, K.D., Quideau, S.A., Kishchuk, B.E. 2006. Forest floor microbial communities in relation to stand composition and timber harvesting in northern Alberta. Soil Biology and Biochemistry 38, 2565-2575.

Hassett, J.E., Zak, D.R. 2005. Aspen harvest intensity decreases microbial biomass, extracellular enzyme activity, and soil nitrogen cycling. Soil Science Society of America Journal 69, 227-235.

Jayasinghe, B.A.T.D., Parkinson, D. 2008. Actinomycetes as antagonists of litter decomposer fungi. Applied Soil Ecology 38, 109-118.

Jentschke, G., Bonkowski, M., Godbold, D.L., Scheu, S. 1995. Soil protozoa and forest tree growth: non-nutritional effects and interaction with mycorrhizae. Biology and Fertility of Soils 20, 263-269.

Knivett, V.A., Cullen, J. 1965. Some factors affecting cyclopropane acid formation in Escherichia coli. Biochemical Journal 96, 771-776.

Köchy, M., Wilson, S.D. 1997. Litter decomposition and nitrogen dynamics in aspen forest and mixed-grass prairie. Ecology 78, 732-739.

Krebs, C. J., 1999. Ecological Methodology. 2nd ed. Addison-Welsey Educational Publishers, Inc., Menlo Park, CA.

Krome, K., Rosenberg, K., Bonkowski, M., Scheu, S. 2009. Grazing of protozoa on rhizosphere bacteria alters growth and reproduction of Arabidopsis thaliana. Soil Biology and Biochemistry 41, 1866-1873.

Kuikman, P.J., Van Veen, J.A. 1989. The impact of protozoa on the availability of bacterial nitrogen to plants. Biology and Fertility of Soils 8, 13-18.

Kuikman, P.J., Jansen, A.G., van Veen, J.A., Zehnder, A.J.B. 1990. Protozoan predation and the turnover of soil organic carbon and nitrogen in the presence of plants. Biology and Fertility of Soils 10, 22-28.

Kuppardt, S., Chatzinotas, A., Kästner, M. 2010. Development of a fatty acid and RNA stable isotope probing-based method for tracking protist grazing on bacteria in wastewater. Applied and Environmental Microbiology 76, 8222-8230.

Lara, E., Acosta-Mercado, D. 2012. A molecular perspective on ciliates as soil bioindicators. European Journal of Soil Biology 49, 107-111.

Lechevalier, H., Lechevalier M.P., 1998. Chemotaxonomic use of lipids – an overview. In: Ratledge, C., Wilkinson, S.G. (Eds.), Microbial Lipids Volume 1. Academic Press, San Diego, 869-902.

Martens, D.A., Frankenberger Jnr, W.T. 1993. Metabolism of tryptophan in soil. Soil Biology and Biochemistry 25, 1679-1687.

McCune, B., Grace, J.B. 2002. Analysis of ecological communities. Oregon.

McGill, W. B., Cannon, K. R., Robertson, J. A. Cook, F. D. 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. Canadian Journal of Soil Science 66, 1–19.

Myers, R.T., Zak, D.R., White, D.C, Peacock, A. 2001. Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Science Society of America Journal 65, 359-367.

Natalia, S., Lieffers, V.J., Landhäusser, S.M. 2008. Effects of leaf litter on the growth of boreal feather mosses: Implication for forest floor development. Journal of Vegetation Science 19, 253-260.

O'Donnell, A.G., Goodfellow, M., Minnikin, D.E. 1982. Lipids in the classification of *Nocardiodes*; reclassification of *Arthobacter simplex* (Jensen) Lochhead in the genus *Nocardiodes* (Prauser) emend. O'Donnell et al. as *Nocardioides simplex* comb nov. Archives of Microbiology 133, 323-329.

Petz, W., Foissner, W., Wirnsberger, E., Krautgartner, W.D., Adam, H. 1986. Mycophagy, a new feeding strategy in autochthonous soil ciliates. Naturwissenschaften 73, 560-562.

Puustjarvi, V. 1973. A rapid method for measuring the structure of a substrate. Meded Fac Landbouwwet Rijksuniv 38, 2051.

Rønn, R., Gavito, M., Larsen, J., Jakobsen, I., Frederiksen, H., Christensen, S. 2002a. Response of free-living soil protozoa and microorganisms to elevated atmospheric CO 2 and presence of mycorrhiza. Soil Biology and Biochemistry 34, 923-932.

Rønn, R., McCaig, A.E., Griffiths, B.S., Prosser, J.I. 2002b. Impact of protozoan grazing on bacterial community structure in soil microcosms. Applied and Environmental Microbiology 68, 6094-6105.

Sampedro, L., Jeannotte, R., Whalen, J.K. 2006. Trophic transfer of fatty acids from gut microbiota to the earthworm *Lumbricus terrestris* L. Soil Biology and Biochemistry 38, 2188-2198.

Thoms, C., Gattinger, A., Jacob, M., Thomas, F.M., Gleixner, G. 2010. Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. Soil Biology and Biochemistry 42, 1558-1565.

Uikman, P.J.K., Jansen, A.G., Van Veen, J.A. 1991. ^{15}N-Nitrogen mineralization from bacteria by protozoan grazing at different soil moisture regimes. Soil Biology and Biochemistry 23, 193-200.

Waksman, S. 1916. Studies on soil protozoa. Soil Science 1, 135-152.Zelles, L., Palojärvi, A., Kandeler, E., Von Lützow, M., Winter, K., Bai, Q.Y. 1997. Changes in soil microbial properties and phospholipid fatty acid fractions after chloroform fumigation. Soil Biology and Biochemistry 29, 1325-1336.

Chapter 5. Chapter summaries and conclusions

5.1 Research objectives

The work contained in this thesis provides an in-depth exploration of how different factors contribute to the composition of microbial communities in the boreal forest floor. The main objective of Chapter 2 was to understand how prescribed burning after harvest affected microbial communities in white spruce, trembling aspen and mixedwood stands. Surprisingly, I found that topography was just as influential in shaping community structure as was composition of the pre-harvest overstory. Topographical effects suggested that differences observed between spruce and aspen microbial communities are linked to how these communities are influenced by moisture.

The work of Chapter 3 was then structured to study how the microbial communities of aspen and spruce forest floor responded to prolonged changes in moisture. From this work, strong links were seen in the aspen forest floor between the moisture treatment, the overall structure of the microbial community and a PLFA biomarker for protozoa. This finding stimulated my interest to investigate effects of protozoa in soil, and led to Chapter 4, in which I determined how protozoa derived from aspen forest floor affected the structure of the microbial community and certain aspects of the soil microbial loop. The culmination of these research topics illustrates how visible components of the forest landscape are linked to and influence microscopic processes invisible to the naked eye.

5.2 Chapter Summaries

Through the research presented in Chapter 2 I attempted to examine how environmental factors, including silvicultural burning, stand type, forest floor nutrients and topographic position, influenced microbial communities at the EMEND research site. The work was defined to answer: 1) what is the effect of slash burning on microbial community structure and function as compared to slash alone?; 2) how does composition of the pre-harvest overstory influence the structure and function of boreal forest floor microbial communities?; and 3) how is the structure and function of forest floor microbial communities influenced by landscape topography? Overall, each objective was successfully addressed.

Harvested sites that had been subjected to prescribed burns exhibited lower total soil microbial biomass and greater NO_3^- concentrations than sites that were only harvested. However, prescribed burning appeared to have no effect on overall forest floor microbial community structure or function. Additionally, coniferous and mixedwood forest floors had similarly structured microbial communities, and these were distinct from deciduous sites. Furthermore, I found that topography was important, and appeared to be equally important to controlling the composition of soil microbial communities as vegetation.

An initial look at the PLFA ordination from the different stand types revealed intriguing patterns, with two apparently separate groups both of which contained spruce, aspen and mixedwood sites. After comparing the groups with a topographical map of EMEND, it became apparent that the sites within each of the two groups corresponded perfectly to two distinct topographical regions on the EMEND landscape. Subsequently, this topographical distinction was added

and the PLFA data were reanalyzed. The new ordination showed that the distinction between coniferous/mixedwood sites and deciduous sites was strongest in the sites with lower elevation and flatter topography. Based on this new insight, an older PLFA dataset from Kirsten Hannam's work at EMEND was reanalyzed as an independent test of the topographical pattern. The lack of a topographical effect in Kirsten's data generated many questions, but finally led to the conclusion that the topographical effect may be seasonal and linked to the amount of forest floor moisture. While Kirsten's sites were sampled in June when the sites are still very wet, my results, as reported in Chapter 2, are based on samples collected in the drier month of August.

Based on these findings I concluded that structural differences in microbial communities observed between sites at higher and lower elevations were linked to seasonal patterns in moisture. During drier months (August sampling), the composition of forest floor microbial communities appeared to be more strongly influenced by topographic position than by stand-related differences. Differences in microclimatic conditions within the forest floors between higher and lower elevations sites may have selected specific microbial groups over others. In particular, one fungal PLFA biomarker (20:1ω9c) was strongly associated with sites at higher elevation, while a biomarker for actinomycetes (10Me19:0) was strongly associated with deciduous sites at lower elevation. The discovery of topographical effects on microbial community structure was unexpected and exciting. The intriguing potential that moisture could be an important factor for microbial community structure of aspen and

spruce gave impetus for further study and led to the rationale and design of Chapter 3.

The work presented in Chapter 3 was aimed to test how, under controlled conditions, forest floor microbial communities in trembling aspen and white spruce respond to the different physical microenvironments generated by manipulating soil moisture content. Forest floor communities were incubated in the laboratory for a period of one month under three distinct moisture contents. As seen in Chapter 2, the origin of the forest floor material had a strong effect on the microbial community and determined how the community structure responded to the moisture treatment. Moisture manipulation caused no apparent change in the microbial community structure in spruce but yielded strong differences in the community structure of aspen. The nature of the moisture manipulation allowed for a comparison of the pore habitats present in the two types of forest floor.

Aspen and spruce habitats were dominated by two types of pores: larger pores that drain once the soil starts to dry following saturation, and smaller pores which may be appropriate bacterial habitats as they stay water-filled under most field conditions but are too narrow for most soil microorganisms. However, aspen litter contained proportionally more pores of an intermediate size than did spruce litter. These intermediate pores are the ones that gradually drain when soil water potential drops from field capacity to the permanent wilting point. Consequently, under the moisture conditions of the experiment, the intermediate pores in aspen forest floor held moisture at the higher moisture contents, but generated different water filled pore habitats at the lower water potentials. This change in pore

habitats was reflected in the structurally and functionally distinct microbial communities among the different moisture treatments. On the other hand, the spruce forest floor habitat, comprised primarily of the narrow pores, stayed relatively similar across all moisture treatments, which resulted in similarly structured microbial communities.

One of the more intriguing results from Chapter 3 was the absence or low concentration of the PLFA 20:4 in aspen samples subjected to the driest moisture treatment. Through a literature review, I found that the PLFA 20:4 was a biomarker for protozoa, and this sparked my interest into the effects of protozoa on forest floor microbial communities, and in particular to investigate the interactions involved in the soil microbial loop. Through a subsequent review of the literature, it became apparent that this biomarker may only represent a certain class of protozoa, the ciliates. Chapter 4 was formulated primarily out of the desire to answer some of the questions generated by the unexpected results of Chapter 3 and the realization of the importance of protozoa in soil processes.

Originally, I designed the work of Chapter 4 to study the soil microbial loop by observing the interactions between forest floor ciliates, forest floor bacteria and aspen seedlings. An exhaustive review of the literature resulted in an elaborate experimental design meant to both control soil water content and prevent protozoa contamination. Unfortunately, complications arose regarding flagellate contamination linked to the maintenance of water content, which confounded the results of the initial experiment. Failure of this earlier experiment led to modifications of the experimental design. However, the second iteration

was also unsuccessful as flagellate contamination was detected prior to the addition of aspen seedlings. The repeated failures prompted a total reevaluation of the entire experiment and resulted in the removal of aspen seedlings and a change of substrate from spruce and aspen forest floor to aspen leaf litter. The failure of the first two iterations, while distressing, was also beneficial as it led to direct counts being used instead of the more problematic MPN approach, and provided the time to learn how to properly measure indole-3-acetic acid.

The final iteration of Chapter 4 focused on interactions between ciliates and other microorganisms isolated from an aspen forest floor. The objectives were to test: 1) if auxin producing soil bacteria would become more prevalent over time in the presence of ciliates 2) if the addition of ciliates would alter the microbial community; and 3) the effectiveness of PLFA analysis as a tool to detect the presence of ciliates.

The work done in Chapter 4 successfully addressed each of these objectives. Ciliate lipid biomarkers taken from the literature were either absent or not correlated with direct counts, a finding which brings into question the robustness of the PLFA analysis as a means of detecting ciliates. Auxin producing bacteria were successfully detected but their growth was neither dependant on nor enhanced by the presence of ciliates. However, ciliates stimulated bacteria that utilize auxin precursors for energy.

Ciliates affected the microbial community mainly through their effects on gram negative bacteria. Gram negative cells undergoing stationary growth were reduced in the presence of ciliates and overall growth of gram negative bacteria

was moderated. Also, moderating gram negative growth allowed for other interactions between microbial groups to develop as the antagonistic interaction between actinomycetes and fungi was only observed in the presence of ciliates. Finally, this work provided evidence suggesting that ciliates isolated from the aspen forest floor were consuming fungal biomass.

Taken together, the body of work contained within this thesis can be used to connect factors that shape microbial community structure in boreal forest floors. Protozoan activity in aspen litter shapes the microbial community through consumption of select bacteria and by promoting the growth of others. However, being aquatic organisms, protozoa can only become active when suitable water filled pore habitat is available. Compared to spruce, aspen forest floor appears to maintain a pore environment suitable for protozoan activity over a larger range of water potentials. Such differences become apparent on the landscape when factors such as topography redistribute moisture. Under these circumstances, spruce microbial communities located at upper and lower slope positions remain relatively similar while aspen communities at different positions exhibit distinct compositions.

5.3 Project Limitations and Future Research

The EMEND sites used in chapter 2 are valuable because they allow researchers the opportunity to monitor the progression of microbial communities over time following human disturbance. Unfortunately, broader conclusions comparing the two silvicultural practices to undisturbed forest could not be made

in Chapter 2 as unharvested sites were not included in the original study design. Future work at EMEND should include unharvested sites as doing so would allow inquiries into the recovery of microbial communities in slash-harvest and slash-harvest followed by prescribed burning. Additionally, links between season and topography should be addressed when designing future studies as topographic differences observed in Chapter 2 were not present in previous research that took place during early summer (Hannam et al., 2006).

Populations of bacteria, fungi and protozoa can stratify by depth in deciduous and coniferous forest floors with more fungi and protozoa occurring in the less decomposed upper litter layers (Šnajdr et al. 2008; Baldrian et al. 2012; Janssen and Heijmans, 1998). However, the forest floor samples studied in Chapter 2 were homogenized so that the entire community was represented within a sample. Further work that tries to determine how prescribed burning impacts forest floor microbial communities would benefit from using samples that are stratified by depth. Such work would show how changes to the forest floor surface caused by burning affect underlying litter layers and would also be able to illuminate the depth of influence that burning has on the forest floor.

Work presented in Chapter 2 also revealed that the forest floor microbial communities of mixedwoods and white spruce were similar in structure. Conifers can have spatially distinct influences on PLFA patterns and concentrations, which had previously been explained by the quality of the organic matter generated under spruce (Saetre, 1999). However, more recently, forest floor moisture has been found to be just as vital in determining microbial community structure and

function in forest ecosystems (Brockett et al., 2012). The factors influencing forest floor microbial communities now appear to be better understood, and yet the exact mechanisms operating within these factors remain elusive, still requiring proper definition and testing.

The forest floor used in Chapter 3 was air dried and sieved after collection from the field. In mineral soils, air drying and sieving can change the composition of the microbial community (Peterson and Klug, 1994; Thomson et al., 2010). However, the change in PLFA community composition of sieved and air-dried samples collected from environments subject to frequent dry/wet cycles is often less severe and supports overall conclusions that are similar to fresh samples (Hamer et al., 2007). To date, the effect of drying on microbial communities has not been adequately tested in the forest floor, an environment which is subject to frequent wet/dry cycles and for which physical properties are heavily modified by sieving. Future work to study the impacts of air drying and sieving on forest floor communities would be valuable as these sample handling techniques are at times necessary due to the facts that study areas in the boreal forest are often remote, and thus timely access to proper facilities can be limited.

Chapter 4 generated many interesting and potentially unique findings. In particular, the role of elevated auxin production in the presence of protozoa warrants further exploration. At present, it appears that elevated auxin production may be related to the activity of amoebae (Krome et al., 2009) and not the activity of flagellates (Ekelund et al., 2009) or ciliates. However, such findings could be species-dependent and need to be confirmed before they can be added as

components of the soil microbial loop. Additionally, promotion of auxin-catabolizing bacteria by ciliates as seen in Chapter 4 has not been previously reported in the literature. Hence, this finding needs to be re-examined and confirmed under a range of conditions before it is built into models of the soil microbial loop. Finally, the potential for fungal grazing by ciliates requires further study, as such a behavior, if common in aspen forest floor, would have broad implications concerning the ecology of fungi in forest ecosystems.

The lack of correlation between PLFA biomarkers and direct counts of ciliates (Chapter 4) should be reconfirmed to support or deny the common use of PLFAs as a broad measure of soil protozoa. Future work comparing direct counts and PLFA concentrations should include other groups of protozoa as well as a larger number of ciliate morphospecies. Also, active and inactive forms of protozoa exist depending on soil conditions; therefore, distinctions between the PLFA of these two forms need to be determined before we can make any meaningful conclusions about protozoan populations at the time of sampling.

Addressing the objectives in Chapter 4 required extreme manipulations in the controlled settings of the laboratory. This could limit the application of the results to a broader context. However, isolating how protozoa affect soil systems requires extreme controls and such studies often have complex experimental designs to prevent contamination and control key factors such as soil moisture (Alphei et al., 1996). Confirming the laboratory results under field conditions would be ideal; yet attempting to do so would require its own set of rigid controls and caveats. Such is the dilemma of studying soil protozoa.

5.4 Research Implications

Boreal landscapes in Alberta are under ever increasing pressure from resource exploitation, particularly in regions associated with oil sands extraction. In some instances, resource extraction results in the complete destruction of large areas of the forest landscape. These areas eventually have to be returned to a self-sustaining forest ecosystem. Unfortunately, we do not fully understand all of the processes which make a forest successful. More importantly, our knowledge is limited when it comes to understanding how these processes are interconnected and how they respond to natural disturbances. I believe that our current dilemma can be expressed with the following question: How do we put humpty dumpty back together again when we did not know what he looked like before he fell?

Chapter two was designed to address the effects of prescribed burning on harvested forests. The application of a prescribed burn appears to have a similar effect from a microbial community perspective as harvesting. Forestry managers wishing to apply a prescribed burn as a management tool can do so without the worry of it altering the microbial community. When reconstructing forest ecosystems, managers will need to take into account that the microbial community structure of these systems is subject to factors, which are still poorly understood, associated with landscape position.

Chapter three highlights how management considerations need to be made for ecosystem processes occurring at the micro-scale. The different responses to moisture by the microbial communities of aspen and spruce are of particular

importance. The organic materials used for reconstructed forest landscapes should potentially be dependent on the type of forest that managers wish to achieve. For example, constructing upland aspen forests with an amendment heavy in peat may result in the establishment of a belowground microbial community that responds to the physical properties of peat rather than the community that would have developed with the physical properties found in an aspen forest floor. However, using an amendment heavy in peat may be suitable for white spruce as it is likely that, due to the high moss content, the physical properties of spruce forest floors are similar to that found in the peat. This question requires further research to determine if the physical properties of the amendment do indeed shape the trajectory and subsequent recovery of reconstructed landscapes to self-sustaining forests.

5.5 Conclusions

Harvesting in aspen, white spruce or mixedwood stands affects much of the boreal landscape in Alberta. In the underlying forest floor of these areas, patterns of microbial community structure are related to the tree species that dominated the overstory prior to harvesting. Community recovery after slash-harvesting was not tested as unharvested stands were not included in the original design; however, prescribed burning after harvesting appears to have no influence on the overall structure of the microbial community in the forest floor. The similarities in community structure between spruce and mixedwood stands is most likely controlled by the presence of spruce trees. However, at certain times of the

year, topography can become the dominant factor that determines microbial community structure in the forest floor, possibly due to the influence it has on the translocation of soil moisture over the landscape.

In addition to their inherent differences in carbon and nutrient availability, and other chemical characteristics, forest floors derived from aspen and spruce provide distinct physical habitats for microbes. The pore habitat of spruce forest floor appears to be dominated by large air-filled pores with bacteria being confined to smaller pores that retain moisture under most field conditions. As a result, microbial communities in spruce litter appear to be relatively similar regardless of the level of soil moisture. Aspen forest floor is also dominated by large and small pores, but includes a higher proportion of intermediate sized pores. These intermediate pores form habitats that change as water content fluctuates. Changes in pore habitat appear to affect the structure of microbial communities in aspen litter. Overall, my work supports the idea that differences in water filled pore habitats under aspen and spruce canopies drives differences commonly seen between aspen and spruce microbial communities.

Ciliates found in aspen forest floor layers can affect the structure of microbial communities by moderating the growth of gram negative bacteria and by potentially grazing on fungi. Their presence allows for observation of interactions between fungi and gram positive organisms that are otherwise obscured by the growth of gram negative bacteria. Ciliate activity promotes bacteria that consume plant auxins and may have broader impacts by affecting plant root growth. However, ciliates are aquatic organisms and are only active

when the water filled pore habitats are available within the forest floor. Thus, their activity within the landscape is dependent on suitable pore habitat generated by the litter of the plant community as well as other factors such as topography and climate which influence the amount of moisture within these pore habitats.

Environmental factors such as overstory composition and topography often account for the differences observed in microbial communities within the boreal plains of Alberta. These factors alone are descriptive and, on their own, do not sufficiently explain the underlying mechanisms affecting the microbial communities within the forest floor. Only by understanding the relationships between factors occurring on both large and small scales will we reveal the mechanisms that ultimately drive the differences that are observed over the landscape.

Literature cited

Alphei, J., Bonkowski, M., Scheu, S. 1996. Protozoa, nematoda and lumbricidae in the rhizosphere of *Hordelymus europaeus* (Poaceae): Faunal interactions, response of microorganisms and effects on plant growth. Oecologia 106, 111-126.

Baldrian, P., Kolaiřík, M., Štursová, M., Kopecký, J., Valášková, V., Větrovský, T., Žifčáková, L., Šnajdr, J., Rídl, J., Vlček, Č., Voříšková, J. 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISME Journal 6, 248-258.

Brockett, B.F.T., Prescott, C.E., Grayston, S.J. 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biology and Biochemistry 44, pp. 9-20.

Ekelund, F., Saj, S., Vestergård, M., Bertaux, J., Mikola, J. 2009. The "soil microbial loop" is not always needed to explain protozoan stimulation of plants. Soil Biology and Biochemistry 41, 2336-2342.

Hamer, U., Unger, M., Makeschin, F. 2007. Impact of air-drying and rewetting on PLFA profiles of soil microbial communities. Journal of Plant Nutrition and Soil Science 170, 259-264.

Hannam, K.D., Quideau, S.A., Kishchuk, B.E. 2006. Forest floor microbial communities in relation to stand composition and timber harvesting in northern Alberta. Soil Biology and Biochemistry 38, 2565-2575.

Janssen, M.P.M., Heijmans, G.J.S.M. 1998. Dynamics and stratification of protozoa in the organic layer of a Scots pine forest. Biology and Fertility of Soils 26, pp. 285-292.

Krome, K., Rosenberg, K., Bonkowski, M., Scheu, S. 2009. Grazing of protozoa on rhizosphere bacteria alters growth and reproduction of *Arabidopsis thaliana*. Soil Biology and Biochemistry 41, 1866-1873.

Petersen, S.O., Klug, M.J. 1994. Effects of sieving, storage, and incubation temperature on the phospholipid fatty acid profile of a soil microbial community. Applied and Environmental Microbiology 60, 2421-2430.

Saetre, P. 1999. Spatial patterns of ground vegetation, soil microbial biomass and activity in a mixed spruce-birch stand. Ecography 22, 183-192.

Šnajdr, J., Valášková, V., Merhautová, V., Herinková, J., Cajthaml, T., Baldrian, P. 2008. Spatial variability of enzyme activities and microbial biomass in the upper layers of Quercus petraea forest soil. Soil Biology and Biochemistry 40, 2068-2075.

Thomson, B.C., Ostle, N.J., McNamara, N.P., Whiteley, A.S., Griffiths, R.I. 2010. Effects of sieving, drying and rewetting upon soil bacterial community structure and respiration rates. Journal of Microbiological Methods 83, 69-73.