Rhizosphere microbial response to predicted vegetation shifts and changes in rhizodeposition in boreal forest soils

by

Sarah Julia Thacker

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Department of Renewable Resources
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ABSTRACT

The boreal forest is the single largest terrestrial store of carbon on Earth. In Canada's boreal forest, approximately 23% of these carbon stocks are found in forest floors and 40% within mineral soils. The rhizosphere, soil under the direct influence of plant roots, is a hotspot for microbial activity and plays an important role in soil carbon dynamics. Rhizodeposits, which contain labile carbon substrates, may result in increased decomposition of soil organic matter: a phenomenon known as priming. With climate change, vegetation shifts are expected in the boreal, and deciduous dominated stands will replace conifers. Increased atmospheric CO_2 with climate change can indirectly affect plant photosynthesis, resulting in increased carbon allocation belowground and increased root biomass. I investigated how these potential vegetation shifts and changes in rhizodeposition could affect: (1) the composition and function of microbial communities, and (2) rhizosphere priming in mineral soils common to the boreal.

To assess the impact of vegetation shifts on microbial communities, I collected rhizosphere samples from the forest floor and compared them to bulk forest floor. Samples were collected at the Ecosystem Management Emulating Natural Disturbance (EMEND) project in northern Alberta, Canada. Phospholipid fatty acid (PLFA) analysis was used to characterize microbial community composition and multiple substrate induced respiration (MSIR) to examine microbial community function. The natural abundance of carbon isotopes in individual PLFAs was used to examine carbon source utilization by microorganisms. I surveyed 17-year-old spruce clear cuts where aspen was naturally regenerating to investigate the effect of aspen replacing former spruce stands. These were compared to mature stands of aspen and spruce, and 17-year-old clear-cuts of aspen. The rhizosphere had a significantly higher proportion of fungi and a higher gram negative to gram positive bacteria ratio compared to bulk soil. Fungi and gram-negative bacteria biomarkers in the rhizosphere showed ^{13}C depletion compared to bulk forest floor, indicating that rhizosphere

microbes were accessing more recently fixed carbon than in bulk soil. Aspen trees exhibited greater influence over their rhizospheres than spruce trees in terms of community composition and function, and aspen rhizospheres showed the highest basal respiration. In less than two decades, aspen regeneration in former spruce stands shifted microbial communities towards aspen stands, with the rhizosphere responding more quickly than bulk forest floor. This study indicates that microbial communities of rhizosphere and bulk forest floor differ in the boreal, and that vegetation shifts have the potential to cause more immediate and profound changes in the rhizosphere.

I investigated priming and microbial uptake of labile carbon in two mineral soils, a Luvisol and Brunisol, commonly found in Canada's boreal. The Luvisol was collected from Cooking Lake Blackfoot Provincial Recreation Area and the Brunisol at the Woodbend Forest University of Alberta research site, both within 50 km of Edmonton, AB. I incubated A and B horizons from the two soils with ^{13}C-labelled glucose as a model root exudate. Glucose was added at three rates relative to microbial biomass carbon: 0.125x, 1x, and 2x. Carbon isotope probing of PLFA biomarkers was used to assess which microbial groups were responsible for uptake and utilization of the added substrate carbon. At the end of the 65 day incubation, no differences in priming were observed between soil types, depth, or glucose treatments. However, in the first hours of the incubation I observed positive priming in B horizons and negative priming in A horizons. Results suggest that the magnitude and direction of priming are both strongly dependent on the timing of glucose addition and measurement. If labile carbon is added regularly, it appears that organic matter would be protected in topsoil but mineralized more quickly in subsoil. If labile carbon additions occur only periodically, our results suggest that organic matter mineralization would not be affected considerably in the long term. Further, I conclude that fungi are an important microbial group in uptaking and utilizing labile carbon added to soil.

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Chapter 1 – INTRODUCTION

1.1. The rhizosphere and soil organic matter

1.1.1. What is the rhizosphere?

The rhizosphere is the interface between aboveground and belowground worlds. The term "rhizosphere" was first coined by Lorenz Hiltner in 1904 (Hartmann et al., 2008). The rhizosphere constitutes soil under the direct influence of plant roots and extends just millimetres from the surface of roots (DeAngelis et al., 2009; Hawkes et al., 2007). Other pioneers in rhizosphere research include R.L. Starkey who used light microscopy to examine rhizosphere bacteria distribution and density, A.G. Lochhead and colleagues, who improved our understanding of the difference between rhizosphere and bulk soil, F.E. Clark who coined the term rhizoplane to describe the root surface, and N.A. Krasilnikov who examined the effect of rhizodeposits on Azotobacter (Rovira, 1991). Since Hiltner's definition in 1904, tens of thousands of scientific papers referencing the rhizosphere have been published. In the early 2000s the number of papers published each year containing the keyword rhizosphere began to skyrocket and since then has increased every year.

The overwhelming interest in the rhizosphere stems from its importance for soil, plant, and ecosystem health. In soil, the rhizosphere provides habitat and an energy source for microorganisms, regulates carbon flux from plants to soil, makes up an important component of soil respiration, and affects mineral weathering. At the individual plant scale, the rhizosphere can improve plant growth via plant growth stimulating bacteria, can hinder plant growth by providing habitat for pathogens, and provides a source for nutrient transfer from soil microorganisms to plant roots. The rhizosphere plays a critical role in ecosystem dynamics through its role in overall plant health and carbon (C) fluxes.

Our ability to study the rhizosphere has improved with the development of sophisticated techniques, such as ^{13}C tracers and ^{13}C phospholipid fatty acid (PLFA) analysis, but there remains a lack of understanding regarding how climate change will affect the rhizosphere and processes at depth. While there is a wealth of studies investigating the rhizosphere, there are fewer looking specifically at climate change effects on microbial soil communities and priming (soil organic matter (SOM) decomposition by microbes using labile C as an energy source; section 1.1.2.) and even fewer focusing on the boreal forest. Boreal priming studies have found varying results. Fan et al. (2013), Lindén et al. (2014), and Karhu et al. (2016) found positive priming while Chigineva et al. (2009) and Linkosalmi et al. (2015) found negative or no priming. All these boreal priming studies took place in Europe, where the dominant tree species differ from those in Canada. This thesis research will help fill the knowledge gap in Canada's boreal forest rhizosphere.

The intimate connection between plant roots and rhizosphere soil makes it unique from bulk soil, soil not under the direct influence of roots. The rhizosphere generally has higher microbial biomass and different microbial community composition than bulk soil. Foster (1988) observed up to $10^{10} – 10^{12}$ microbes per gram in the rhizosphere and less than 10^{8} in bulk soil. Bodelier et al. (1997) reported that the rhizosphere houses 19 to 32 times more microorganisms than bulk soil. Chaudhary et al. (2012) found that rhizosphere and bulk soil showed different microbial communities for two biofuel crops. Soil mineralogy differs between rhizosphere and bulk soil (Courchesne and Gobran, 1997), indicating that the rhizosphere not only has biological significance but also pedogenic significance. Courchesne and Gobran (1997) investigated a Podzolic soil and found that mineral composition in the rhizosphere differed from bulk soil, with less amphiboles and expandable phyllosilicates and more oxalate-extractable Al and Fe in the rhizosphere compared to bulk soil. While two discreet zones, the rhizosphere and bulk soil are

interlinked. Bulk soil microbial community composition plays a role in determining rhizosphere community composition (de Ridder-Duine et al., 2005; Bakker et al., 2015).

Rhizosphere soil differs from bulk soil in that it receives rhizodeposits from plant roots, which are a source of carbon for microorganisms. Rhizodeposits include exudates, root secretions, mucilage, lysates, and gases (Kuzyakov, 2002; Table 1.1.). Rhizodeposits are comprised primarily of low molecular weight molecules, which are primarily root exudates (Cheng and Gershenson, 2007). The specific composition of root exudates varies depending on plant species, but exudates are generally composed of a combination of sugars, organic acids, amino acids, enzymes, and nucleotides and flavonones (Rovira, 1965). There are many factors that affect root exudation: plant species, plant age, temperature, light, plant nutrition, microbes, medium that roots are grown in, soil moisture, and root damage (Rovira, 1964).

Soils on Earth release approximately 60 Gt of carbon to the atmosphere each year via soil respiration (Giardina et al., 2014; IPCC, 2007a). Soil respiration is balanced by inputs of carbon, but with global climate change the balance may be shifting (Giardina et al., 2014). There are two main processes of belowground carbon dioxide (CO_2) flux: (1) rhizosphere respiration which includes root respiration and microbial respiration and (2) soil organic matter (SOM) decomposition performed by microorganisms (Cheng and Gershenson, 2007). These processes are distinct but interlinked, and becoming more so with global climate change.

1.1.2. The rhizosphere and priming

Rhizodeposits can be used by microorganisms in the rhizosphere as an energy source for degrading SOM; this process is called the rhizosphere priming effect (RPE) and the result is an increased CO_2 flux to the atmosphere (Cheng and Gershenson, 2007; Kuzyakov, 2010). The

rhizosphere is the most important microbial hotspot for priming (Kuzyakov, 2010). However, a suppressive effect has also been observed (Lindén et al., 2014).

There are seven proposed mechanisms for the rhizosphere priming effect (Kuzyakov, 2002), which include:

1. Drying effect or drying/rewetting hypothesis: Water uptake by plants creates drier conditions that limit SOM decomposition (negative priming). However, drying-rewetting that occurs under cultivation has also caused increased SOM decomposition (positive priming).
2. Aggregate destruction hypothesis: Growing roots break apart soil aggregates, making SOM that was previously protected now susceptible to decomposition by microbes (positive priming).
3. Root uptake of soluble organic substances: If roots re-uptake much of the released exudates, then there is less carbon available for microorganisms, resulting in less SOM decomposition (negative priming).
4. Enhanced microbial turnover due to faunal grazing: Release of CO_2 is due to the death of microorganisms predated by soil fauna, not SOM decomposition (neutral/negative priming).
5. Competition for nitrogen between plant roots and rhizosphere microorganisms: Because microbes in the rhizosphere are nitrogen limited, uptake of nitrogen by plants increases competition. This in turn reduces microbial growth and therefore SOM decomposition (negative priming).
6. Preferential substrate utilization: Exudates are more easily available than SOM, so microbes prefer to utilize exudates, resulting in a decrease in SOM decomposition, at least initially when exudates are introduced to the rhizosphere (negative priming).

7. Microbial activation: Labile substances released by roots stimulate microbial growth in the rhizosphere leading to increased SOM decomposition either to access nitrogen or as an unintentional co-metabolic decomposition of SOM (positive priming).

According to Kuzyakov (2002), the most important priming mechanisms are microbial activation, preferential substrate utilization, and competition for nitrogen between plants and rhizosphere microorganisms.

Positive priming effects could reduce the carbon sequestration potential of soils (Hungate et al., 2003). Carney et al. (2007) concluded that altering microbial communities can convert soils from carbon sinks to sources. Climate change, through alterations in plant carbon allocation, has the potential to increase priming and change rhizosphere microbial communities—both of which could alter soil carbon dynamics. If we wish to understand how climate change will affect our planet, it is critical that we study the rhizosphere within the world's largest terrestrial store of carbon (Watson et al., 2000): the boreal forest.

In priming studies, researchers typically incubate soils with a labile C substrate and evaluate whether addition of the substrate increased SOM mineralization. A common substrate used in such studies is glucose (Kuzykov, 2010). Researchers have found that priming effects can be dependent on substrate addition relative to soil microbial biomass carbon (MB-C) (Karhu et al., 2016; Blagodatskaya and Kuzyakov, 2008). Blagodatskaya and Kuzyakov (2008) described three different scenarios. When substrate carbon added was less than 15% of microbial biomass C, primed CO_2 increased linearly with the amount of substrate C. At substrate C additions greater than 50% of microbial biomass, primed CO_2 decreased exponentially with added substrate carbon. With additions 200-500% of MB-C, priming was zero or negative.

1.1.3. Mechanisms of SOM stabilization

While priming can destabilize SOM, there are mechanisms of SOM stabilization that may prevent mineralization of native organic matter. SOM stabilization in subsoil is important for carbon storage as deep soil horizons can contain over half of total soil C stocks and may be a more important carbon sink than topsoil (Rumpel and Kögel-Knabner, 2011). There are four main sources of organic matter (OM) in subsoil: plant roots, root exudates, dissolved organic carbon, and bioturbation (Rumpel and Kögel-Knabner, 2011). Another source of OM may be translocation of particulate OM and clay-bound OM (Rumpel and Kögel-Knabner, 2011).

The three main processes of SOM stabilization for deep soil horizons as discussed by Rumpel and Kögel-Knabner (2011) include physical protection, physico-chemical interaction, and stable chemical structure.

1. Physical protection: Particulate and clay-associated OM can be aggregate protected, preventing destabilization. The location of OM within aggregates physically separates OM from microbes responsible for SOM decomposition.
2. Physio-chemical interaction: Association with soil minerals may stabilize OM as clay and poorly crystalline minerals have been found to protect OM from oxidation (Hosking, 1932; Singer and Huang, 1993).
3. Stable chemical structure: Chemical recalcitrance is a controversial mechanism. Chemical recalcitrance suggests that litter inputs that are difficult to degrade (for example, roots which may be recalcitrant due to high lignin and aliphatic material content) will not be decomposed easily in subsoil, thus becoming part of the SOM (Rasse et al., 2005).

Subsoil SOM can be destabilized by microorganisms. Microbial biomass and activity generally decline with depth (Taylor et al., 2002; Andersen and Domsche, 1989; Ekklund et al., 2001; Fang

and Moncrieff, 2005). However, the carbon source for microbes at greater depth increasingly was found to be older SOM, suggesting that microbes can utilize carbon found in older SOM (Kramer and Glexiner, 2008). The availability of deep SOM to microbes is affected by environmental conditions such as temperature, moisture, and nutrient availability (Rumpel and Kögel-Knabner, 2011). Stabilized C has a heterogeneous distribution in soil due to soil structure and texture, plant cover, and the different SOM sources into soil (Rumpel and Kögel-Knabner, 2001; Chevallier et al., 2000). Rumpel and Kögel-Knabner (2011) state that spatial separation of microbes and degradable substrates could be one of the most important factors in subsoil C dynamics.

1.2. The boreal forest of Canada

The boreal forest is one of Canada's greatest natural resources and the largest terrestrial store of carbon (Watson et al., 2000) on Earth. The managed boreal is a carbon sink storing 28 Tg C per year and in total stores 28 Pg of C in biomass, organic matter, and soil (Kurz et al., 2013). The boreal forest extends across Canada and is a circumpolar vegetation zone comprised mainly of forested land dominated by cold tolerant tree species (Brandt et al., 2013; Figure 1.1.). Canada has 28% of the world's boreal zone, which totals 552 million ha in Canada, and of that, 307 million ha is forested (Natural Resources Canada, 2016). Tree species in the Canadian boreal are mainly from the following genera: *Abies*, *Larix*, *Picea*, *Pinus*, *Populus*, and *Betula* (Brandt et al., 2013).

The Canadian boreal zone consists of numerous ecozones. In Alberta, aspen parkland, boreal plains, and taiga plains are the three main ecozones with boreal plains covering the largest area (Price et al., 2013; Figure 1.2.). The boreal plains ecozone has high carbon density at about 230 Mg of C per ha and has the largest total C stock of all the ecozones in Canada at about 8 Pg of carbon (Kurz et al., 2013). The taiga plains in Canada has a carbon density of about 150 Mg of

C per ha and a total C stock of approximately 3 Pg of carbon (Kurz et al., 2013). The boreal forest in Alberta serves an important role in carbon storage, given the ecozones found in the province.

Kurz et al. (2013) state that soil organic matter in mineral soil accounts for approximately 40% of total carbon in Canada's boreal forest (when also considering aboveground biomass, belowground biomass, dead wood, and forest floor material/litter), and ~23% is stored in the forest floor layer. Of the carbon sources identified, soil organic matter also accounts for the greatest carbon density at approximately 78 Mg of C per ha (Kurz et al., 2013). When considering the top 100 cm of mineral soil alone, 50% of soil organic carbon is stored from 0-20 cm, 25% from 20-40 cm, 13% from 40-60 cm, and 12% from 60-100 cm (Jobbágy and Jackson, 2000). Upper soil horizons may be generally more carbon rich, but subsoil has the potential to play an important role in the soil carbon balance with climate change. It is important to consider both the forest floor and mineral soil horizons to gain a more complete understanding of boreal carbon dynamics.

1.3. The boreal forest under climate change

Anthropogenic climate change refers to changes in the global atmosphere as the result of direct or indirect human activity (IPCC, 2007b), whereas climate change in general may refer to natural variation or human-induced changes. Here we refer to anthropogenic climate change simply as climate change.

1.3.1. General trends already observed

Global temperatures have been increasing, with a 0.7°C increase in the last 150 years (Brandt et al., 2013). From 1950 to 2003, the western boreal experienced 2.0°C mean annual temperature increases while mean annual temperature in the east increased 0.5°C (Lemprière et al., 2008), highlighting the importance of investigating climate change impacts in western Canada, in provinces such as Alberta. Throughout much of the boreal, annual precipitation has increased

10 to 20% since 1900, except in southern forests and parklands in prairie provinces where dry conditions exist due to drought (Price et al., 2013).

1.3.2. General trends predicted to occur

Even with conservative estimates, an average warming of 2.0ºC (relative to about the year 2000) is likely for the boreal by 2050 (Price et al., 2013). Larger temperature increases are expected during the winter in the northern boreal compared to southern areas; regardless of latitude, these warming trends will lead to longer growing seasons and longer frost free periods (Price et al., 2013). Predictions for precipitation are not as clear, but greater summer moisture deficits at mid latitudes are expected (Price et al., 2013). Predictions for extreme events are even more unclear. However, frequency and intensity of extreme events—drought, wind, flooding, and convective storms that result in lightening-induced fires—are expected to increase and have been linked with climate change (Meehl et al., 2007). Increases in insect outbreaks and occurrence of large fires are expected with climate change (Price et al., 2013). Balshi et al. (2009) state that in the western boreal the area burned could increase 3.5 to 5.5 times by 2100. For Canada, Canadian general circulation model (GCM) results suggest a 75% increase in fire occurrence by 2100, while the Hadley GCM suggests a 140% increase (Price et al., 2013).

1.3.3. Vegetation shifts

Using the A1B emissions scenario, Loarie et al. (2009) found that the boreal biome is moving northward 0.43 km/year while the rates for temperate broadleaf and coniferous forests are 0.35 km/year and 0.11 km/year, respectively. Clearly the boreal biome is moving relatively faster than other biomes, which should make the boreal a priority for climate change research. The northward move of the boreal could mean that several trees species, in particular conifers such as *Picea glauca* (Moench) Voss (white spruce), become replaced by more southerly species such as

Populus tremuloides Michx. (trembling aspen). In Alberta, vegetation shifts in the Central Mixedwood and Boreal Highlands will be influenced by elevation and moisture deficits (Schneider, 2013). The Central Mixedwood and Boreal Highlands will both be characterized by aspen outcompeting conifers as conditions become drier and hotter (Schneider, 2013).

Climate sensitive species will first disappear at their southern boundaries (Price et al., 2013). Boreal species, especially trees, may become maladapted to new climate conditions that result from climate change. An example of maladaptation in Canada is the drought-caused dieback of aspen in the southern boreal and aspen parkland from 2001 to 2003 (Price et al., 2013). Increased evaporative demand will, in most cases, cause the climate moisture index (CMI) to decrease. This means that even though precipitation has increased, conditions will become drier (Price et al., 2013). CMI for the aspen parkland is predicted to become more negative than it currently is, and CMI for the boreal plain is expected to shift from positive to negative by 2100.

By 2100, climate zones suitable for boreal conifer species will no longer exist in their southern boundaries across much of Canada (McKenney et al., 2007, 2011). Southern regions of the Boreal Plain will likely become more climatically similar to the current day aspen parkland within a few decades. With Alberta becoming drier, increased fire risk and expansion of the aspen parkland makes studying the effects of aspen replacing spruce important. In an example from southwestern Yukon, warming and drying led to low regeneration of spruce forests after fire. This resulted in a shift towards aspen forests on south facing slopes and low elevations (Johnstone et al., 2011). In some places in the Yukon, spruce forests have been replaced by aspen clones interspersed with grassland (in scattered locations), resembling vegetation of the parkland (Hogg and Wein, 2005).

1.3.4. Elevated atmospheric CO_2

Kurz et al. (2013) state that scientists have observed increases in productivity in the boreal due to warming and longer growing seasons, elevated atmospheric CO_2 concentration, and increased nitrogen availability (Magnani et al., 2007; Briffa et al., 2008; Hickler et al., 2008). However, large scale productivity increases likely will not be able to offset carbon emissions from increased disturbance and heterotrophic respiration (Kurz et al., 2013) because increased photosynthetic productivity with climate change will not necessarily result in increased biomass (carbon sequestration) (Brandt et al., 2013). Enhanced plant growth in response to elevated atmospheric CO_2—termed carbon fertilization—will be limited in much of the boreal due to nutrient limitations (Price et al., 2013).

While increased photosynthesis may not result in large carbon storage gains due to greater aboveground biomass, it may affect belowground processes. For example, Körner and Arnone (1992) found that elevated atmospheric CO_2 increased fine root production and soil respiration without increases in aboveground biomass, resulting in a loss of soil carbon. Elevated atmospheric CO_2 can result in increased C allocation belowground via root exudation and turnover, due to increased photosynthetic rates (Coûteaux et al., 1999). Olszyk et al. (2003) found that an increase in both atmospheric CO_2 and temperature resulted in increased percent sugar in fine roots. The effect of elevated CO_2, however, may be species specific (Asshoff et al., 2006). Increased root production could increase organic matter accumulation in soil. Alternatively, new areas of soil become exposed to rhizodeposits which could increase soil organic matter (SOM) decomposition (priming). Warmer temperatures could lead to increased microbial activity, contributing further to SOM decomposition.

1.4. The rhizosphere under climate change

1.4.1. Changes in rhizodeposition

Quality and quantity of rhizodeposits vary depending on plant species (more importantly group of species, for example grass versus woody plant), plant age, soil texture, soil nitrogen, and the presence of rhizosphere microbes (Cheng and Gershenson, 2007). Differences in rhizodeposition with plant species and groups could affect composition and function of the rhizosphere microbes if vegetation communities shift. Plants may secrete fewer rhizodeposits with age; however, the authors note that this conclusion comes mostly from experiments on annual plants (Cheng and Gershenson, 2007). Rhizodeposition can increase when soils have up to 15% clay content, but decreases when nitrogen availability is higher (Cheng and Gershenson, 2007). Compared to sterile cultures, rhizosphere microorganisms increase rhizodeposition (Cheng and Gershenson, 2007).

1.4.2. Changes in rhizosphere size and respiration

Increases in fine root biomass and root exudation have been observed with increased atmospheric CO_2 and temperature, potentially increasing the volume of rhizosphere soils in the future (Yin et al., 2013; Leppälammi-Kujansuu et al., 2013, 2014; Norby et al., 2004; Pendall et al., 2004; Curtis and Wang, 1998). Rhizodeposition quantity is primarily controlled by plant carbon allocation, which means that environmental factors affecting plant carbon allocation affect rhizodeposition and in turn rhizosphere microorganisms (Cheng and Gershenson, 2007). Increases in atmospheric CO_2 will likely have little direct effect on soil microbial communities because CO_2 concentration in the pore space of active soil is 2000 to 38 000 ppm—much higher than in the atmosphere (Drigo et al., 2008). The greatest impact of elevated atmospheric CO_2 in soil will be due to changes in plant photosynthesis (Drigo et al. 2008), which can increase allocation of carbon

belowground via root exudation and turnover, leading to changes in microbial community size and activity (Coûteaux et al. 1999; Körner 2000; Rillig et al. 2001). It is key that we study the rhizosphere under climate change, as microbes associated with plants are likely to be more affected than those in the bulk soil.

Elevated atmospheric CO_2 concentrations may greatly increase rhizosphere respiration compared to plant biomass. For example, three studies using different plant species found that even though plant biomass increased only 15 to 26%, rhizosphere respired carbon increased 56 to 74% under elevated CO_2 compared to ambient CO_2 (Cheng and Johnson, 1998; Hungate et al., 1997; Lekkerkerk et al., 1990). Increases in plant biomass are unlikely to compensate for increased rhizosphere respiration (Cheng and Johnson, 1998; Hungate et al., 1997; Lekkerkerk et al., 1990). The major consequence of increased rhizosphere respiration is the negative effect on organic C storage in soil.

1.5. Methods of studying microbial communities and carbon

1.5.1. Phospholipid fatty acid (PLFA) analysis

Soil microbial communities can be broadly characterized by analysis of PLFAs extracted from soil (Frostegård et al., 2010). PLFAs are present in cell membranes and vary in carbon chain length, unsaturation, and branching. Variability in PLFA structure allows researchers to assess microbial community composition and identify microbial groups within soil (Frostegård et al. 2010; Frostegård and Bååth, 1996). Microbial groups include fungi, gram negative bacteria (contain an outer cell membrane), gram positive bacteria (lack an outer cell membrane), actinomycetes, and protists.

Previous research has found differences in boreal forest soil microbial communities with aboveground vegetation using PLFA techniques (Hannam et al., 2004, 2006; Swallow and

Quideau, 2013). Researchers have used PLFA analysis to identify differences between rhizosphere and bulk soil in agroforestry systems (Guo et al, 2015; Chaudhary et al., 2012). Distinction between rhizosphere and bulk soil in forest soils is less common and has used DNA-based techniques (Fonseca et al., 2018) and is unstudied in Canada's boreal forest. We still lack an understanding of how vegetation shifts could affect microbial communities, and if differences exist in rhizosphere and bulk soil microbial communities in the boreal forest floor.

1.5.2. ^{13}C PLFA techniques

PLFAs can be used as biomarkers in stable carbon isotope ^{13}C analysis to assess carbon flow and microbial community functioning in soils. Biochemical processes, such as photosynthesis, tend to favour ^{12}C atoms over heavier ^{13}C atoms. Photosynthesis involves carbon isotope discrimination, resulting in plant tissue being depleted in ^{13}C compared to atmospheric CO_2 (Farquhar et al., 1989). During soil organic matter decomposition, as plant material is microbially processed over time, isotopic fractionation results in residual soil becoming enriched with ^{13}C (Natelhoffer and Fry, 1988). Isotope fractionation makes it possible to observe differences in carbon source acquisition of microorganisms by analyzing the natural abundance of ^{13}C in PLFA biomarkers. Addition of ^{13}C-labelled substrates to soil allows researchers to track carbon flow and microbial uptake of the substrate. Many researchers have followed ^{13}C-labelled substrate incorporation into PLFAs (Boschker et al., 1998; Boschker and Middelburg, 2002; Evershed et al., 2006; Jin and Evans, 2010; Chaudhary and Dick, 2016), and this technique is useful for studying priming effects. Fewer studies have assessed variations in the natural abundance of ^{13}C in PLFAs. Watzinger (2015) recently reviewed isotopic PLFA analysis methods, discussing current understanding in the area and avenues for further research.

1.5.3. Multiple substrate induced respiration (MSIR) analysis

MSIR is a method of determining microbial community function by measuring the response of the microbial community to different carbon substrates (Peham and Bruckner, 2012). Swallow and Quideau (2015) modified the technique of Degens and Harris (1997) for a whole-soil approach. Substrates are added to soil samples and the microbial response, measured as respiration rate, is analyzed. Hannam et al. (2006) used SIR to assess differences in microbial community function with tree canopy species in the boreal forest, but did not separate rhizosphere and bulk soil. MSIR can be used to test preference for specific substrates or gain insight into the overall function of microbial communities.

1.6. Research questions and hypotheses

The focus of this thesis is to investigate potential effects of climate change on the rhizosphere in boreal forest soils of Alberta, Canada. This research focuses on two climate change effects on microorganisms in the rhizosphere: (1) vegetation shifts and (2) changes in root exudation and root depth which can affect priming. Chapter 2 investigates how predicted climate change induced vegetation shifts impact microbial communities in the forest floor, and the implications for soil carbon fluxes. Chapter 3 focuses on quantifying priming in mineral soil horizons via addition of a ^{13}C labeled model root exudate to examine the effects of predicted changes in rooting depth and root exudation.

1.6.1. Overall research questions

In this thesis I work to answer the following questions:

1. How could rhizosphere microorganisms be affected by climate change in Alberta, and what could this mean for boreal carbon fluxes?

2. Can we observe differences in rhizosphere and bulk soil microbial communities of forest floor material from the boreal forest, and if differences are observed, what are the implications?

1.6.2. Chapter 2 study overview and research questions

The overall objective of Chapter 2 was to investigate the effects of climate change-associated shifts in vegetation on carbon stability in boreal forest soils by assessing forest floor microbial communities in the rhizosphere and bulk soil. Research was conducted in boreal forest stands in northern Alberta at the Ecosystem Management Emulating Natural Disturbance (EMEND) research site. The present study characterized microbial community composition and function within four site types (mature stands of spruce and aspen, clear-cut stands of spruce and aspen) and two sample types (rhizosphere and bulk soil) and used natural abundance ^{13}C PLFA analysis to assess carbon source acquisition between rhizosphere and bulk soil microorganisms. Clear-cut stands left to naturally regenerate allow a unique look into forest succession. Classic forest succession in the boreal mixedwood sees early colonization by aspen trees, which are later replaced by white spruce. Clear-cut spruce stands, where aspen encroaches into stands formerly dominated by spruce, were used as a proxy for climate change induced vegetation shifts.

The majority of previous studies looking at ^{13}C concentrations in microbial PLFAs have followed the fate of a ^{13}C-labeled substrate; alternatively, they followed ^{13}C fractionation during anaerobic decomposition to track changes in microbial isotopic ratios (e.g.; Gomez et al., 2014; Ahad and Pakdel, 2013; Watzinger et al., 2008). Our study differs in that we compare ^{13}C natural abundance in individual PLFAs between rhizosphere and bulk soil samples (hence under aerobic conditions), with no substrate addition. PLFAs with a peak intensity between 100 to 300 mV were included in the ^{13}C PLFA analysis but should be interpreted with caution: 14:0i, 14:0, 15:0, 15:1i

ω6c, 16:0i, 16:1 ω5c, 17:0a, 17:0cy ω7c, 18:0, 19:0cy ω7c, and 19:0. Results from PLFAs 15:0i, 15:0a, 16:0, 18:2 ω6c, 18:1 ω9c, and 18:1 ω7c are considered reliable (>300 mV).

I answered two main research questions:

1. *Do microbial communities in the rhizosphere and bulk soil differ, and does the dominant overstory tree species affect the trends observed in rhizosphere and bulk soil?* I hypothesized that the rhizosphere and bulk soil would have different microbial community composition and function in both aspen and spruce stands. I expected that the rhizosphere would have more fungi and gram negative bacteria than bulk soil, and that rhizosphere microbes would utilize root exudates preferentially in community function analysis. Additionally, I expected PLFAs from the rhizosphere to be depleted in ^{13}C and bulk soil PLFAs to be relatively enriched.
2. *How will vegetation shifts affect microbial communities?* I compared mature aspen and spruce stands, and investigated clear-cut aspen and spruce stands where aspen is naturally regenerating. Clear-cut spruce stands were used to investigate the vegetation shift from former spruce stand to current aspen stand. I compared clear-cut spruce stands with clear-cut aspen stands (both of which were dominated by regenerating aspen of the same age), but in one case growing into a forest floor previously formed under spruce, and in the other case formed under aspen. I hypothesized that clear-cut spruce stands would more closely resemble aspen stands than mature spruce stands due to aspen regeneration.

1.6.3. Chapter 3 study overview and research questions

I answered two main research questions:

1. *Does priming differ in boreal forest soil types, comparing three different glucose addition rates, a coarse-textured Brunisol and fine-textured Luvisol, and A and B soil horizons?* For A

horizons we hypothesized that priming will be greatest with the 0.125x MB-C glucose treatment, priming will be positive for the 1x glucose treatment but lower than in the 0.125x treatment, and priming will be negative for the 2x glucose treatment. For the B horizon, increasing exudation rate (glucose amount) will increase priming, stimulating microbes by providing labile C. Priming will be greater in coarse textured soils compared to fine textured soils, because the lower C content of the coarser soil means microbes will be stimulated to a greater degree by the introduction of labile C. Greater priming will occur lower in the soil profile as microbes will be stimulated by introduction of a substrate in areas where they previously did not have access to labile C.

2. *Does microbial utilization of the substrate carbon differ with microbial groups, and do changes in microbial community composition occur?* ^{13}C stable isotope probing of PLFAs was used to assess which microbial groups most effectively utilized the added glucose, and PLFA analysis used to assess microbial community composition. We hypothesized that PLFAs associated with fungi and gram negative bacteria would be most enriched with the ^{13}C label, as these microbial groups are considered responsible for priming (Nottingham et al., 2009). Additionally, microbial communities will shift by the end of the incubation to have a higher proportion of fungi and gram negative bacteria.

1.7. Tables and Figures

Table 1.1. Composition of rhizodeposits. Adapted from: Kuzyakov, 2002.

Component	Description
Exudates	Water soluble; low molecular weight sugars and organic acids
Secretions	High molecular weight substances such as carbohydrates proteins, lipids
Mucilage	Coats roots; high molecular weight polysaccharides and polygalacturonic acids
Lysates	Sloughed off cells from cell autoylsis
Gases	Carbon dioxide and ethylene from roots; not considered rhizodeposits by all researchers

Figure 1.1. Extent of the boreal forest in Canada. Source: Natural Resources Canada, 2016.

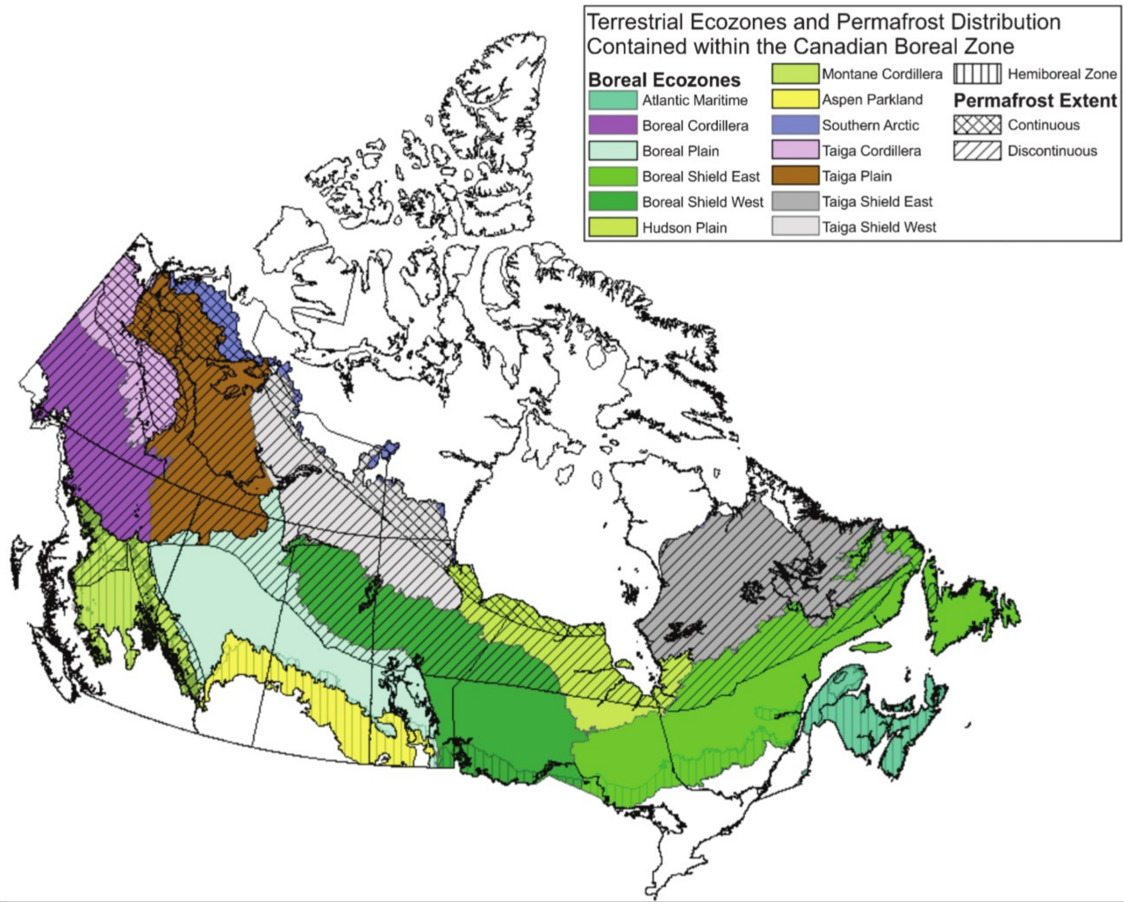

Figure 1.2. Extent of boreal ecozones in Canada. Source: Price et al., 2013.

Chapter 2 – RHIZOSPHERE RESPONSE TO PREDICTED VEGETATION SHIFTS IN BOREAL FOREST SOILS

2.1. Introduction

The boreal forest is the largest terrestrial sink of carbon on Earth (Watson et al., 2000). Global estimates of boreal carbon stocks average 1095 Pg (Bradshaw and Warkentin, 2015), and about a quarter of this carbon is stored in the forest floor alone (Kurz et al., 2013), which is also the site of the highest microbial activity in soil (Foster and Bhatti, 2006; Coleman et al., 2004), making it a critical part of the boreal ecosystem. With climate change, increased temperature and evapotranspiration will increase drought stress, resulting in species maladaptation (Price et al., 2013). Loarie et al. (2009) estimated that the boreal biome is moving northward at a rate of 0.43 km/year, and within the main forest, deciduous trees will replace conifers (Bradshaw et al., 2009). In the mixedwood boreal forest of Western Canada, this will mean a shift from *Picea glauca* (white spruce) to *Populus tremuloides* (trembling aspen) stands. Forest floors from white spruce and trembling aspen stands have distinct properties and microbial communities, and aspen forest floors are composed of higher quality litter with a higher pH and lower carbon storage (Hannam et al., 2004, 2006; Swallow and Quideau, 2013; Laganière et al., 2017). However, the time it will take for these expected vegetation shifts to translate to changes in boreal forest floors remains unknown.

The rhizosphere—soil under the direct influence of plant roots—differs from bulk soil in that it receives root exudates comprised of sugars and organic acids, providing a labile carbon source to rhizosphere microorganisms (Kuzyakov, 2002; Cheng and Gershenson, 2007). Microbes in the rhizosphere can use this labile carbon to degrade more recalcitrant soil organic matter (a process known as priming) which releases CO_2 to the atmosphere (Blagodatskaya and Kuzyakov, 2008; Kuzyakov, 2010). The potential contribution of rhizosphere microbes to atmospheric CO_2 concentration makes studying these communities important if one is to understand boreal forest

carbon dynamics. The rhizosphere has been well studied in agricultural settings, but less so in forests (Maul et al., 2014; Chaudhary et al., 2012; Cui et al., 2015). Previous research in northern Alberta has found differences in microbial communities of aspen and spruce stands (Hannam et al., 2004, 2006; Swallow and Quideau, 2013), but did not investigate differences in rhizosphere and bulk soil.

Two techniques for studying microbial community composition and function are phospholipid fatty acid (PLFA) analysis and multiple substrate induced respiration (MSIR). PLFAs, present in cell membranes, are variable in structure, and this variability can be utilized to assess microbial community composition and identify microbial groups within soil (Frostegård et al. 2010; Frostegård and Bååth, 1996). MSIR measures the response of the microbial community to different carbon substrates (Peham and Bruckner, 2012) to gain insight into the community's functional diversity. These methods can be used to assess differences in microbial communities due to vegetation shifts, and differences between rhizosphere and bulk soil.

Another useful tool for assessing differences in the functioning of soil microbial communities between rhizosphere and bulk soil is the stable carbon isotope (^{13}C) analysis of microbial biomarkers, namely PLFAs. Watzinger (2015) recently reviewed the use and current understanding of isotopic PLFA analysis. There have been numerous studies that followed ^{13}C-labelled substrate incorporation into PLFAs and papers discussing the use of these techniques (e.g.; Boschker et al., 1998; Boschker and Middelburg, 2002; Evershed et al., 2006; Jin and Evans, 2010; Chaudhary and Dick, 2016), but much fewer that utilized variations in the natural abundance of ^{13}C in PLFAs. A related natural abundance study was conducted by Churchland et al. (2007), who found that as distance from trees increased fungal and bacterial PLFAs became more ^{13}C enriched; their study, however, did not involve separation of samples into rhizosphere and bulk soil.

Similarly, Veresoglou et al. (2012) studied the rhizosphere with natural abundance ^{13}C PLFA techniques at a fertilization experiment in a temperate grassland in Greece, but did not compare rhizosphere and bulk soil samples. Other researchers have investigated differences between rhizosphere and bulk soil PLFA carbon sources using ^{13}C labelled substrates or ^{13}C labelled CO_2 (Chaudhary et al., 2012, 2016; Lu et al., 2007; Paterson et al., 2007; Butler et al., 2003), but here we were interested in testing if distinct carbon sources in the rhizosphere and bulk soil would result in measurable variations in the natural abundance of carbon isotopes in individual PLFAs.

The overarching objective of this research was to elucidate the effects of climate change-associated shifts in vegetation on carbon stability in boreal forest soils. Clear-cut spruce stands, where aspen encroaches into stands formerly dominated by spruce, were used as a proxy for climate change induced vegetation shifts in this study. We characterized microbial community composition (PLFA) and function (MSIR) within four site types (mature stands of spruce and aspen, clear-cut stands of spruce and aspen) and two sample types (rhizosphere and bulk soil) and used natural abundance ^{13}C PLFA analysis to assess carbon source acquisition between rhizosphere and bulk soil microorganisms. Research was conducted in boreal forest stands in northern Alberta, focusing on rhizosphere microbial communities.

2.2. Materials and methods

2.2.1. Study area

Soil sampling was conducted in June 2016 at the Ecosystem Management Emulating Natural Disturbance (EMEND) site (56° 46' 13" N, -118° 22' 28" W), approximately 90 kilometres northwest of Peace River, Alberta, Canada (Figure 2.1). EMEND is located in the Clear Hills Upland Ecoregion within the Boreal Plains Ecozone (Wiken, 1986; EcoRegions Working Group, 1989; Kischuck, 2004). The area has mean January and July temperatures of -16.9ºC and 15.0ºC,

respectively, and average annual precipitation of 436.2 mm (Environment Canada, 2016). Elevation at the study area ranges from 677 to 880 m asl (Kishchuk, 2004). Soils are dominantly fine-textured Luvisols (IUSS Working Group WRB, 2015; Soil Classification Working Group, 1998) derived from glaciolacustrine, glacial till, and lacustro-till deposits (Kishchuk, 2004).

EMEND is a long term, fully replicated factorial experimental site unique to western Canada allowing researchers to investigate how harvesting practices affect the boreal ecosystem in terms of soils, vegetation, fauna, and hydrology. EMEND began in 1998 and is expected to remain for an entire stand rotation, which is approximately 80 to 100 years. EMEND covers a 1000 ha area, divided into 10 ha compartments. For our study, we focused on two stand types at EMEND: deciduous dominated (trembling aspen overstory) and coniferous dominated (white spruce overstory). Common understory vegetation at EMEND includes low bush cranberry (*Viburnum edule* (Michx.) Raf.), prickly rose (*Rosa acicularis* Lindl.), Canada buffaloberry (*Sheperdia canadensis* (L.) Nutt.), green alder (*Alnus crispa* (Ait.) Turrill), and river alder (*Alnus tenufolia* Nutt.). Aspen forest floors have been classified as Mormoders, and spruce forest floors as Humimors (Hannam et al., 2006). Clear-cut stands at EMEND were harvested in winter 1998/1999 to minimize disturbance. After harvest no site preparation occurred, allowing clear-cut stands to naturally regenerate. Our study was conducted in clear-cut stands and mature stands (no known disturbance since last fire event).

2.2.2. Sampling sites

Sample collection was performed in compartments with either clear-cut or mature forest under two stand types: deciduous dominated (more than 70% deciduous species) or conifer dominated (greater than 70% white spruce). To achieve spatial heterogeneity, sampling was conducted across the EMEND landscape. There were four site types in total: mature aspen, mature

spruce, clear-cut aspen, and clear-cut spruce. Aspen trees were specifically targeted in mature and clear-cut aspen as well as clear-cut spruce sites, where aspen is naturally regenerating, and white spruce trees were targeted in the mature spruce site type. To target a specific tree, soil sampling was conducted within the critical rooting zone of the desired species and an approximate 15 m distance from a non-target tree species was maintained. Each site type included nine replicates, resulting in a total of 36 sampling sites. A distance of at least 50 m between sampling sites was maintained to ensure spatial independence. No significant difference in elevation or slope was found between sites, making all sites suitable comparisons with one another.

2.2.3. Vegetation survey and soil sampling

The 36 sampling sites were described and sampled in June 2016. At each sampling site a representative tree was selected (aspen or spruce, depending on the site type) as a centre point and four vegetation subsampling areas were marked 5 metres in each cardinal direction, resulting in a 100 m^2 plot. The vegetation subsampling areas were 0.25 m^2. At each subsampling area, a variety of measurements were taken: leaf area index (LAI); dominant tree, shrub and forb species; and ground cover (%) of shrubs, lichen, moss, leaf litter, spruce needles, and coarse woody debris (Royer and Dickinson, 2007). LAI, described as the total surface area of leaves per unit of ground area (Marshall and Waring, 1986), was measured on understory and overstory vegetation using a LI-COR® LAI-2200C plant canopy analyzer. The following measurements were taken within the 100m^2 plots: slope, aspect, and dominant tree diameter at breast height (DBH). Slope percent and degrees at the four plot corners were measured using a Suunto PM-5 clinometer. Diameter at breast height (DBH) was measured on the central tree and those located at the four cardinal directions. Age was estimated from time since fire for the different compartments. Fire events occurring in 1837, 1895, and 1977 have resulted in stands ranging in age from 121 to 179 years (Bergeron,

2012). Mature stands of both aspen and spruce were affected by each fire event, therefore both site types contain trees ranging from 121 to 179 years of age.

Forest floor material was collected in this study, down to a maximum depth of 10 cm. Rhizosphere and bulk soil samples were collected approximately at the centre tree's dripline, as this is considered the tree's critical root zone (UC Berkeley Forest Pathology and Mycology Lab, no date), in each cardinal direction (Appendix 1). The four rhizosphere and bulk soil samples from each sampling site were homogenized to yield one of each sample type from each site. To sample, a 20 cm diameter by 10 cm deep cylindrical core was excavated and forest floor was separated from mineral soil and shaken on a 4 mm sieve. The forest floor material that remained on the sieve was considered rhizosphere, as it consisted of material clinging to roots; forest floor material that fell through the sieve was considered bulk soil. Bulk soil material was collected for analysis at all mature aspen and spruce sites and from three clear-cut aspen sites and four clear-cut spruce sites, while rhizosphere soil was collected at each site (total of 36 rhizosphere and 25 bulk soil samples). Additionally, at each of the four vegetation subsampling locations, a sample for bulk density (BD) and water content was taken by excavating a 10 cm^3 cube of soil, removing mineral soil, and measuring the length of the sides of the cube. Forest floor samples for pH, total organic carbon (TOC), and total nitrogen (TN) were also taken at each of the vegetation subsampling locations and homogenized. Soil temperature was recorded at a depth of 5 cm.

All samples collected in the field were kept on ice until taken back to the in-field laboratory each day where they were thoroughly mixed, subsampled into whirlpack bags for phospholipid fatty acid (PLFA) and multiple substrate induced respiration (MSIR) analyses, and placed in a -20°C freezer. The rest of the samples were allowed to air dry. When back at the University of

Alberta, MSIR samples were placed in a -20ºC freezer while PLFA samples were placed in a -80ºC freezer until further analysis.

2.2.4. General laboratory analysis

Water content and BD were determined by oven drying samples at 65ºC for 48 hours. pH was determined in triplicate on 10 g of air-dried forest floor with a 1:4 ratio of soil to 0.01 *M* $CaCl_2$ (Kalra and Maynard, 1991; Carter and Gregorich, 2006). Samples for TOC, TN, and $\delta^{13}C$ analyses were oven-dried at 65ºC for 48 hours and ground using a Retsch MM200 ball mill grinder. TOC and TN concentrations (%wt) were determined using a Shimadzu TOC-V CHS/CSN Model Total Organic Carbon Analyzer (Shimadzu Corporation, Kyoto, Japan). For $\delta^{13}C$ analysis samples were analyzed on a ThermoFinnigan Delta Advantage Continuous Flow Isotope Ratio Mass Spectrometer (Thermo Finnigan Corp, Bremen, Germany). Results were expressed in δ notation (‰) from the Pee Dee Belemnite (PDB) standard.

2.2.5. PLFA analysis

PLFA analysis was conducted following the Quideau et al. (2016) protocol. All site and sample types were analyzed for PLFA (n=61). In short, forest floor samples were freeze-dried and stored in the dark at room temperature prior to analysis until 0.7 gram of each sample was added to centrifuge tubes. PC(19:0/19:0) nonadecanoate surrogate standard was used to assess PLFA recovery. Samples were extracted with a modified Bligh and Dyer (1959) extractant. Lipid fractionation was performed using solid phase extraction (SPE) silica columns (Agilent Technologies, Wilmington, DE, USA). Lipid methylation was performed with an alkaline methanolysis to form fatty acid methyl esters (FAMEs). Solvent was evaporated under compressed N_2, and samples stored in the freezer until gas chromatograph analysis.

FAMEs were quantified with an Agilent 16 6890N Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE, USA). An internal standard of MeC10:0 (methyl decanoate) was used to assess gas chromatograph performance. FAMEs were prepared by dissolving sample residues in internal standard solution. Sherlock Microbial Identification System Version 6.3 (MIDI, Inc., Newark, DE) was used to identify fatty acid peaks. PLFAs were described using the standard X:YωZ nomenclature. X indicates the number of carbon atoms, Y the number of double bonds, and Z the location of the first double bond from the aliphatic (ω) end of the molecule. Suffixes 'c' and 't' denote cis and trans isomers. Prefixes 'a', 'i' and Me indicate anteiso and iso branching and presence of methyl groups.

Specific PLFAs were designated to microbial groups according to the literature as follows: fungal PLFAs included 18:2ω6c, 18:1ω9c, 20:1ω9c, and 18:3ω6c (Esperschütz et al., 2011; Frostegård et al., 2010; Hamman et al., 2007; Myers et al., 2001); gram negative PLFAs included 17:1ω8c, 18:1ω7c, 16:1ω7c, 16:1ω9c, and 15:1ω6c (Derrien et al., 2014; Esperschütz et al., 2011; Elfstrand et al., 2008; Kramer and Gleixner, 2008; Myers et al., 2001, Frostegård and Bååth, 1996); gram positive PLFAs included 14:0i, 15:0i, 15:0a, 16:0i, 17:0i, 17:0a, 18:0i (Frostegård et al., 2010; Myers et al., 2001), and 10Me PLFAs were considered actinomycetes (Fernandes et al., 2013; Myers et al., 2001; O'Donnell et al., 1982). Protists were 20:4 ω6c (Myers et al., 2001).

The natural abundance $\delta^{13}C$ values of individual PLFAs (‰) were obtained on a GC-IRMS (Thermo Fisher Scientific) with the GC linked to the IRMS through a GC Isolink unit. The $\delta^{13}C$ values of PLFAs were corrected for the carbon atom added during methylation. Isotopic ratios of PLFAs were calibrated against three 20:0 isotope standards—USGS70, USGS71, and USGS72 (Indiana University CRMS) (Schimmelmann et al., 2016)—run at the start and end of the sample set, with the standards run in triplicate each time. The calibration was periodically checked (at the

start of each sample set and approximately every eight samples throughout the analysis) using a mixture of eight FAMEs certified and purchased from Indiana University (F8-3 mixture, Indiana University CRMS).

2.2.5. MSIR analysis

MSIR analysis only included mature sites (n=36). MSIR was performed using the MicroResp™ method with the modified 24-well system (Cameron, 2007; Swallow and Quideau, 2015), which allows for analysis of larger forest floor samples. Frozen samples were placed in the dark at 4ºC two weeks prior to analysis. Soil moisture was adjusted to 40% water holding capacity one week before analysis. One day prior to analysis, samples were transferred to an incubator with soda lime and deionized water to remove CO_2 emitted by the samples. Detection agar was prepared according to the MicroResp™ Technical Manual (Cameron, 2007) and 500 µL were pipetted into each well of a 24-well plate.

Seven substrates, along with deionized water, were used for MSIR analysis. D-glucose, L-threonine, L-lysine, malonic acid, and malic acid were chosen as they are considered root exudates and D-cellobiose and syringic acid because they are not root exudates (Campbell et al., 1997). Following addition of 250 µL of substrate and soil, deepwell plates (in triplicate) were sealed with a silicone gasket and a detection plate placed on top; the system was incubated for 1.5 hours in the dark at 21ºC (Swallow and Quideau, 2015). Change in absorbance of detection agar was measured on a BioTek® Synergy™ HT multi-detection microplate reader with BioTek® Gen5™ microplate data collection and software (BioTek® Instruments, Inc, Vermont, USA). Absorbance was converted to a CO_2 rate using calculations from the MicroResp™ technical manual and calibration and regression curves to correct for 24 instead 96-well plates (Swallow and Quideau, 2015).

2.2.6. Data analysis

Four different site types were considered (clear-cut aspen, clear-cut spruce, mature aspen, mature spruce) and two different sample types (rhizosphere and bulk soil). One-way permutational analysis of variance (permANOVA) followed by Tukey's Honest Significant Difference was used to investigate patterns of environmental variables with site type. Two-way (site type x sample type) permANOVA followed by Tukey's Honest Significant Difference were used to analyze total PLFA (nmol g^{-1}), PLFA microbial groups (mol %), basal respiration (μg CO_2-C g^{-1} forest floor h^{-1}), total respiration (μg CO_2-C g^{-1} forest floor h^{-1}), and catabolic evenness. Significance was defined at α=0.1. All statistical analyses were performed using R Version 3.3.3 (R Core Team, 2017). Total PLFA was calculated as the sum of all fatty acids with carbon chains 14 to 20 carbon atoms in length. Basal respiration was calculated from the CO_2 production rates after water addition. Total respiration was calculated by summing respiration response of all substrates and water for the sample. Catabolic evenness (uniformity of substrate use by the microbial community) was calculated from CO_2 production rates of each substrate divided by the catabolic response of all the substrates. Catabolic evenness describes the range of functions within microbial communities (Degens et al., 2001).

To investigate the response of microbes to individual substrates, respiration response was separated by site type. We used pairwise t-tests followed by manual p-value adjustment with the Holm method to analyze rhizosphere versus bulk soil response within aspen and spruce stands, and overall response of aspen versus spruce stands. P-value adjustment was used to avoid making a Type I error (McCune and Grace, 2002). When comparing rhizosphere versus bulk response, syringic acid was log transformed for aspen stands and for spruce stands syringic acid and L-threonine were square root transformed.

The structural (PLFA) and functional (MSIR) composition patterns of the forest floor microbial communities were investigated by ordinating PLFAs (mol %, Hellinger transformed) and MSIR response to the seven substrates (µg CO_2-C g^{-1} forest floor h^{-1}, with Wisconsin double standardization) with non-metric multidimensional scaling (NMDS) using the MetaMDS function from the vegan package in R (Oksanen et al., 2017). PLFAs with chain length 14 to 20 were included in analysis. In total, 110 PLFAs were identified after data manipulation. Hellinger transformation favours zero-inflated data and gives low weight to rare PLFAs in the NMDS solution thus avoiding grouping sites containing zeros as being more similar in the ordination. Wisconsin double standardization first standardizes substrates by their maximum and then by sample total. The Sorensen (Bray-Curtis) distance was used in both analyses. Correlation vectors were fitted to ordination using the envfit function from the vegan package in R (Oksanen et al., 2017). Ordinations were followed by permutational multivariate analysis of variance (permMANOVA) (Appendix 2) and subsequent pairwise comparisons, with Bonferroni adjustment for multiple inference, using vegan and RVAideMemoire R packages (Oksanen et al., 2017; Hervé, 2017).

Originally, we obtained $\delta^{13}C$ values for 24 PLFAs, but seven were rejected due to low peak intensity (<100 mV) or poorly resolved peaks resulting in unreliable results. While there are nine replicates of each site type, some of the PLFA data do not include all nine replicates due to low peak intensity (Appendix 3). Permutational ANOVA followed by Tukey's Honest Significant Difference was performed to check for significant differences between rhizosphere and bulk soil for each PLFA in mature aspen or spruce stands, and to assess differences in $\delta^{13}C$ values between mature aspen and spruce (α=0.1).

2.3. Results

2.3.1. Vegetation characteristics

Trembling aspen was the dominant overstory species in mature aspen, clear-cut aspen, and clear-cut spruce sites, due to natural regeneration of aspen in both clear-cut site types (Table 2.1). The aspen and spruce clear-cut sites were similar in term of ground cover, shrub and total live cover. Nine dominant understory vegetation species were described in both clear-cut aspen and spruce stands, and these stands shared six out of the nine species: fireweed (*Chamerion angustifolium* (L.) Holub), prickly rose (*Rosa acicularis* Lindl.), lowbush cranberry (*Viburnum edule* (Michx.) Raf.), palmate-leaved coltsfoot (*Petasites palmatus* (Ait.) Cronq.), twinflower (*Linnaea borealis* L.), and grasses (Poaceae spp.). Mature aspen stands did not differ from clear-cut aspen or clear-cut spruce in terms of moss, spruce needle, leaf litter, and lichen ground cover, or shrub cover. Mature aspen stands had significantly higher total live ground cover than clear-cut aspen and clear-cut spruce stands ($p<0.001$, $p=0.01$, respectively).

Mature spruce stands, the only site type with a conifer-dominated canopy, were markedly different in terms of vegetation characteristics compared to both clear-cut spruce and mature aspen sites. Mature spruce stands had the highest total live cover (85%), moss ground cover (64%), and spruce needle cover (6%). Mature spruce had significantly higher moss ground cover ($p<0.001$), spruce needle ground cover ($p<0.001$), total live cover ($p<0.001$) than clear-cut spruce. Clear-cut spruce stands had significantly higher leaf litter cover ($p<0.001$) and shrub cover ($p=0.10$) than mature spruce.

When comparing mature spruce and aspen stands, spruce stands exhibited significantly higher moss ground cover ($p<0.001$), spruce needle ground cover ($p<0.001$), and total live ground cover ($p=0.03$). Mature aspen stands had significantly higher leaf litter cover ($p<0.001$) and shrub cover ($p=0.06$). Mature spruce and aspen stands did not differ in lichen ground cover. Across all

site types, no significant differences were found for site LAI, coarse woody debris ground cover, or lichen cover.

2.3.2. Forest floor soil characteristics

Mature spruce sites, in addition to having different vegetation characteristics from the other three site types, had different forest floor physical and chemical properties (Table 2.2). Mature spruce forest floors were thicker ($p=0.08$) than mature aspen forest floors, with lower bulk density ($p=0.01$) and pH ($p=0.004$), and a higher C:N ratio ($p<0.001$). The pH of clear-cut spruce stands differed from both mature and clear-cut aspen ($p=0.03$, $p=0.008$, respectively) but did not differ from mature spruce sites ($p=0.80$). The C:N ratio of clear-cut spruce sites was significantly higher than at clear-cut aspen sites ($p=0.04$). Natural abundance $\delta^{13}C$ values were significantly less negative in mature spruce sites compared to both aspen-dominated site types ($p<0.001$). Clear-cut spruce site $\delta^{13}C$ values were also significantly less negative than the mature and clear-cut aspen sites ($p=0.002$, $p<0.001$, respectively). No significant differences were found for total carbon, ^{15}N, or moisture content.

2.3.3. Microbial community composition

In total, 110 PLFAs were included in the calculation of total PLFAs, with averages ranging from 6751 nmol g^{-1} in clear-cut aspen forest floors to 11873 nmol g^{-1} in mature aspen forest floors (Table 2.3). Total PLFAs did not differ significantly between rhizosphere and bulk soil samples ($p=0.64$) or among site types. There was a large amount of variability in total PLFAs for the mature stands (Appendix 4); however, site types clustered together well in the ordination (Figure 2.3).

Microbial community composition data were visualized with an ordination. Non-metric multidimensional scaling analysis of mol % PLFA data produced a 3-dimensional solution with a final stress of 8.8% after 20 iterations (Figure 2.3). Axis one and two are shown in the NMDS as

they explained the greatest variability in the data. A significant difference in microbial community composition was found for rhizosphere and bulk soil ($p=0.001$) with all site types combined. When site types were analyzed separately, it became evident that rhizosphere and bulk soil differences were driven by the mature aspen site type, which had the smallest p-value ($p=0.004$), but all site types showed significant differences between rhizosphere and bulk soil ($p=0.05$, $p=0.08$, $p=0.08$ for mature spruce, clear-cut aspen, and clear-cut spruce, respectively).

Figure 2.2 indicated visually that microbial communities of the clear-cut spruce rhizosphere were more closely clustered with mature and clear-cut aspen stands than the clear-cut spruce bulk soil. Pairwise permutational MANOVA revealed that while the clear-cut spruce rhizosphere remained significantly different from the aspen stands, indeed the difference was more prominent in the bulk soil ($p=0.02$ for clear-cut spruce rhizosphere versus aspen stands; $p=0.003$ for clear-cut spruce bulk versus aspen stands).

Overall, when combining rhizosphere and bulk soil data, mature spruce stands were significantly different in terms of PLFA community from the other three site types ($p=0.006$), all of which currently have an aspen canopy. Mature aspen differed from clear-cut aspen ($p=0.07$) and clear-cut spruce ($p=0.006$), but the strength of difference was greater between mature aspen and clear-cut spruce. The two clear-cut stands differed ($p=0.06$), but not as strongly as compared to mature sites. Mature spruce stands had the most variable microbial community of the four site types (Figure 2.2). Environmental variable correlation vectors suggest that separation in microbial community composition was driven by greater pH ($R^2=0.28$, $p=0.001$) and leaf litter ($R^2=0.80$, $p=0.001$) in mature and clear-cut aspen sites and clear-cut spruce sites, as well as higher C:N ratio ($R^2=0.52$, $p=0.001$), $\delta^{13}C$ values ($R^2=0.71$, $p=0.001$), moss ($R^2=0.62$, $p=0.001$) and needle ($R^2=0.52$, $p=0.001$) cover in mature spruce sites (Figure 2.2).

In terms of microbial groups, the rhizosphere was found to have significantly more fungi compared to bulk soil ($p<0.001$; Table 2.4). Bulk soil had significantly more gram positive bacteria ($p<0.001$) and actinomycetes ($p=0.001$) than the rhizosphere. The gram negative:gram positive bacteria ratio was higher in rhizosphere compared to bulk soil ($p<0.001$), however this did not hold true in mature spruce stands (Table 2.4). No significant difference was found among site types for gram negative bacteria or protists when comparing rhizosphere and bulk samples.

Within the rhizosphere, mature spruce stands had significantly lower amounts of gram negative bacteria than the clear-cut sites ($p=0.05$ for clear-cut aspen, $p=0.1$ for clear-cut spruce), but no difference was found between the two mature sites. A significant difference was found for gram positive bacteria between mature spruce and the other three site types ($p=0.03$ for mature aspen, $p=0.07$ for clear-cut aspen, and $p=0.04$ for clear-cut spruce, respectively), with spruce having a higher proportion of gram positive bacteria. No differences were observed for fungi, actinomycetes, or protists. Within bulk soil, mature aspen had significantly more actinomycetes than mature and clear-cut spruce ($p=0.09$, $p=0.08$, respectively). No difference in fungi, gram negative or gram positive bacteria, or protists were found across site types within bulk soil.

2.3.4. Microbial community function

MSIR was only used to characterize microbial community function (respiration response across all substrates) of mature stands. Non-metric multidimensional scaling analysis of MSIR data ($\mu g\ CO_2\text{-}C\ g^{-1}$ forest floor h^{-1}) produced a 2-dimensional solution with a final stress of 13.4% (Figure 2.3) after 20 iterations. Microbial community function of aspen and spruce stands differed from one another ($p=0.004$) using permutational MANOVA. The difference between stand types was driven by the rhizosphere because, when analyzed separately, the rhizosphere produced a difference between stand types but the bulk soil did not. Similarly to the microbial community

composition results (Figure 2.2), mature spruce stands were more variable in function than aspen stands (Figure 2.3). Function of rhizosphere microbial communities differed from bulk soil communities in aspen stands only ($p=0.043$).

We further investigated the response of microbial communities to individual substrates. Because we found a strong difference between mature aspen and spruce stands, we chose to separate the data by site type. Response of microbes to individual substrates are shown in Figure 2.4. Respiration was significantly higher in aspen stands compared to spruce stands for the following substrates (when combining rhizosphere and bulk soil data): glucose, malic acid, malonic acid, and syringic acid ($p=0.05$, $p=0.01$, $p=0.008$, $p=0.13$, respectively). Within the aspen and spruce stands we checked for differences between rhizosphere and bulk soil for each substrate. We found weakly significant differences within aspen stands for malonic acid, syringic acid, and L-lysine ($p=0.13$, $p=0.14$, $p=0.13$, respectively). No significant differences within spruce stands were found.

The rhizosphere of mature aspen stands had significantly higher basal respiration than both spruce rhizosphere and bulk soil ($p=0.001$, $p=0.02$, respectively; Table 2.3). Basal respiration from the rhizosphere of mature aspen stands was also significantly higher than that of bulk soil in aspen stands ($p=0.018$), but this trend was not seen in the spruce stands. Basal respiration in the bulk soil of aspen and spruce stands did not differ. Total respiration was significantly higher in the mature aspen rhizosphere compared to the aspen bulk soil and both mature spruce sample types ($p=0.08$, $p=0.02$, $p=0.02$, respectively; Table 2.3).

The spruce rhizosphere had a significantly lower catabolic evenness than both the aspen rhizosphere and bulk soil ($p<0.001$; Table 2.3). The spruce rhizosphere also had a significantly lower catabolic evenness than the spruce bulk soil ($p=0.002$), while there was no measurable

difference between the bulk and rhizosphere samples in aspen stands. Bulk soil in aspen and spruce stands did not differ.

2.3.5. Carbon isotopic composition ($\delta^{13}C$) of PLFAs

In general, PLFAs from aspen stands had more negative $\delta^{13}C$ values than spruce stands (Figure 2.5), with average values ranging from -35.58‰ to -25.87‰ for aspen and -31.95‰ to -24.9‰ for spruce. Significant differences between aspen and spruce were observed for these $\delta^{13}C$ values, with the exception of 19:0 and 15:1i ω6c. Figure 2.5 also shows a trend of rhizosphere PLFA $\delta^{13}C$ values being more negative than their bulk soil counterparts, by 0.36‰ on average. Significant differences between rhizosphere and bulk soil PLFA $\delta^{13}C$ values were found for four PLFAs in mature aspen stands, and six in mature spruce stands (Table 2.5). Aspen and spruce stands shared three PLFAs that exhibited significant differences between rhizosphere and bulk soil $\delta^{13}C$ values: 18:1ω7c, 18:1ω9c, and 18:2ω6c. Of the significant PLFAs, one was associated with gram negative bacteria, one with gram positive bacteria, and three with fungi (Table 2.5).

2.4. Discussion

2.4.1. Rhizosphere versus bulk forest floor

Even in the forest floor where carbon availability and microbial biomass are high, clear differences were observed between rhizosphere and bulk soil in terms of both microbial community composition and function. In mature stands, PLFA communities and MSIR profiles both differed between rhizosphere and bulk soil. Differences were also observed between rhizosphere and bulk soil PLFA communities within each clear-cut stand. Microbial biomass (total PLFAs) did not differ between rhizosphere and bulk soil, which is contrary to other research findings (e.g.; Bodelier et al., 1997; Foster, 1998; Guo et al., 2015). However, the studies noted here were not conducted in forest floor material. Surface soil layers such as the forest floors that

were analyzed in our study have a high microbial activity (Lavahun et all., 1996; Karhu et al., 2016), which could have resulted in similar total PLFA amounts that we observed for bulk and rhizosphere samples.

Three MSIR substrates showed a weakly significant difference between rhizosphere and bulk soil respiration response in aspen stands (malonic acid, L-lysine, and syringic acid) but not spruce stands. These results differed from our hypothesis that rhizosphere microbes would prefer substrates that were known root exudates. Malonic acid and L-lysine are root exudates that represent carboxylic acids and amino acids, respectively. Syringic acid is a by-product of lignin (Bergbauer 1991) and is an aromatic compound. In the aspen stands, it is possible that rhizosphere microbes preferred syringic acid more than bulk soil microbes because it is a compound they did not previously have access to. This would be an example of the priming effect *versus* home field advantage. Under priming, I would predict that the addition of a labile carbon source stimulates organic matter decomposition, or increases microbial activity in the case of apparent priming (Blagodatskaya and Kuzyakov, 2008). The home field advantage is the concept that microbes will prefer substrates they are accustomed to over new, unfamiliar substrates (Gholz et al., 2000).

When focusing on specific microbial groups, rhizosphere samples had more fungi and a higher gram negative:gram positive bacteria ratio than bulk soil, while bulk soil had significantly more gram positive bacteria. Numerous ^{13}C labelling studies using ^{13}C-enriched CO_2 have found that fungi and gram negative bacteria PLFAs become more enriched with the label than gram positive bacteria, indicating their activity in the rhizosphere (Nottingham et al., 2009; Paterson et al., 2007; Treonis et al. 2004; Butler et al. 2003). Lu et al. (2007) separated rhizosphere and bulk soil and found that gram negative bacteria and fungi were actively utilizing root derived carbon, whereas gram positive bacteria were more active in bulk soil.

The $\delta^{13}C$ values of individual PLFAs revealed that rhizosphere PLFAs were generally depleted compared to bulk soil PLFAs, further indicating a difference in rhizosphere and bulk soil microbial communities. The $\delta^{13}C$ depletion of rhizosphere PLFAs indicates use of more labile plant material (rhizodeposits) from rhizosphere microbes, while bulk soil microbes are likely using the more $\delta^{13}C$ enriched, microbially processed carbon in forest floor material (Kohl et al., 2018; Flanagan et al., 1999; Natelhoffer and Fry, 1988). Some of the PLFAs showing significant ^{13}C depletion in the rhizosphere compared to bulk soil were fungi and gram negative bacteria, microorganisms thought to be responsible for priming (Nottingham et al., 2009). In ^{13}C enrichment studies, microbes actively using rhizodeposits—fungi and gram negative bacteria—were found to become readily enriched with the label (Nottingham et al., 2009; Lu et al., 2007; Paterson et al., 2007; Treonis et al. 2004; Butler et al. 2003). In the absence of a ^{13}C label, microbes utilizing rhizodeposits as a carbon source should be ^{13}C depleted compared to bulk soil, as microbial processing and decomposition result in ^{13}C enrichment in soil organic matter (Flanagan, Kubien, and Ehleringer, 1999; Kohl et al., 2018). We observed similar findings in our study, although differences in the $\delta^{13}C$ values between rhizosphere and bulk soil PLFAs were small (on average 0.36‰).

The difference between rhizosphere and bulk soil microbial communities has been well documented by other researchers in agricultural and agroforestry settings (Maul et al., 2014; Guo et al, 2015; Chaudhary et al., 2012; Cui et al., 2015) but less so in native forest soils (Fonseca et al., 2018). Our study confirms that in forest floor material, which has high microbial activity and dense root systems, rhizosphere and bulk soil microbial communities differed from one another. This research is the first to our knowledge to demonstrate different carbon source utilization

between rhizosphere and bulk soil, and the dependence of fungi and gram negative bacteria on rhizodeposits, using the natural abundance ^{13}C PLFA technique.

2.4.2. Legacy effect of spruce after aspen regeneration

Trembling aspen was the main overstory tree species in mature aspen, clear-cut aspen, and clear-cut spruce stands. The similarity of clear-cut spruce sites to both aspen sites in terms of their vegetation characteristics suggests that clear-cutting resulted in a shift back to an earlier successional stage. MacDonald and Fenniak (2007) found aspen to be an indicator of clear-cutting in both aspen and spruce forests. Clear-cut spruce stands have likely followed the classic successional trajectory from deciduous to coniferous vegetation in the past, leaving behind aspen seed and bud banks from which aspen could regenerate after the 1998-1999 harvest. Aspen seed is wind dispersed which could be another mechanism of aspen regeneration on clear-cut spruce sites. Climate change induced vegetation shifts may come about via conifer dieback due to maladaptation (Price et al. 2013), not necessarily fire. If an aspen seed or bud bank persists in the soil or there is a source for wind-dispersed seed nearby, it is likely that aspen regeneration will occur on former spruce sites.

Differences in vegetation characteristics went hand-in-hand with different forest floor characteristics. Sites with an aspen overstory showed different forest floor characteristics than sites with a white spruce overstory (Table 2.2). Mature spruce forest floors had significantly lower bulk density than the other three site types, which is consistent with other research at EMEND (Hannam et al., 2004, 2006). A thicker forest floor and a low bulk density were consistent given observation that the spruce forest floor was composed largely of decaying moss. C:N ratio was highest in mature spruce stands, followed by clear-cut spruce, mature aspen, and clear-cut aspen (Table 2.2). Four years after harvest, Hannam et al. (2006) observed the same trend. The bulk $\delta^{13}C$ values of

mature and clear-cut aspen stands were statistically more negative than the spruce stands, with clear-cut spruce $\delta^{13}C$ values between the aspen stands and mature spruce (Table 2.2). While clear-cut spruce stands resembled aspen stands in terms of vegetation and most soil characteristics, the pH of the clear-cut spruce forest floor remained similarly low to the mature spruce forest floor. Seventeen years after harvest there is still a legacy effect of spruce in the clear-cut spruce forest floor. Catabolic evenness was lowest in the mature spruce rhizosphere. Soils with lower catabolic evenness may be more resistant to disturbance (Degens et al., 2001), which could explain why 17 years after harvest clear-cut spruce stands are still showing a legacy effect of spruce in terms of microbial community composition.

2.4.3. Vegetation shifts and microbial communities

Vegetation shifts with climate change have the potential to alter microbial communities. Mature aspen and spruce stands exhibited clear differences in microbial community composition and function. Other research has confirmed that soil microbial communities differ under aspen and white spruce canopies (Hannam et al., 2004, 2006; Swallow and Quideau, 2013). Mature spruce stands displayed the greatest variability in microbial community composition, which has been observed by other researchers (Hannam et al., 2006; Norris et al., 2013). Variability in spruce stands may be driven by moss cover, which is comprised of numerous different bryophytes providing different materials and microhabitats.

In terms of MSIR analysis, it is difficult to know which of the root exudates used in this study would be found in aspen and spruce rhizospheres, because much of the data on root exudate compounds comes from studies of agricultural systems (Campbell et al., 1997; Ma et al., 2001; Walker et al., 2003; Carvalhais et al., 2011). Therefore, while a substrate may be a root exudate, it is not necessarily a root exudate from aspen or spruce trees. Studies of exudate carbon from aspen

trees have been performed (Karst et al., 2017), but individual exudate compounds were not identified. Additionally, exudation is affected by multiple factors such as: plant species, plant age, temperature, light, plant nutrition, microbes, medium that roots are grown in, soil moisture, and root damage (Rovira, 1964). Tuason and Arocena (2009) found malonic and oxalic acids to predominate in white spruce root exudates, confirming that malonic acid is an appropriate MSIR substrate. Regardless, the variety of substrates used in this study effectively demonstrated differences between aspen and spruce microbial community function.

Focusing on PLFA analysis, mature spruce stands harboured a distinct microbial community, likely because this was the only site type with a dominant conifer canopy. Figure 2.3 shows that needles and moss were associated with spruce canopies, while leaf litter was associated with aspen canopies. The different origin of the forest floor material, combined with possible species-specific root exudation (Lesuffleur et al., 2007; Sandnes et al., 2005), is likely driving the difference in community composition. The $\delta^{13}C$ value of bulk forest floor material was -27.8 ‰ for undisturbed aspen and -26.6 ‰ for undisturbed spruce (Table 2.2). It follows that $\delta^{13}C$ values of PLFAs were more depleted in aspen than spruce stands. Our results are consistent with Hannam et al. (2005) who also found that un-cut aspen stands at EMEND were more depleted in ^{13}C than un-cut spruce stands. Differences in $\delta^{13}C$ values between PLFAs from aspen and spruce stands are likely due to the origin of the material (leaves versus needles and moss). Additionally, lignin from conifers is dominated by guiacyl monomers while lignin of deciduous trees is dominated by syringal monomers (Wagner et al., 2015). Goñi and Eglinton (1996) found that syringic phenols were ^{13}C depleted compared to other lignin phenols.

Aspen regeneration in former spruce stands resulted in microbial communities more comparable to mature and young aspen stands than mature spruce stands in less than two decades,

with the rhizosphere responding more quickly to vegetation shifts than the bulk soil. While mature spruce stands had a distinct microbial community, all site types were found to be significantly different from one another. The clear-cut spruce PLFA profile differed from the mature aspen and spruce stands. However, Figure 2.3 shows that the rhizosphere of clear-cut spruce samples was more closely grouped with the aspen stands than the mature spruce. Clear-cut spruce bulk soil communities separated out between the aspen stands and mature spruce stands in the NMDS (Figure 2.3), indicating a shift away from mature spruce even in the bulk soil. While both clear-cut spruce rhizosphere and bulk soil differed from the aspen stands, the difference was smaller in the rhizosphere. Aspen regeneration altered both rhizosphere and bulk soil microbial communities away from mature spruce towards existing aspen communities, with vegetation shifts more quickly altering rhizosphere communities.

There was an effect of tree age, as community composition in clear-cut and mature aspen stands differed. However, age of aspen trees affected the microbial community less so than whether the canopy was aspen versus spruce. Clear-cut spruce more closely resemble clear-cut aspen than mature aspen or mature spruce. Using DNA sequencing techniques, Zhang et al. (2016) found that 35 years after clear-cutting, bacterial communities of the disturbed, secondary forest remained distinct from the mature, primary forest. However, the case at EMEND is likely different because Zhang et al. (2016) found that dominant species in the secondary forest were not the same as in the primary forest. While aspen regeneration has not yet altered clear-cut spruce communities to be indistinguishable from aspen stands, likely due to the remaining legacy effect spruce in these stands, it could be that with more time these communities will converge.

2.4.4. Tree species influence over the rhizosphere

Aspen trees exhibited greater influence over their rhizosphere microbial communities than spruce trees. This trend can be observed in both the MSIR and PLFA data. Microbial community function differed between rhizosphere and bulk soil with mature aspen and spruce data combined; when the two site types were separated, there was only a distinction between rhizosphere and bulk soil in aspen stands. The mature aspen rhizosphere had significantly higher basal respiration than the aspen bulk soil and both spruce soils (Table 2.3). Our basal respiration results are in contrast with Hannam et al. (2006) who found that spruce stands had higher basal respiration than aspen stands. Our results indicate that, with a shift from spruce to aspen vegetation, the rhizosphere could be an important source of carbon release to the atmosphere, more so than bulk soil.

When comparing rhizosphere and bulk soil microbial communities, the strongest difference was found in mature aspen stands. Mature aspen may exert more control over the rhizosphere than younger trees, as plant development stage affects rhizodeposition (Gransee and Wittenmayer, 2000). Additionally, while the gram negative:gram positive bacteria ratio was higher in the rhizosphere compared to bulk soil with all site types combined, when separated we found no difference in mature spruce stands (the only site type with a spruce canopy). Aspen trees could be exerting greater influence over their rhizosphere due to the mycorrhizal associations they support. Aspen roots can host both ectomycorrhizae (EM) and arbuscular mycorrhizae (AM), while white spruce hosts only EM fungi (Malloch et al., 1980; Malloch and Malloch, 1981; Godbout and Fortin, 1985; Cripps and Miller, 1993). Ectomycorrhizal roots release more rhizodeposits than arbuscular mycorrhizae (Smith, 1976; Phillips and Fahey, 2005; Yin et al., 2014). Even though AM are less leaky, both can contribute substantial labile C to soil (Phillips and Fahey, 2005). Possibly, the combination of EM and AM causes greater exudation from aspen, resulting in aspen's greater influence over the rhizosphere. Understory vegetation could also play

a role. While tree roots were targeted during sampling, it was not always possible to separate tree roots from shrub or herbaceous plant roots. While mature spruce stands had the highest total live ground cover, it was dominated by moss, which can host AM fungi; mature aspen stands had greater shrub cover, which can host EM fungi. Perhaps the understory vegetation was colonized by EM fungi which resulted in greater rhizodeposition, allowing greater influence over the rhizosphere microbes which rely on plant roots for carbon.

2.5. Conclusion

Even in forest floor materials where microbial biomass is typically high, there were clear differences between rhizosphere and bulk soil, with aspen trees exerting more influence over their rhizosphere than spruce. Higher proportion of fungi and a higher gram negative:gram positive bacteria ratio in the rhizosphere compared to bulk soil, combined with higher basal respiration in the aspen rhizosphere, indicated both the importance of rhizosphere soil and of a shift to aspen vegetation for boreal carbon fluxes. Basal respiration data suggested that the mature aspen rhizosphere presents the potential to release CO_2 to the atmosphere, which would be a negative consequence of climate change caused by vegetation shifts. In less than two decades, vegetation shifts from white spruce to trembling aspen changed forest floor microbial communities to more closely resemble those under aspen vegetation, with changes occurring more quickly in the rhizosphere. Higher basal respiration of the rhizosphere and a faster community composition response to vegetation shifts highlight the importance of considering the rhizosphere in climate change studies. A legacy effect of spruce persists in stands where aspen has regenerated, and future research should investigate how long this effect remains. Future studies should also investigate the rhizosphere within the mineral component of boreal soils.

2.6. Tables and Figures

Table 2.1. Main site and vegetation characteristics from mature and clear-cut spruce and aspen sites. Values are means (n=9) with standard deviation in parentheses. Lowercase letters (p values < 0.1; Tukey's test) are for interpretation. Further information on EMEND compartments can be found on The EMEND Project website (http://www.emendproject.org).

Site type	EMEND compartment	Stand age (yr)	Dominant tree species	Dominant tree DBH (cm)	Dominant understory species	Site LAI (m^{-2} m^{-2})	Leaf litter ground cover (%)	Spruce needle ground cover (%)	Moss ground cover (%)	Lichen ground cover (%)	Shrub ground cover (%)	Total live ground cover (%)	CWD ground cover(%)
Mature spruce	D:889, G:915, G:918	121, 139, 179	White spruce	29.7a (6.3)	*Cornus canadensis* *Poaceae spp.* *Equisetum pratense* *Chamerion angustifolium* *Rosa acicularis* *Hylocomium splendens* *Ptilium crista-castrensis*	11.6a (0.4)	12.6b (9.6)	6.1a (3.4)	63.9a (28.3)	1.7a (3.5)	6.8b (7.4)	85.1a (16.7)	1.9a (3.7)
Clear-cut spruce	C:892	17	Trembling aspen	7.0b (0.9)	*Cornus canadensis* *Chamerion angustifolium* *Rosa acicularis* *Viburnum edule* *Shepherida canadensis* *Petasites palmatus* *Poaceae spp.* *Linnaea borealis* *Rubus ideaus*	10.4a (0.7)	98.3a (5.0)	0.0b (0.0)	0.1b (0.3)	0.0a (0.0)	16.4a (7.8)	44.4c (12.2)	1.1a (2.1)
Mature aspen	A:852, B:862, I:940	121, 139, 179	Trembling aspen	27.0a (4.8)	*Cornus canadensis* *Chamerion angustifolium* *Rosa acicularis* *Viburnum edule* *Poaceae spp.* *Rubus ideaus* *Fragaria virginiana* *Petasites palmatus* *Lathyrus ochroleucus*	11.8a (3.5)	92.1a (16.2)	0.0b (0.0)	2.5b (3.0)	0.0a (0.0)	17.6a (9.8)	66.1b (11.5)	2.9a (3.1)
Clear-cut aspen	A:850, B:864, I:941	17	Trembling aspen	6.8b (1.0)	*Chamerion angustifolium* *Rosa acicularis* *Viburnum edule* *Poaceae spp.* *Mertensia paniculata* *Linnaea borealis* *Petasites palmatus* *Actaea rubra* *Pyrola spp.*	8.8a (4.4)	99.6a (1.3)	0.0b (0.0)	0.3b (0.8)	0.0a (0.0)	14.0ab (9.3)	32.6c (14.0)	0.9a (1.2)

Table 2.2. Main forest floor characteristics from mature and clear-cut spruce and aspen sites. Values are means (n=9) with standard deviation in parentheses. Lowercase letters (p values < 0.1; Tukey's test) are for interpretation.

Forest floor	Bulk density (g cm^{-3})	Forest floor thickness (cm)	pH	Total carbon (mg g^{-1})	Total nitrogen (mg g^{-1})	C:N ratio	$\delta^{13}C$ (‰)
Mature spruce	0.061b (0.01)	9.0a (1.0)	5.1b (0.4)	413.9a (20.5)	14.2c (1.6)	29.5a (4.1)	-26.6a (0.6)
Clear-cut spruce	0.077a (0.01)	9.0a (1.1)	5.3b (0.3)	397.8a (24.9)	17.2b (0.8)	23.2b (1.5)	-27.1b (0.4)
Mature aspen	0.082a (0.02)	7.3b (1.5)	5.7a (0.4)	391.9a (20.4)	18.3ab (0.9)	21.5bc (1.7)	-27.8c (0.3)
Clear-cut aspen	0.077a (0.01)	8.0ab (2.0)	5.7a (0.3)	391.5a (21.9)	19.7a (1.8)	19.9c (1.3)	-28.1c (0.2)

Table 2.3. Main forest floor characteristics for bulk soil and rhizosphere samples from spruce and aspen clear-cuts and uncut controls (mature). Values are means (n=9 for rhizosphere samples, n=3 for aspen clear-cut bulk soil, n=4 for spruce clear-cut bulk soil) with standard deviation in parentheses. Lowercase letters (p values < 0.1; Tukey's test) are for interpretation.

Forest floor soil	Sample type	Total PLFA (nmol g^{-1})	Total respiration (µg CO2-C g^{-1} hr^{-1})	Basal respiration (µg CO_2-C g^{-1} hr^{-1})	Catabolic evenness (E)
Mature spruce	Bulk	7808.0a (5325.6)	11549.7b (5780.6)	476.4b (380.4)	6.130a (0.385)
	Rhizosphere	8869.0a (4670.8)	11408.5b (4257.3)	271.1b (308.9)	4.968b (1.106)
Clear-cut spruce	Bulk	6618.3a (430.4)	-	-	-
	Rhizosphere	8057.7a (989.5)	-	-	-
Mature aspen	Bulk	11873.1a (9703.7)	13053.7b (3421.9)	440.0b (306.8)	6.205a (0.260)
	Rhizosphere	11047.5a (5880.7)	19248.0a (6743.9)	1152.8a (634.1)	6.376a (0.393)
Clear-cut aspen	Bulk	6751.2a (1103.7)	-	-	-
	Rhizosphere	7517.7a (1599.0)	-	-	-

Table 2.4. Proportion of PLFA microbial groups (mol %) in the different forest floor site types and sample types. Values are means (n=9 for rhizosphere samples, n=3 for aspen clear-cut bulk soil, n=4 for spruce clear-cut bulk soil) with standard deviation in parentheses. Different capital letters indicate significant differences among forest floor site type within either rhizosphere or bulk soil of PLFA mol % data (p values < 0.1; Tukey's test). Different lowercase letters indicate significant differences between rhizosphere and bulk soil of PLFA mol % data (p values < 0.1; Tukey's test).

Forest floors	Sample type	Fungi (mol%)	Gram negative bacteria (mol%)	Gram positive bacteria (mol%)	Actinomycetes (mol%)	Gram negative:gram positive ratio
Mature spruce	Bulk	18.8 (2.0)Ab	19.1 (1.8)Aa	15.0 (1.1)Aa	4.8 (0.5)Ba	1.3 (0.1)Aa
	Rhizosphere	23.2 (2.0)Aa	18.5 (1.5)Ba	14.0 (1.3)Ab	4.2 (0.6)Ab	1.3 (0.1)Ba
Clear-cut spruce	Bulk	20.2 (1.9)Ab	20.4 (1.2)Aa	14.3 (1.1)Aa	4.6 (0.3)Ba	1.4 (0.1)Ab
	Rhizosphere	24.0 (3.4)Aa	20.1 (1.4)Aa	12.5 (0.8)Bb	4.0 (0.5)Ab	1.6 (0.2)Aa
Mature aspen	Bulk	19.2 (1.1)Ab	20.1 (1.2)Aa	14.7 (0.7)Aa	5.3 (0.4)Aa	1.4 (0.1)Ab
	Rhizosphere	24.9 (1.4)Aa	19.6 (1.1)Ba	12.4 (1.2)Bb	4.4 (0.4)Ab	1.6 (0.2)Aa
Clear-cut aspen	Bulk	19.6 (0.6)Ab	20.6 (1.3)Aa	14.4 (0.4)Aa	5.0 (0.4)ABa	1.4 (0.1)Ab
	Rhizosphere	24.5 (3.3)Aa	20.3 (1.6)Aa	12.6 (1.1)Bb	4.5 (0.7)Ab	1.6 (0.2)Aa

Table 2.5. Results of a permutational ANOVA to test for differences between rhizosphere and bulk soil δ13C (‰) values for individual PLFAs.

PLFA	Site type	Microbial group	p-value
18:1 ω7c	Aspen Spruce	Gram negative bacteria	0.07 0.09
18:1 ω9c	Aspen Spruce	Fungi	0.04 0.14
18:2 ω6c	Aspen Spruce	Fungi	0.13 0.04
16:1 ω5c	Aspen	Arbuscular fungi/gram negative bacteria	0.08
16:0	Spruce	-	0.006
17:0a	Spruce	Gram positive bacteria	0.003
18:0	Spruce	-	0.0001

Figure 2.1. Boreal forest of Canada. Research site indicated by red star. Adapted from: Natural Resources Canada, 2016; Natural Regions Committee, 2006.

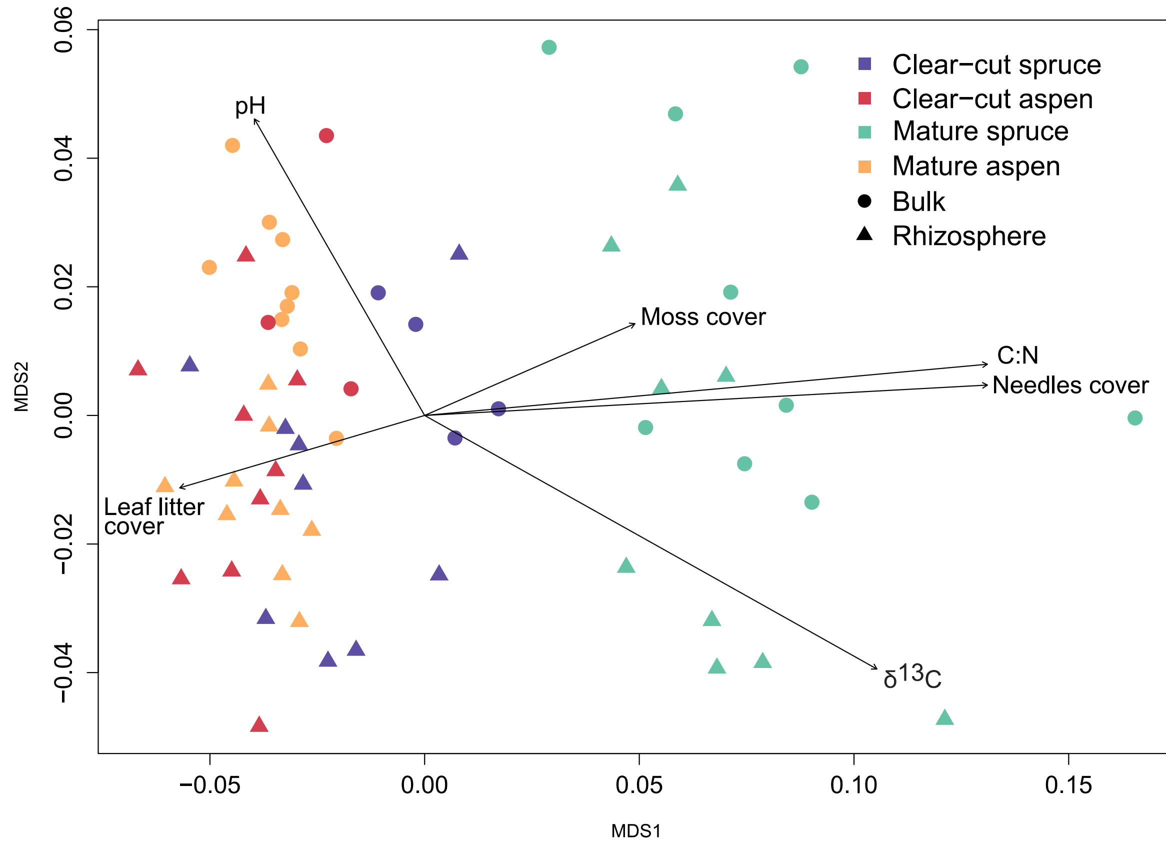

Figure 2.2. MetaMDS ordination of PLFAs (Hellinger transformed, mol %) for mature and clear-cut spruce stands and mature and clear-cut aspen stands, with rhizosphere and bulk soil samples indicated by different shapes. Vectors indicate significant environmental variables (p values < 0.05).

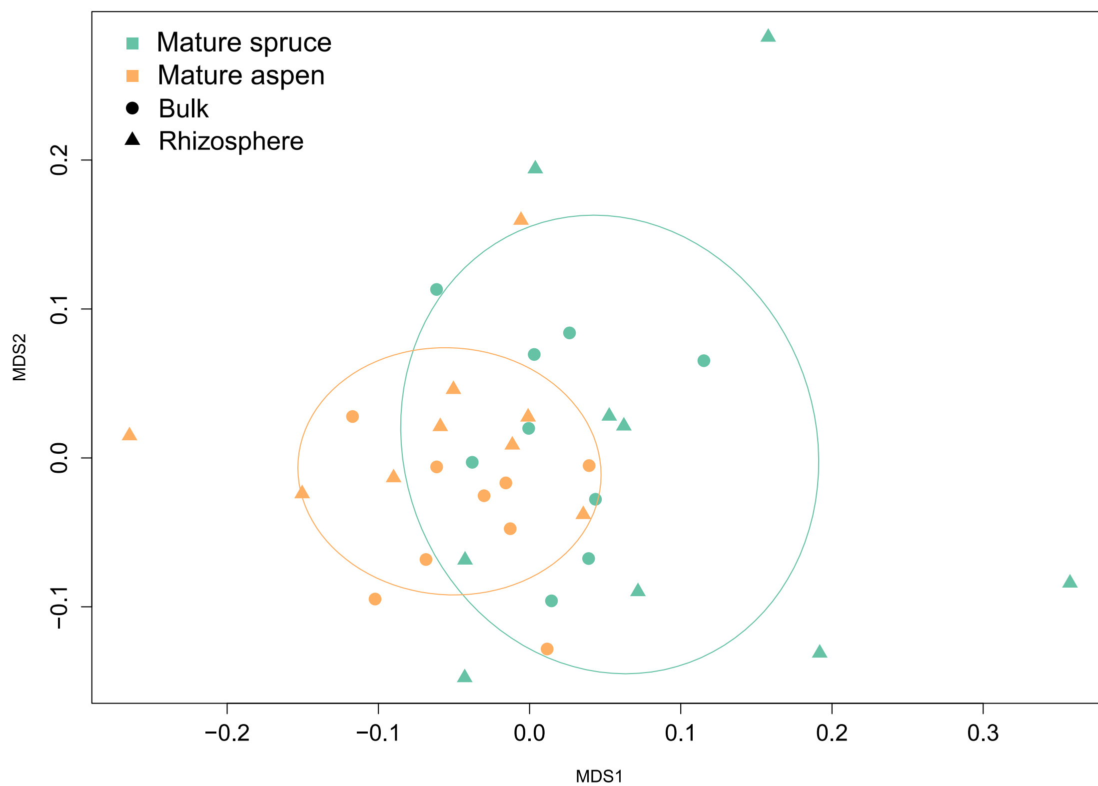

Figure 2.3. MetaMDS ordination of MSIR (Wisconsin double standardization, µg CO_2-C g^{-1} forest floor h^{-1}) data from the mature spruce and aspen rhizosphere and bulk soil.

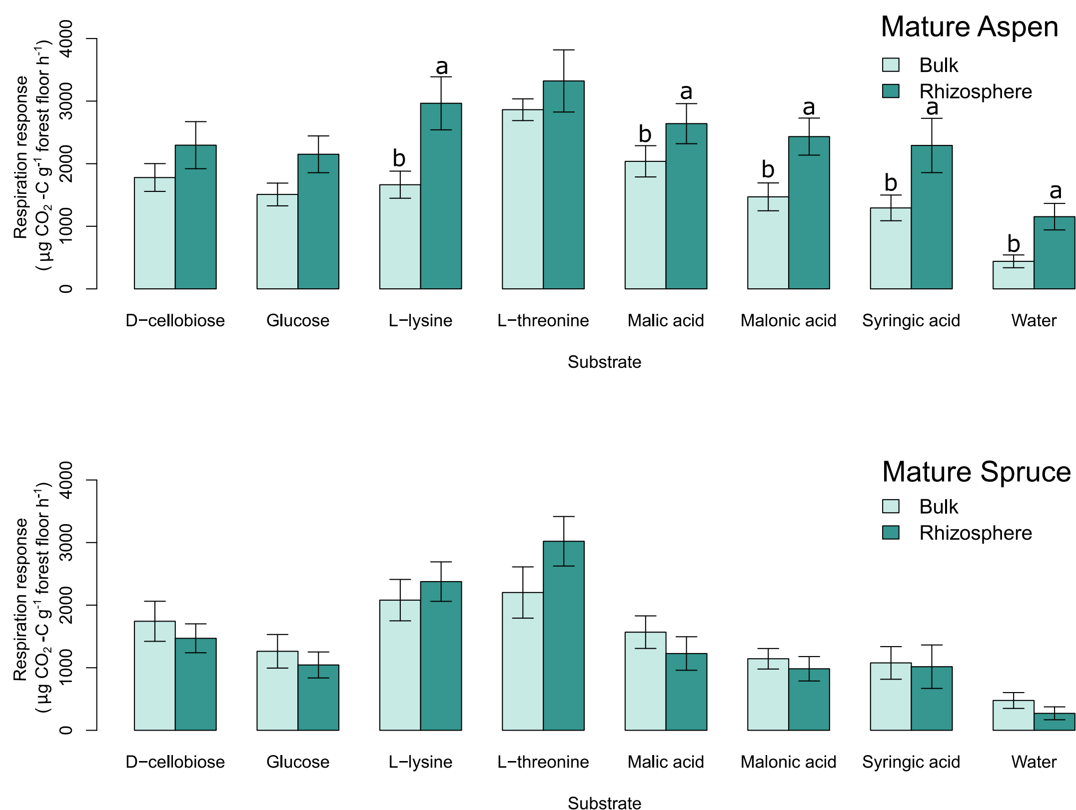

Figure 2.4. Microbial respiration response (µg CO_2-C g^{-1} forest floor h^{-1}) to substrate addition for mature aspen and spruce rhizosphere and bulk soils. Different lowercase letters indicate significant differences between rhizosphere and bulk soil (p values < 0.1; t-test followed by Holm adjustment).

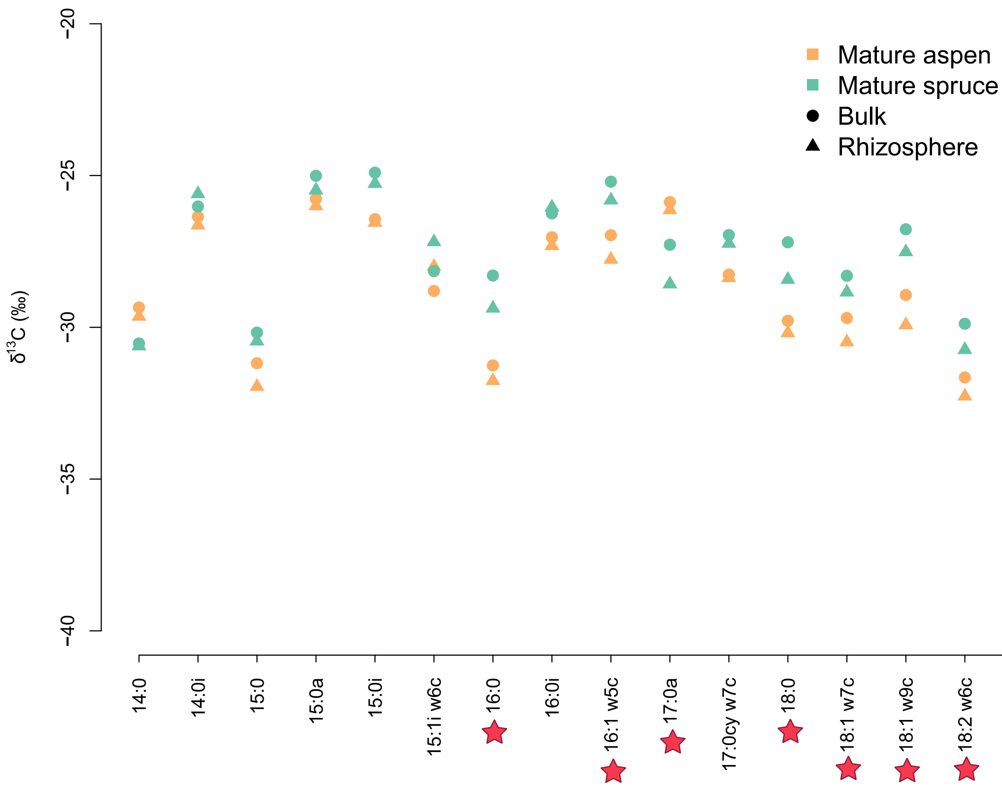

Figure 2.5. Carbon isotopic composition ($\delta^{13}C$, ‰) of individual PLFAs from rhizosphere or bulk soil of mature aspen and spruce stands. Red stars indicate PLFAs for which there was a significant difference between rhizosphere and bulk soil (p values < 0.1; Tukey's test).

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Chapter 3 – PRIMING EFFECTS IN BOREAL SOILS DEPEND ON SOIL DEPTH AND TIME OF MEASUREMENT

3.1. Introduction

The boreal forest is one of Canada's greatest natural resources and the largest terrestrial store of carbon on Earth (Watson et al., 2000). Canada's managed boreal is a carbon sink storing 28 Pg of C in biomass, organic matter, and soil; soil organic matter in mineral soil down to one metre stores approximately 40% of the ecosystem carbon (Kurz et al., 2013). While upper soil horizons are generally more carbon rich, subsoil is an important part of the soil carbon balance. When considering the top 100 cm alone, 50% of soil organic carbon is stored from 0-20 cm, 25% from 20-40 cm, 13% from 40-60 cm, and 12% from 60-100 cm (Jobbágy and Jackson, 2000). Laboratory studies have shown that decomposition of subsoil carbon (C) can be stimulated by the addition of labile C (Rumpel and Kögel-Knabner, 2011; Fontaine et al., 2007) and suggest that subsoil C stability can be maintained in the absence of a labile C source for microbes (Fontaine et al., 2007).

An average 2.0˚C temperature increase (relative to about the year 2000) is likely for the boreal by 2050 (Price et al., 2013), and this warming trend may shift boreal forest soils from carbon sinks to carbon sources (DeLuca and Boisvenue, 2012). Climate change also has the potential to alter the carbon balance of the boreal forest through indirect changes in plant photosynthesis (Drigo et al., 2008) causing increased allocation of C belowground via root exudation and turnover (Coûteaux et al., 1999), which could lead to changes in microbial community size and activity. Given that root production and exudation have been shown to increase in response to elevated temperature and atmospheric CO_2 concentration (Yin et al., 2013; Leppälammi-Kujansuu et al., 2013, 2014; Pendall et al., 2004; Norby, et al. 2004; Curtis and Wang, 1998), the rhizosphere may come to play an increasingly important role in the subsoil. The residence time of subsoil carbon

can be thousands of years (Rumpel and Kögel-Knabner, 2011), and if root exudates stimulate subsoil microorganisms, this ancient carbon could be lost to the atmosphere, further exacerbating climate change effects. Priming, the enhanced decomposition of soil organic matter by microorganisms due to availability of a labile carbon substrate, has been studied in both agricultural and forest soils (Fontaine et al., 2007; Lindén et al., 2014; Fan et al., 2013; Blagodatskaya et al., 2007), but less so in subsoil horizons of forest soils (Karhu et al., 2016; Ohm et al., 2007), and even less so in Canada's forests (Norris et al., 2016; Tan and Chang, 2007). Priming has the potential to reduce the carbon sequestration potential of soils (Hungate et al., 2003). Numerous mechanisms for explaining observed positive or negative priming have been proposed (Kuzyakov, 2002), with some important mechanisms being microbial nitrogen (N) mining, stoichiometric decomposition, and preferential substrate utilization (Chen et al., 2014; Blagodatskaya and Kuzyakov, 2008). Microbial N mining may occur in low N environments, when microbes utilize added labile C to degrade SOM and access nitrogen, resulting in positive priming (Fontaine et al. 2004, Blagodatskaya and Kuzyakov, 2008, Chen et al., 2014). The stoichiometric decomposition theory predicts that when inputs of C and N match the requirements of microorganisms for growth, positive priming occurs when SOM is decomposed by the extracellular enzymes released by microbes feeding on the labile C (Blagodatskaya and Kuzyakov, 2008, Chen et al., 2014). Preferential substrate utilization corresponds to negative priming, when SOM decomposition is reduced as microbes preferentially take up more easily degradable labile C. Short-term "apparent" positive priming effects may also occur when microbial turnover is accelerated following labile C addition, without a significant increase in degradation of SOM (Blagodatskaya and Kuzyakov, 2008). Thus, triggered microbial activity or a shift in the composition of communities have also been suggested as a necessary condition to priming. Focus

has been mostly on r-strategist microbes, which have the ability to grow quickly under an abundant supply of C and N, and K-strategists, which grow more slowly but are more competitive in degrading SOM (Fontaine et al. 2004, Blagodatskaya and Kuzyakov, 2008; Chen et al., 2014). Stimulating r-strategists would match the theory of stoichiometric decomposition, while increased activity of K-strategists would be in accordance with N mining theory (Chen et al., 2014).

The amount of priming may also depend on the amount of labile C added to the soil. Blagodatskya and Kuzyakov (2008) found three different scenarios depending on the amount of labile C added relative to microbial biomass. When substrate carbon added was less than 15% of microbial biomass C, the magnitude of positive priming (measured as CO_2) increased linearly with the amount of substrate C. At substrate C additions greater than 50% of microbial biomass, primed CO_2 decreased exponentially with added substrate C. With additions 200-500% of microbial biomass C, priming was generally zero or negative. Karhu et al. (2016) found these scenarios to be approximately true for organic and surficial mineral horizons, but that in B horizons priming increased with increased rate of glucose addition up to 2x microbial biomass carbon.

A useful technique for studying microbial communities is phospholipid fatty acid (PLFA) analysis. PLFAs are present in cell membranes, and variability in their structure can be utilized to assess microbial community composition and identify microbial groups within soil (Frostegård et al., 2010; Frostegård and Bååth, 1996). Stable carbon isotope (^{13}C) analysis of microbial biomarkers, namely PLFAs, provides insight into microbial substrate utilization (Watzinger, 2015). Stable isotope probing of PLFAs is useful for soil priming studies as it allows separation of carbon sources from the labile substrate and from the native soil organic matter (e.g.; Boschker et al., 1998; Boschker and Middelburg, 2002; Evershed et al., 2006; Jin and Evans, 2010; Chaudhary and Dick, 2016).

This research aims to: (1) quantify the priming effect caused by the introduction of labile carbon in various horizons of common boreal soil types, and (2) investigate microbial uptake of labile carbon and changes in microbial community composition. Priming of SOM was investigated in A and B soil horizons of a coarse-textured Brunisol and a fine-textured Luvisol, typical soils in the boreal forest. We added glucose as a model root exudate (Kuzyakov, 2010) relative to microbial biomass carbon (MB-C) as suggested by Blagodatskya and Kuzyakov (2008) at three different rates: 0.125x MB-C, 1x MB-C, and 2x MB-C. We investigated microbial utilization of substrate carbon and changes in microbial community composition using ^{13}C PLFA analysis.

3.2. Materials and Methods

3.2.1. Study areas

Soil sampling was conducted at two sites within 50 kilometres of Edmonton, Alberta. The first site was within Cooking Lake Blackfoot Provincial Recreation Area (PRA; 53° 30' 13" N, 112° 56' 10" W). Cooking Lake Blackfoot PRA is located within a pocket of the Dry Mixedwood Subregion of the Boreal Forest Natural Region, surrounded by Central Parkland. The area has mean January and July temperatures of -12.0°C and 17.0°C, respectively, and average annual precipitation of 482.4 mm (Environment Canada, 2017). Elevation ranges from 225 to 1225 m (Natural Regions Committee, 2006). Parent material is generally moderately fine textured glacial till (Natural Regions Committee, 2006). We sampled a Gleyed Gray Luvisol.

The second site was located within the Woodbend Forest University of Alberta research site near Devon, Alberta (53° 23' 25" N, 113° 45' 11" W). The Woodbend Forest is located within the Central Parkland subregion of Alberta (Natural Regions Committee, 2006; Alberta Parks, 2015). The area has mean January and July temperatures of -11.1°C and 16.4°C, respectively, and average annual precipitation of 508.0 mm (Environment Canada, 2017). The Central Parkland

subregion has elevations ranging from 500 to 1250 m (Natural Regions Committee, 2006). Parent material is generally medium to fine textured glacial till (Natural Regions Committee, 2006). However, sand dunes are present near Devon due to wind blown sand deposition after the drainage of Glacial Lake Edmonton (Godfrey, 1993), and we sampled an Eluviated Dystric Brunisol.

3.2.2. Site characterization

At both Cooking Lake and Woodbend, sampling was conducted under aspen vegetation to allow comparison between sites. One sampling area was selected in each of the two sites, at least 50 m from any pathways or roads. At the Cooking Lake and Woodbend sites, one $100m^2$ plot was delineated around a central soil pit. Vegetation subsampling plots were established in each cardinal direction 5 m from the central pit. The vegetation subsampling areas were 0.25 m^2. At each subsampling area, a variety of measurements were taken: leaf area index (LAI); percent cover of vegetation species; and ground cover (%) of lichen, moss, leaf litter, spruce needles, and coarse woody debris (Royer and Dickinson, 2007). LAI, described as the total surface area of leaves per unit of ground area (Marshall and Waring, 1986), was measured on overstory vegetation using a densiometer. The following measurements were taken within the $100m^2$ plots: slope, aspect, and tree diameter at breast height (DBH). Slope percent and degrees at the four plot corners were measured using a Suunto PM-5 clinometer. Diameter at breast height (DBH) was measured on all aspen trees within the plot.

Clear similarities and differences between the Cooking Lake and Woodbend sites can be observed. Both sites had a dominant aspen canopy with comparable tree height, DBH, and canopy cover. Lichen, moss, needles, leaf litter, coarse woody debris, and shrub ground cover were all similar between the two sites. The greatest difference was observed in total live cover (52.3% for Cooking Lake and 63.3% for Woodbend). The two sites did not share any of the same dominant

understory species. The Cooking Lake sampling site had two dominant understory vegetation species: *Corylus cornuta* (L.) and *Rubus pubescens* Raf.1811. Cooking Lake had a total live ground cover (averaged across the four vegetation subsampling plots) of 52% and average total shrub ground cover of 24%. Leaf litter ground cover was 100%, needles ground cover 0%, moss ground cover 0.5%, lichen ground cover 1.0%, and course woody debris cover 7.0%. Average DBH of all aspen trees in the $100m^2$ plot was 11.2 cm with an average height of 11.2 m and canopy cover of 85.7%. Average slope at the site was 1.8%. At the Woodbend sampling site there were four dominant understory vegetation species: *Rosa acicularis* Lind*., Populus balsamifera* (L.), *Amelanchier alnifolia* (Nutt.), *and Petasites palmatus* (L.) Fr. The Woodbend sampling plot had an average total live ground cover of 63% and average total shrub ground cover of 29%. Leaf litter ground cover was 100%, needles ground cover 0%, moss ground cover 0.5%, lichen ground cover 0.3%, and course woody debris cover 4.5%. Average DBH of all aspen trees in the $100m^2$ plot was 11.2 cm with an average height of 12.6 m and canopy cover of 85%. Average slope at the site was -1.6%.

3.2.3. Soil sampling and analysis

The central soil pits were described using the Canadian System of Soil Classification (Soil Classification Working Group, 1998) and horizons sampled separately (Appendix 5). Forest floor material and all mineral soil horizons down to 100 cm depth were collected, but only the Ae and B horizons were used for this project (four soils used for the project). The Ae horizon is a surficial mineral soil horizon showing signs of clay eluviation in the Luvisol and iron eluviation in the Brunisol (Appendix 5), while the B horizon is considered subsoil. Two soil cores (442 cm^3) were collected from each mineral soil horizon for field water content and bulk density determination. Samples for pH, particle size analysis, TOC, TN, and $\delta^{13}C$ were collected from each horizon and

air dried in the laboratory. Samples from the A horizons and top 20 cm of the B horizons were collected for the incubation experiment and accompanying chloroform fumigation extraction (CFE). Samples were kept on ice during transportation. In the laboratory, each horizon was homogenized and placed in a -20ºC freezer until further analysis. Prior to chloroform fumigation extraction and the incubation, soils were thawed, sieved (<2mm) and kept in the dark at 4°C.

Field water content and bulk density were determined by oven drying samples at 105ºC for 24 hours. pH was determined in triplicate on 10 g of air-dried soil with a 1:2 mass ratio of soil to 0.01 *M* $CaCl_2$ (Carter and Gregorich, 2006). Particle size distribution was determined using the hydrometer method with Calgon® (sodium hexametaphosphate) dispersion pre-treatment (Carter and Gregorich, 2006). Samples for TOC, TN, and $\delta^{13}C$ analyses were oven-dried at 105ºC for 24 hours and ground using a Retsch MM200 ball mill grinder. TOC and TN concentrations (%wt) were determined using a Shimadzu TOC-V CHS/CSN Model Total Organic Carbon Analyzer (Shimadzu Corporation, Kyoto, Japan). For $\delta^{13}C$ analysis samples were analyzed on a ThermoFinnigan Delta Advantage Continuous Flow Isotope Ratio Mass Spectrometer (Thermo Finnigan Corp, Bremen, Germany). Results were expressed in δ notation (‰) from the Pee Dee Belemnite (PDB) standard using the following equation where R represents the ratio of ^{13}C to ^{12}C atoms:

$$\delta = \left[\left(\frac{R_{sample}}{R_{standard}} - 1\right)\right] * 1000$$

Microbial biomass was determined using the chloroform fumigation extraction method (Carter and Gregorich, 2006). Soil water content was adjusted to -33 kPa, determined with pressure plates, and soils were pre-incubated for five days at room temperature. Each sample had four replicates (four unfumigated, four fumigated) of 40 grams each. Samples were extracted with 80

mL of 0.5M K_2SO_4 and NPOC and TN measured on a Shimadzu TOC-V CHS/CSN Model Total Organic Carbon Analyzer (Shimadzu Corporation, Kyoto, Japan).

The two sites were very different in terms of soil texture (Table 3.1). The Cooking Lake Ae horizon texture was classified as a loam, and the Bt horizon a clay loam. The Woodbend Ae horizon was a loamy sand and the Bm horizon was sand. The Cooking Lake soil was classified as a Gleyed Gray Luvisol. The Woodbend soil was classified as an Eluviated Dystric Brunisol. Among the four soil horizons, the Woodbend Ae horizon had the highest carbon content, microbial biomass, and C:N ratio (Table 3.1). For each soil, initial microbial biomass C represented between 0.85% and 0.95% of total soil organic C.

3.2.4. Incubation experiment

Four different soil horizons were incubated: Luvisolic Ae, Luvisolic Bt, Brunisolic Ae, and Brunisolic Bm. Five days before the start of the incubation, 20 grams (oven dried equivalent) of soil were weighed into 250 mL jars, moisture content was adjusted to 50% of -33 kPa, and jars were sealed and kept at room temperature. To yield a glucose stock solution of 1.5 atm%, 99% ^{13}C rich glucose (Cambridge Isotope Laboratories, Inc, MA, USA) was mixed with unlabelled glucose in water. On the first day of the experiment, ^{13}C labelled glucose was added to soils. There were three glucose treatments relative to microbial biomass carbon (MB-C) as determined by chloroform fumigation extraction (0.125x MB-C, 1x MB-C, and 2x MB-C) and one control treatment in which only water was added (Table 3.1). In terms of absolute glucose addition (µg glucose C g^{-1} soil), some treatments had a similar glucose addition (Table 3.1): (1) approximately 100 µg/g for Cooking Lake Ae and Bt 2x treatments and Woodbend Ae 1x treatment and (2) approximately 10 µg/g for Woodbend Ae 0.125x and Woodbend Bm 1x treatments. Water and

glucose solutions were added to bring the soil water content to -33 kPa. Each treatment had four laboratory replicates.

The jars were sampled for CO_2 (ppm) and δ^{13} CO_2-C (‰) using a Picarro G2201-i cavity ringdown spectrometer with attached small sample isotope module (Picarro Inc, Santa Clara, CA, USA). The jars were sampled 3hr, 24hr, 48hr, 4 days, 10 days, and 14 days after glucose addition. After 14 days, sampling was conducted once or twice weekly until day 65. To sample, 20 mL of air was drawn from each jar into a syringe. After sampling the jars were opened for 10 minutes, moisture content adjusted, and sealed. After 65 days, the incubation was stopped and soil was sampled for TOC, δ^{13}C, and PLFA analyses. TOC/δ^{13}C samples were treated as described in section 3.2.3. PLFA samples were placed in a -80ºC freezer until further analysis.

3.2.5. PLFA and ^{13}C PLFA analysis

PLFA analysis was conducted following the Quideau et al. (2016) protocol on soils from day 65 of the incubation. Soil samples were freeze-dried and stored in the dark at room temperature prior to analysis until 6 grams of each sample was added to centrifuge tubes. PC(19:0/19:0) nonadecanoate surrogate standard was used to assess PLFA recovery and samples were extracted with a modified Bligh and Dyer (1959) extractant. Lipid fractionation was performed using solid phase extraction (SPE) silica columns (Agilent Technologies, Wilmington, DE, USA). Lipid methylation was performed with an alkaline methanolysis to form fatty acid methyl esters (FAMEs). Solvent was evaporated under compressed N_2, and samples stored in the freezer until gas chromatograph analysis.

FAMEs were quantified with an Agilent 16 6890N Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE, USA). An internal standard of MeC10:0 (methyl

decanoate) was used to assess gas chromatograph performance. FAMEs were prepared by dissolving sample residues in internal standard solution. Sherlock Microbial Identification System Version 6.3 (MIDI, Inc., Newark, DE) was used to identify fatty acid peaks. PLFAs were described using the standard X:YωZ nomenclature. X indicates the number of carbon atoms, Y the number of double bonds, and Z the location of the first double bond from the aliphatic (ω) end of the molecule. Suffixes 'c' and 't' denote cis and trans isomers. Prefixes 'a', 'i' and Me indicate anteiso and iso branching and presence of methyl groups.

The $\delta^{13}C$ values of individual PLFAs (‰) were obtained on a GC-IRMS (Thermo Fisher Scientific) with the GC linked to the IRMS through a GC Isolink unit. The $\delta^{13}C$ values of PLFAs were corrected for the carbon atom added during methylation. Isotopic ratios of PLFAs were calibrated against three 20:0 isotope standards: USGS70, USGS71, and USGS72 (Indiana University CRMS) (Schimmelmann et al., 2016). The standards were run at the beginning of the control sample set and at the beginning of the enriched sample set, with the standards run in triplicate each time. The calibration was periodically checked (at the start of each sample set and approximately every eight samples throughout the analysis) using a mixture of eight FAMEs certified and purchased from Indiana University (F8-3 mixture, Indiana University CRMS).

Specific PLFAs were designated to microbial groups. Fungal PLFAs were 18:2 ω6c, 18:1 ω9c, 20:1 ω9c, and 18:3 ω6c (Esperschütz et al., 2011; Frostegård et al., 2010; Hamman et al., 2007; Myers et al., 2001). Gram negative PLFAs were 17:1 ω8c, 18:1 ω7c, 16:1 ω7c, 16:1 ω9c, and 15:1 ω6c (Derrien et al., 2014; Esperschütz et al., 2011; Elfstrand et al., 2008; Kramer and Gleixner, 2008; Myers et al., 2001, Frostegård and Bååth, 1996). Gram positive PLFAs were 14:0i, 15:0i, 15:0a, 16:0i, 17:0i, 17:0a, 18:0i (Frostegård et al., 2010; Myers et al., 2001). 10Me PLFAs

were considered actinomycetes (Fernandes et al., 2013; Myers et al., 2001; O'Donnell et al., 1982). Protists were 20:4 ω6c (Myers et al., 2001).

3.2.6. Calculations and data analysis

Total CO_2-C (mol) from the glucose-treated soils was separated in SOM-derived C (SOM C) and glucose-derived C (glucose C) using the following equations:

$$glucose\ C = total\ C \times \frac{A_{treated} - A_{control}}{A_{glucose} - A_{control}}$$

$$SOM\ C = total\ C\ \times (1 - \frac{A_{treated} - A_{control}}{A_{glucose} - A_{control}})$$

where total C (mol) is the total CO_2-C emissions from the jar, $A_{treated}$ is the atom% ^{13}C of glucose treated soil CO_2, $A_{control}$ is atom% ^{13}C of control soil CO_2 (ranging between 1.06 and 1.07% depending on soil horizon), and $A_{glucose}$ is the atom% ^{13}C of the added glucose (1.50%). We assumed that the CO_2 derived from the mineralization of soil organic matter in the glucose-treated soil had the same isotopic composition as the CO_2 respired from the control treatment over time.

The priming effect (μmol) was calculated with the following equation:

$$PE = \ SOM\ C_{treated} - SOM\ C_{control}$$

where SOM $C_{treated}$ is the SOM-derived CO_2-C emitted in soils treated with glucose and SOM $C_{control}$ is the SOM-derived CO_2-C emitted in the control treatment. To calculate a standard error for priming we assumed no variability in initial CO_2 concentration or delta value and no variability in CO_2 concentration or delta value of controls when calculating priming effects within glucose treatments. Cumulative primed C relative to controls (%) was calculated by dividing primed C by SOM-derived CO_2-C emitted from the respective control soils. Permutational ANOVA followed

by Tukey's Honest Significant Difference test was used to assess statistical differences between soils and treatments for CO_2-C and priming data. Significance was defined at $\alpha=0.05$. One replicate of the Brunisolic Bm horizon was removed from analysis as an outlier. All statistical analyses were performed using R Version 3.3.3 (R Core Team, 2017).

Total PLFAs was calculated as the sum of all fatty acids with carbon chains 14 to 20 carbon atoms in length. For total PLFAs and microbial groups, permutational ANOVA followed by Tukey's Honest Significant Difference test was used to assess statistical differences between glucose treatments within one soil, and between the different soil horizons for a given glucose treatment. Ordination was used to visualize patterns of microbial community composition (PLFA) of the different soils and glucose treatments. PLFAs (mol %, Hellinger transformed) were ordinated with non-metric multidimensional scaling (NMDS) using the MetaMDS function from the vegan package in R (Oksanen et al., 2017). PLFAs with chain length 14 to 20 were included in analysis. In total, 79 PLFAs were identified after data manipulation. Hellinger transformation favours zero-inflated data and gives low weight to rare PLFAs in the NMDS solution thus avoiding grouping sites containing zeros as being more similar in the ordination. Permutational multivariate ANOVA (MANOVA) followed by pairwise permutational MANOVAs and Bonferroni adjustment for multiple inference was used to assess differences in microbial community composition between soils and treatments using vegan and RVAideMemoire R packages (Oksanen et al., 2017; Hervé, 2017).

PLFA $\delta^{13}C$ values were obtained for 14 microbial PLFAs (Appendix 6). Originally we obtained values for 23 PLFAs, but 9 were rejected due to low peak intensity (<100 mV) or poorly resolved peaks resulting in unreliable results. PLFAs with a peak intensity between 100 to 300 mV were included in the analysis but should be interpreted with caution: 14:0, 15:0, 16:1 ω5c, 16:1

ω7c, 17:0cy ω7c, 17:0i, 18:0, and 18:2 ω7c. Results from PLFAs 15:0a, 15:0i, 16:0, 18:1 ω7c (except interpret Luvisolic Bt with caution), 18:1 ω9c (except interpret 0.125x treatment with caution), and 19:0cy ω7c (except interpret 0.125x treatment with caution) are considered reliable (>300 mV). Permutational ANOVA followed by Tukey's Honest Significant Difference was performed to check for significant differences in enrichment between the different soils for each PLFA, and to assess differences in $\delta^{13}C$ values between treatments within each soil (α=0.1). To compare ^{13}C enrichment across soils, we used the difference between the $\delta^{13}C$ (‰) value of the enriched sample and the average of the control samples. To assess the average $\delta^{13}C$ value of total PLFAs among soils at the end of incubation we accounted for the mol % each PLFA represented in the soil samples and the number of carbon atoms in each PLFA.

3.3. Results

3.3.1. Incubation experiment

In general, SOM was mineralized more quickly in the Brunisol (Woodbend) than in the Luvisol (Cooking Lake, Figure 3.1). In the absence of glucose addition, the fraction of initial SOM mineralized after two months was higher in the Brunisol than in the Luvisol, and generally higher in the A horizons than in the B horizons for both soils (Figure 3.1). The addition of glucose increased the respiration rate compared to controls within hours to one day in all soils. The glucose-treated soils maintained an elevated respiration rate compared to the controls for approximately four days (Appendix 7).

Figure 3.2 shows that for all soils, the 0.125x glucose treatment respired proportionally less of the initial glucose C compared to the other treatments. A mass balance based on CO_2 data showed that between 49% and 63% of the introduced glucose-C had been fully mineralized as CO_2 at the end of the incubation in the 2x glucose treatment (Table 3.4). This mass balance was

corroborated by the $\delta^{13}C$ values of bulk soil material at the end of the incubation (Table 3.3). In the Luvisol, the A horizon respired more glucose than the B horizon for the 1x and 2x treatments, while the B horizon respired more at the 0.125x treatment; the opposite trend was observed in the Brunisol (Figure 3.2).

At the end of the two-month incubation, there was no statistically significant difference in cumulative SOM-derived CO_2-C (μmol g carbon^{-1}) between the control and glucose treated samples for the four soils investigated (Figures 3.1). At this stage, the addition of glucose had not resulted in any positive or negative net priming effect since the start of the experiment, whatever the soil horizon or rate of glucose addition. Trends in cumulative primed C, expressed as a fraction of the SOM-derived CO_2 emission measured in the control samples, varied among glucose treatments and soils (Figure 3.3). For the A horizons priming was negative after three hours of incubation with glucose. With the Luvisolic Ae, cumulative priming became less negative over the course of the incubation for all glucose applications. Cumulative priming peaked at positive values for the Brunisolic Ae on day four, and then decreased until day 65. For the B horizons, priming was generally positive after three hours (with the exception of the Brunisolic Bm 0.125x MB-C treatment) and became slightly negative at the end of incubation.

3.3.2. PLFA and ^{13}C PLFA

The Brunisolic Ae had significantly higher total PLFAs than the Luvisolic Ae for control, 0.125x, and 2x treatments ($p=0.03$, $p=0.02$, and $p=0.005$, respectively). The Brunisolic Bm had lower total PLFAs compared to the other soils for all treatments. Total PLFAs of controls at the end of incubation matched trends in microbial biomass determined by CFE at the beginning of the experiment, where the Brunisolic Ae showed the highest microbial biomass followed by the Luvisolic Bt and Ae, and then Brunisolic Bm (Figure 3.4). Ordination was used to visualize

microbial community composition of the Brunisolic (Woodbend) and Luvisolic (Cooking Lake) soil horizons and three different glucose treatments (control, 0.125x MB-C, and 2x MB-C; Figure 3.5). The 1x MB-C glucose treatment was not included in PLFA analysis due to large variability in soil respiration measurements. Microbial communities of all soils differed from one another ($p=0.006$). The Brunisolic Ae horizon had weakly significantly different microbial communities in the control treatment compared to the 2x glucose treatment ($p=0.06$). Within the Luvisolic Ae and Bt and Brunisolic Bm horizons, there was no significant difference between microbial communities in the different glucose treatments.

In terms of microbial groups (Appendix 8), the Luvisolic Ae horizon had a significantly higher proportion of fungi in the 0.125x and 2x glucose treatments compared to the control ($p=0.001$ and $p<0.001$, respectively) and there was no significant difference for gram negative bacteria between treatments. The Luvisolic Bt horizon had significantly more fungi in the 0.125x glucose treatment compared to the 2x treatment ($p=0.006$) and there were no significant differences in the proportion of gram negative bacteria between treatments. In the Brunisolic Ae horizon the 0.125x treatment had a significantly higher proportion of fungi than the control and 2x treatment ($p=0.02$ and $p=0.001$ respectively), but there was no significant difference for gram negative bacteria. The Brunisolic Bm horizon showed no significant differences between treatments for the proportion of fungi or gram negative bacteria. When combining all glucose treatments, the Brunisolic Bm had less fungi than the Luvisolic Bt and Brunisolic Ae ($p=0.05$ and $p=0.03$, respectively).

The $\delta^{13}C$ values of individual PLFAs showed that for all soils the 2x glucose treatment showed significant ^{13}C enrichment compared to the controls for the PLFAs investigated (Figure 3.6). Two of the PLFAs showing the highest level of enrichment across the soils were fungal

biomarkers 18:2 ω6c and 18:1 ω9c (Figure 3.6). A summary of significant ^{13}C enrichment values between soils for the 2x glucose treatment is shown in Table 3.2. Considering only the PLFAs for which there were significant differences in ^{13}C enrichment among soils, the Luvisolic Ae was the most enriched for the 2x glucose treatment for four PLFAs (16:1 ω5c, 17:0cy ω7c, 17:0i, and 18:0), Luvisolic Bt for three PLFAs (18:1 ω9c, 18:2 ω6c, and 19:0cy ω7c), Brunisolic Ae for one PLFA (14:0), and Brunisolic Bm for three (15:0a, 15:0i, and 16:1 ω7c). The Luvisolic Ae had significantly greater enrichment compared to the other horizons for fungal PLFA 16:1 ω5c and gram positive PLFA 17:0i; the Bt showed greater enrichment than the other horizons for two fungal PLFAs (18:1 ω9c and 18:2 ω6c) and one gram positive PLFA (15:0a). Gram negative PLFA 18:1 ω7c was significantly more enriched in the Luvisol compared to Brunisol. Gram negative PLFA 16:1 ω7c was significantly more enriched in A horizons compared to the Luvisolic Bt, but was most enriched in Brunisolic Bm. Gram positive PLFAs 15:0a and15:0i were significantly more enriched in the Brunisolic Bm compared to the other soils. The Luvisolic Bt horizon was significantly more enriched than the other soils (Table 3.2).

3.4. Discussion

This research aimed to: (1) quantify the priming effect caused by the introduction of labile carbon at different rates in various horizons of common boreal soil types, and (2) investigate microbial uptake of the labile carbon source and changes in microbial community composition. First, we investigated how SOM mineralization differed between soils in the absence of glucose. Priming mechanisms at play in the incubation, microbial groups involved in labile C uptake, and glucose mineralization and how it related to carbon use efficiency were analyzed. Finally, implications for future studies are discussed.

3.4.1. Organic matter stability as a function of pedological parameters

SOM mineralization in the absence of glucose occurred more quickly in the Brunisolic soils compared to Luvisolic soils (Figure 3.1). This is likely linked to the clay content of the Luvisol, which was 3 to 7 times higher than the Brunisol (Table 3.1). Rumpel and Kögel-Knabner (2011) define three main mechanisms of SOM stabilization: physical protection, whereby particulate and clay-associated OM are physically separated from microbes; physico-chemical interaction, where association with soil minerals, in particular clay, may protect SOM from oxidation; and stable chemical structure, which is considered a controversial mechanism. The higher clay content in the Luvisol suggests that SOM was protected due to its physical and physio-chemical association with clay particles.

SOM mineralization occurred more quickly in the A than in the B horizons (Figure 3.1). In the Luvisol, this can be explained by higher clay content in the Bt horizon compared to the Ae horizon, resulting in SOM protection due to association with clays. However, in the Brunisol, slower SOM mineralization in the Bm horizon is likely linked to the microbial population. Subsoil SOM can be degraded by microbes, but their ability to degrade organic matter is affected by environmental conditions and spatial separation from carbon sources (Rumpel and Kögel-Knabner, 2011). Due to a lack of labile C inputs, subsoil microbes generally feed on older SOM (Kramer and Glexiner, 2008). Microbial biomass C in the Brunisolic Bm horizon was the lowest of all soils. Low microbial biomass and a lack of labile C likely caused slower SOM mineralization in the Brunisolic Bm compared to Ae horizon.

3.4.2. Priming mechanisms at play in the long and short term

Given the low C content of the soils and high microbial C:N ratio, we expected to see positive priming in all soils by the end of incubation. Instead, microbes tended to utilize glucose

and did not mineralize additional native soil organic matter. Positive priming at the end of the incubation was expected for the Brunisolic Ae given that it had the highest C:N ratio (15:1) of the soils investigated. One mechanism of rhizosphere priming is microbial activation whereby labile carbon released by roots stimulates microbes leading to increased SOM decomposition (Kuzyakov, 2002). A possible cause of microbial activation is the N mining hypothesis, which states that microbes utilize labile C from roots to degrade SOM to access nitrogen locked in organic matter (Kuzyakov, 2010; Chen et al., 2014). High C:N soils could result in N mining in the presence of labile C, as microbes starved for nitrogen use rhizodeposits to degrade SOM. Previous research has used microbial N mining to explain observed priming effects under low N availability (Karhu et al., 2016; Qiao et al., 2016); however this did not appear to occur in the Brunisolic Ae by the end of the incubation. Microbial N mining also unexpectedly did not appear to occur in the Luvisolic Bt which had the highest microbial biomass C:N ratio (30.3:1).

Another priming mechanism known as stoichiometric decomposition theory explains a scenario where priming occurs indirectly when SOM is decomposed by extracellular enzymes released by microbes feeding on the labile C substrate (Chen et al., 2014). Razanamalala et al. (2018) proposed that priming in colder climates is generated by stoichiometric decomposition, while in warmer climates the driving mechanism is N mining. Perhaps positive priming observed only in the beginning of our experiment in the B horizons was the result of stoichiometric decomposition. In the current study, soil respiration rates surged soon after glucose addition and remained elevated for approximately one week, indicating activation of the microbial communities. Stoichiometric decomposition could have occurred during this time of high microbial activity, and then faded as microbes became less active. Kuzyakov (2010) ascribes increases in respiration shortly after substrate addition to apparent priming effects (changes in

microbial metabolism and turnover resulting in increased respiration fluxes without increases in SOM decomposition). Apparent priming is another plausible explanation for positive priming observed early in our experiment.

An important mechanism for negative priming is preferential substrate utilization, whereby more easily degradable rhizodeposits are preferred by microorganisms resulting in a decrease in SOM decomposition (Kuzyakov, 2002). Negative priming observed during the experiment was likely caused by preferential substrate utilization, considering the conceptual model of Blagodatskaya and Kuzyakov (2008). Given that the C content of soils used in this experiment was generally low, microbes may have preferred to use glucose over more recalcitrant SOM. Figure 3.1 shows that, with the exception of the Brunisolic Bm, the 1x and 2x glucose treatments generally respired less SOM-derived CO_2-C than controls, indicating possible preferential substrate utilization of glucose. However, the lack of statistical significance in SOM-derived CO_2-C between control and glucose treated soils suggests that glucose addition had little effect on net SOM dynamics by the end of incubation. These findings contradict our hypotheses that different glucose addition rates would stimulate positive priming depending on soil horizon depth. Boreal priming studies have found varying results with addition of labile C. Fan et al. (2013), Lindén et al. (2014), and Karhu et al. (2016) observed positive priming, while Chigineva et al. (2009) and Linkosalmi et al. (2015) found negative or no priming. Contrary to Karhu et al. (2016) and Blagodatskaya and Kuzyakov (2008), our results did not show that priming could be influenced by the input of labile C relative to microbial biomass. While Karhu et al. (2016) found that the relative magnitude of priming was greater in deeper soil horizons, we observed no clear difference between A and B horizons at the end of the incubation. Other researchers have explained differences in priming with soil depth by a lack of labile C in deeper soil horizons limiting the

ability of microbes to degrade SOM (Karhu et al., 2016; Fontaine et al., 2007). This could be the case in our study given observations at the beginning of the incubation.

Priming effects three hours following glucose addition were negative in the A horizons and positive in the B horizons, with the exception of the Brunisolic Bm 0.125x MB-C treatment (Figure 3.3). Positive priming in the B horizons is likely the result of apparent priming. Negative priming in the Luvisolic Ae horizon attenuated over time, remaining negative until the end of the incubation. In the Brunisolic Ae horizon, priming became positive four days after glucose addition, then became negative again on day seven. The contrasting results between the two A horizons could be due to shifts in the microbial community composition that PLFA analysis after two months was not able to capture. Overtime the cumulative priming effect for the Brunisolic Ae attenuated, becoming near neutral (Figure 3.3), as glucose-derived CO_2-C emissions decreased while SOM-derived CO_2-C remained relatively constant (Figure 3.2, 3.1). In the A horizons at this early stage of the incubation, it appears that preferential substrate utilization was the dominant mechanism affecting priming. The flush of labile C resulted in a temporary decrease in the mineralization of more recalcitrant SOM. While total CO_2 respiration rate increased in the early stages of the incubation for all soils, in the A horizons this was likely caused by a triggering effect that increased microbial metabolism without increased SOM mineralization (Blagodatskaya and Kuzyakov, 2008). We did not observe differences between glucose addition rates which Blagodatskaya and Kuzyakov (2008) suggested would result in activation of different priming mechanisms.

3.4.3. Microbial carbon use efficiency and labile C uptake

While we found no effect of labile C addition rate on priming, there did appear to be an effect on microbial carbon use efficiency. Carbon use efficiency refers to how well microbes utilize

C to increase biomass compared to losses due to respiration, and essentially it is the ratio of microbial C uptake used for growth versus C that is respired (Sinsabaugh et al., 2013; Spohn et al., 2016). A lower fraction of glucose was mineralized as CO_2 in the 0.125x treatment across the soils (Figures 3.2), which could be due to greater carbon use efficiency or adsorption on the mineral matrix. With labile C additions less than MB-C, microbes may more efficiently use the substrate. More than 30% of glucose C initially added to the soil was respired as CO_2 within the first week across soils and glucose treatments (Figure 3.2). After this period, glucose mineralization slowed considerably. Calculations based on the amount of $^{12}CO_2$ and $^{13}CO_2$ respired over time and $\delta^{13}C$ values of bulk soil at the end of the incubation suggest that between 37% to 50% of glucose C remained in the soil after two months. However, the form in which glucose C remained at the end of the incubation is unknown. We estimated the fraction of glucose C incorporated into microbial biomass at the end of the incubation by assuming (1) that the average PLFA isotopic signature reflected the isotopic signature of the bulk microbial biomass, (2) that the average isotopic signature of PLFAs from the control at the end of the incubation was similar to the signature prior to glucose addition, and (3) that the microbial biomass at the end of the incubation in the 2x glucose treatment was similar to the one initially measured by chloroform fumigation extraction. Based on these calculations, only about 1% of the initial glucose C was incorporated into microbial biomass by the end of the incubation (Appendix 9). The remaining glucose C was likely in a non-bioavailable form due to interaction with soil minerals or had been previously utilized by microorganisms and existed in a low energy form that could not be metabolized further. For example, Fontaine et al. (2007) suggested that C in deep soil horizons does not provide enough energy to support microbial communities and enzyme production. In our study, microbial biomass

in both B horizons incorporated more of the ^{13}C label than A horizons (Appendix 9), indicating that subsoil had lower carbon availability and thus prompted microbes to utilize glucose.

At the end of the incubation, percent fungi had increased with glucose addition for both A horizons compared to the controls and fungal biomarkers 18:2 ω6c and 18:1 ω9c had the highest ^{13}C enrichment. This is in contrast to Chigineva et al. (2009), who found that incubation with sucrose led to negative priming with a decrease in fungal diversity and increase in the dominance of r-strategists. Like Chigineva et al. (2009), other researchers have found that labile C addition increased the activity of r-strategists but not K-strategists (Blagodatskaya et al., 2007; Hamer and Marschner, 2005; Fontaine et al., 2003). Quick growing r-strategists can immobilize nutrients and therefore supress K-strategists able to decompose more complex SOM compounds, resulting in negative priming (Hobbie, 2005; Fontaine et al., 2003).

In our study, fungi were a key microbial group utilizing labile C, either feeding directly on glucose or indirectly on dead bacterial biomass that had incorporated the ^{13}C label. Our findings could not show a shift from K- to r-strategist dominated microbial communities as PLFA analysis was conducted only at the end of the incubation, but support other research that has found that fungi uptake labile C, becoming strongly enriched with the ^{13}C label (Nottingham et al., 2009; Paterson et al., 2007; Treonis et al., 2004; Butler et al., 2003). It is interesting that the Brunisolic Ae had a high C:N ratio compared to the other soils and higher percent fungi in the 0.125x glucose treatment compared to the control but did not exhibit priming, as Chen et al. (2014) have ascribed N mining under low nitrogen conditions to K-strategists such as fungi. Further research is required to gain a clearer understanding of which microbial groups utilize labile C and contribute to priming, and under what conditions.

3.4.4. Implications

Further study of the effect of labile C addition rate on priming is needed. Blagodatskaya and Kuzyakov (2008) suggested that the magnitude of priming decreases at C addition rates high compared to microbial biomass (two to five times MB-C). Karhu et al. (2016) found this to be true for upper soil horizons, but glucose additions up to two times microbial biomass carbon stimulated greater priming in subsoil horizons compared to the lower glucose addition rates. Paterson and Sim (2013) found that higher glucose addition rate increased SOM-derived CO_2 fluxes in three of the four soils studied. Ohm et al. (2007) conducted a two month incubation where small additions of labile C were made weekly for four weeks, after which there was one large labile C addition. The magnitude of positive priming was greatest after the large substrate addition. Our results contradict these studies, showing no clear effect of addition rate on priming. Larger labile C additions appear to stimulate greater priming (Ohm et al., 2007), at least in subsoil, and may better simulate increases in root exudation with climate change.

Given the lack of quantitative data on current root exudation in the boreal forest, we defined the amount of glucose added in each soil relative to microbial biomass measured prior to glucose introduction. Adding labile C in accordance with actual rhizodeposition rates of native vegetation could tease apart the complexity of how labile C addition affects SOM decomposition. Additionally, we used glucose as a model root exudate, but exudates are much more complex than a single compound. Other researchers have found acids such as palmititc acid to be important components of rhizodeposits (Melnitchouck et al., 2005), and have combined sugars with amino and organic acids to create model root exudates (Paterson et al., 2007). Phillips et al. (2007) developed a technique for collecting root exudates in the field in forest soils. Using such a

technique to learn the chemical composition of root exudates from naturally growing forests would allow us to more closely mimic root exudates in future priming studies.

While cumulative priming at the end of the incubation revealed neutral priming and no difference in horizon depths, quite opposite conclusions can be drawn from priming just three hours after glucose addition. Our experiment was conducted to mimic the effect of root exudates on SOM decomposition, and we monitored the effect of a single glucose addition for over two months. However, root exudation is a continuous, or at least semi-continuous, process and regular glucose applications might affect priming. For example, if glucose was added regularly with less than 24 hours between additions, our results suggest that SOM would be protected in A horizons but degraded more quickly in B horizons. If peaks in root exudation occurred once or twice per month, our results suggest that SOM degradation would not be significantly affected in the long term. This explanation does not consider how microbial communities may adapt to regular labile C inputs, and therefore care should be taken when extrapolating these results. Ohm et al. (2007) found that one large input of labile C resulted in greater positive priming than multiple small additions, in contrast to our results. However, Zhang et al. (2017) stress the importance of evaluating the temporal dynamics of priming in the long term.

3.5. Conclusion

This work aimed to evaluate whether destabilization of soil organic matter would occur in boreal forest soil types following increased rhizodeposition, an expected consequence of climate change. Our results suggest that a single input of labile C would not result in any significant positive priming in the long-term. We demonstrated however that the magnitude and direction of priming effects are strongly dependent on the time of measurement. Priming early in the incubation suggested difference in priming with soil depth, but by the end of the 65 day incubation we saw

no significant different in priming with depth. The frequency in which glucose is added could play a role in the direction of priming. If labile C is added regularly, it appears that SOM would be protected in topsoil but mineralized more quickly in subsoil. If labile C additions occur only periodically, our results suggest that SOM mineralization would not be affected considerably in the long term. More frequent analysis of the microbial community would help to further explain soil organic carbon dynamics following labile C addition. Further priming research in Canada, specifically in the boreal forest which is threatened by climate change, must be done if we are to understand how soil carbon flux may change in years to come.

3.6. Tables and Figures

Table 3.1. Soil horizon physical, chemical, and biological characteristics for the Cooking Lake (Luvisol) and Woodbend (Brunisol) sites, and glucose addition amounts for the soil incubation.

													Glucose addition (μg glucose C g^{-1} soil)		
Site	Horizon	Depth (cm)	pH	Sand(%)	Silt(%)	Clay(%)	TOC(%)	TN(%)	C:N ratio	MB-C* (μg g-1 soil)	MB-N* (μg g-1 soil)	MB* C:N ratio	0.125x MB-C	1x MB-C	2x MB-C
Cooking Lake	Ae	4.5 - 13	4.45	45	40	15	0.43	0.05	8.3	39.3	2.2	18.0	4.9	39.3	78.6
	Btgj	13 - 92	4.37	44	20	35	0.55	0.06	8.7	50.2	1.7	30.3	6.3	50.2	100.3
Woodbend	Ae	7 - 22	4.52	87	7	6	0.80	0.05	14.7	86.0	5.5	15.6	10.8	86.0	172.1
	Bm	22 - 96	5.23	89	6	5	0.17	0.02	7.3	14.5	0.6	23.2	1.8	14.5	29.0

* MB-C (microbial biomass carbon) and MB-N (microbial biomass nitrogen) as determined via chloroform fumigation extraction.

Table 3.2. The $\Delta\delta^{13}C$ (‰) enrichment of PLFAs in 2x MB-C* glucose treated soils compared to their controls, and the mean $\Delta\delta^{13}C$ (‰) value for each 2x MB-C glucose treated soil. Values are averages (n=4) where different lowercase letters indicate significant differences among soils (p values < 0.1; Tukey's test). Values in parentheses indicate standard deviation. While there are four replicates of each soil and glucose treatment combination, some of the PLFAs to be interpreted with caution additionally did not have all four replicates due to low peak intensity (Appendix 6).

PLFA	Luvisolic Ae 2x	Luvisolic Bt 2x	Brunisolic Ae 2x	Brunisolic Bm 2x
	Δδ13C (‰)			
14:0	23.6b	18.8c	28.6a	
15:0	11.7a	10.0a	9.3a	
15:0a	15.4c	39.0b	16.0c	52.1a
15:0i	17.1bc	19.0b	15.9c	23.2a
16:0	30.9a	32.3a	27.5a	
16:1 ω5c	41.1a	15.5b	17.3b	
16:1 ω7c	24.3b	20.0c	26.2b	31.4a
17:0cy ω7c	43.2a	34.7b	27.3c	
17:0i	15.7a	12.3b	12.3b	
18:0	22.6a	13.9b	16.3b	
18:1 ω7c	24.9a	25.8a	14.9b	
18:1 ω9c	46.6b	54.3a	48.8b	37.1c
18:2 ω6c	76.6b	101.5a	65.5c	
19:0cy ω7c	26.3b	32.7a	20.4c	
	Mean Δδ13C (‰)			
	24.9 (1.3)b	31.9 (1.9)a	24.8 (1.1)b	23.4 (3.5)b

* MB-C (microbial biomass carbon) as determined via chloroform fumigation extraction.

Table 3.3. The $\delta^{13}C$ (‰) values of soil from Cooking Lake (Luvisol) and Woodbend (Brunisol) on day 65 of the incubation. Values are means (n=3) with standard deviations in parentheses. Lowercase letters indicate significant differences within soil horizons.

Soil	Horizon	Treatment	$\delta^{13}C$ (‰) of soil
Luvisol	Ae	Control	-25.9 (0.1)c
	Ae	0.125x	-25.7 (0.1)c
	Ae	1x	-24.4 (0.1)b
	Ae	2x	-23.3 (0.1)a
	Bt	Control	-25.6 (0.1)c
	Bt	0.125x	-25.3 (0.1)c
	Bt	1x	-24.1 (0.2)b
	Bt	2x	-22.3 (0.2)a
Brunisol	Ae	Control	-26.7 (0.1)c
	Ae	0.125x	-26.5 (0.1)c
	Ae	1x	-25.1 (0.2)b
	Ae	2x	-24.1 (0.2)a
	Bm	Control	-25.4 (0.3)c
	Bm	0.125x	-25.2 (0.1)c
	Bm	1x	-24.1 (0.2)b
	Bm	2x	-23.4 (0.3)a

Table 3.4. Mass balance of glucose on day 65 of the incubation for each soil horizon with 2x MB-C glucose addition. Values are means (n=4 for Luvisolic horizons and Brunisolic Ae; n=3 for Brunisolic Bm) with standard deviations in parentheses. Lowercase letters indicate significant differences between soil horizons.

Horizon	Amount of glucose added (mg glucose-C jar^{-1})	Cumulative glucose-C respired (% of glucose added)
Luvisolic Ae	1.57	58.85 (0.38)b
Luvisolic Bt	2.01	49.78 (1.37)c
Brunisolic Ae	3.44	59.48 (4.05)ab
Brunisolic Bm	0.58	63.14 (1.85)a

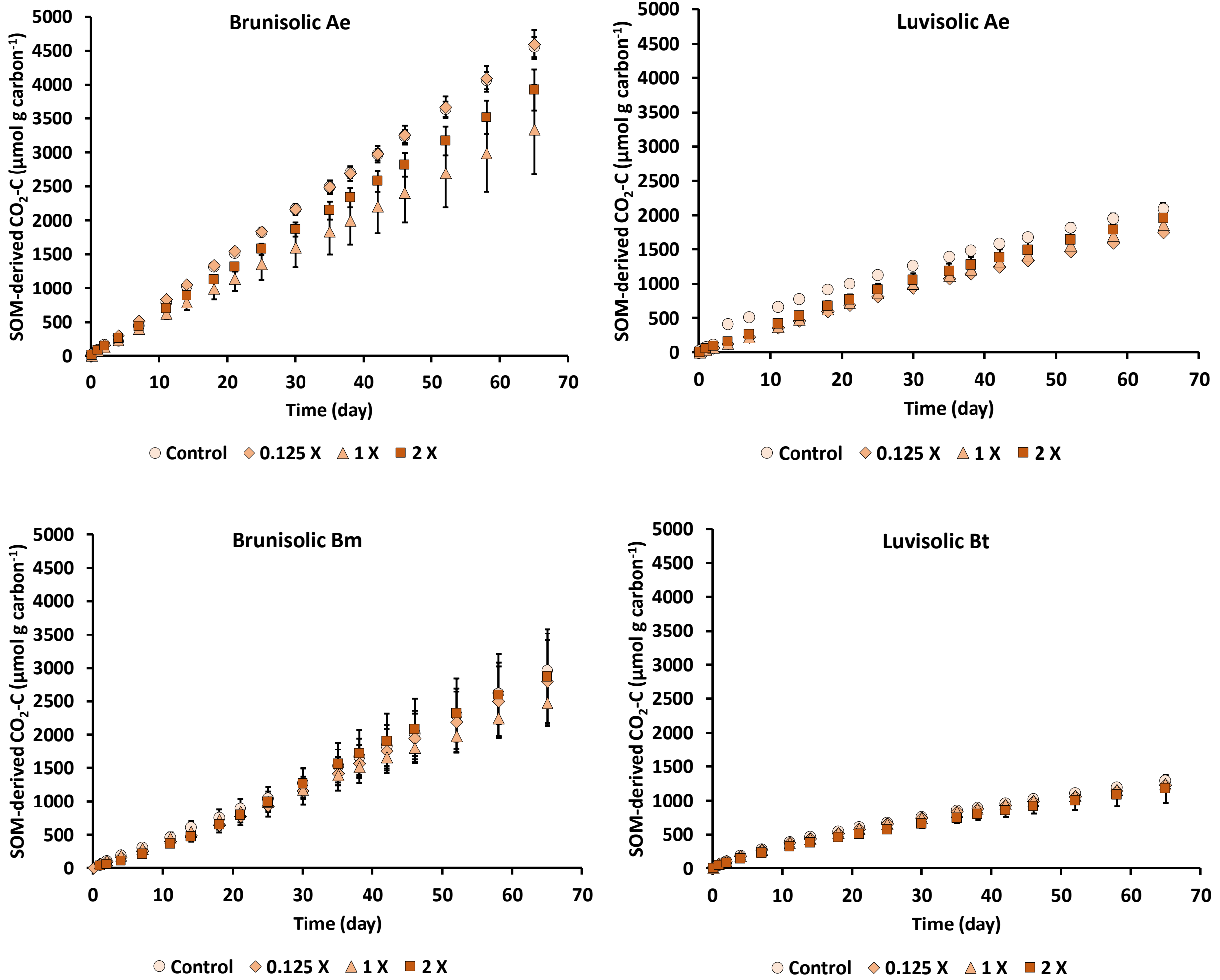

Figure 3.1. Cumulative soil organic matter (SOM) derived CO_2-C (µmol g_{carbon}^{-1}) emitted over the 65 day incubation from the Woodbend (Brunisol) Ae and Bm and Cooking Lake (Luvisol) Ae and Bt horizons. Values are means (n=4 for control, 0.125x, and 1x treatments; n=3 for 2x treatment). Error bars represent standard error.

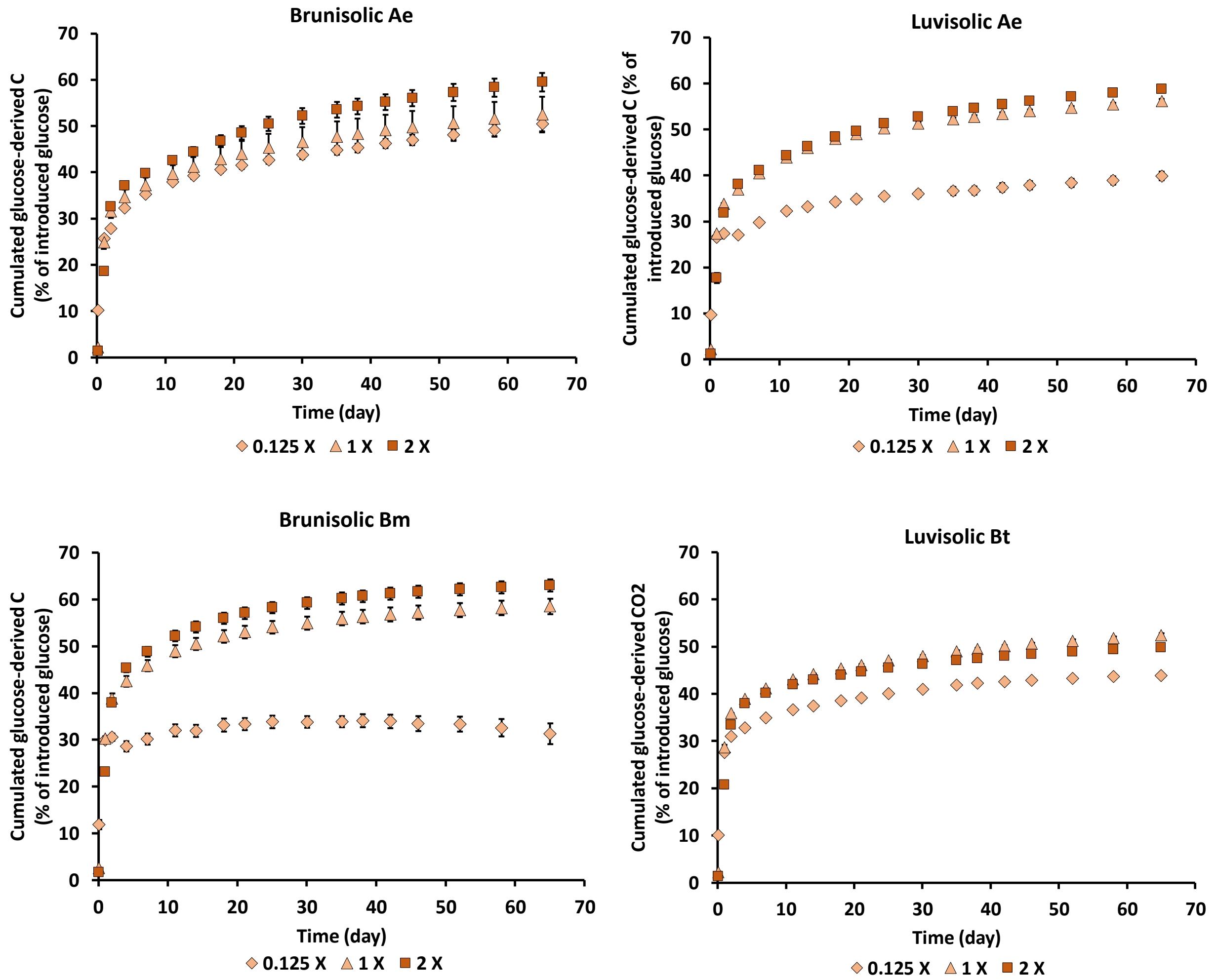

Figure 3.2. Cumulative glucose-derived C (carbon) relative to the glucose introduced at the beginning of the experiment (%) for the Woodbend (Brunisol) Ae and Bm and Cooking Lake (Luvisol) Ae and Bt horizons amended with glucose at three different rates (0.125x MB-C, 1x MB-C, and 2x MB-C). Values are means (n=4 for 0.125x and 1x treatments; n=3 for 2x treatment). Error bars represent one standard error from the mean.

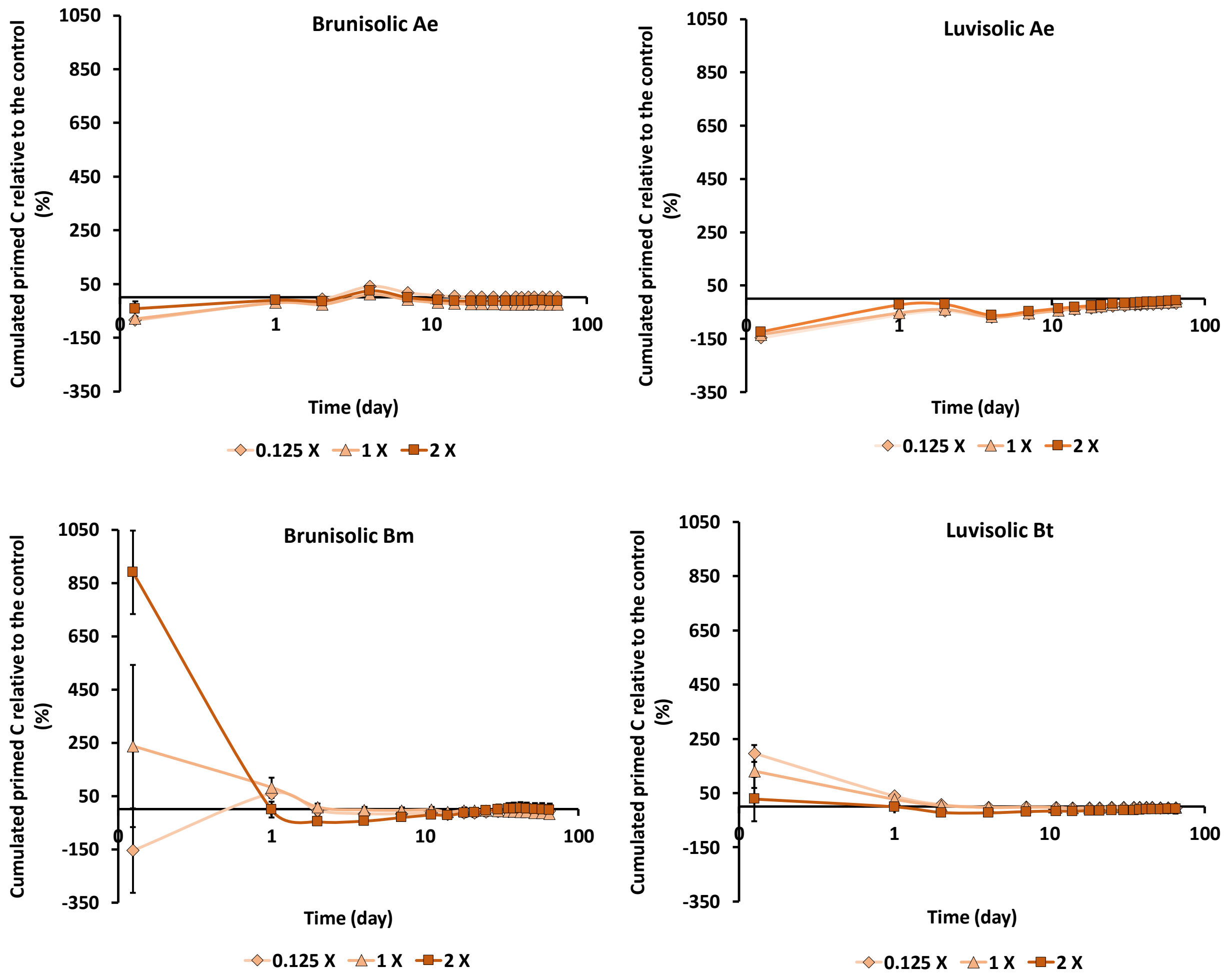

Figure 3.3. Cumulative primed C (carbon) relative to the control (%) for the Woodbend (Brunisol) Ae and Bm and Cooking Lake (Luvisol) Ae and Bt horizons amended with glucose at three different rates (0.125x MB-C, 1x MB-C, and 2x MB-C). Time is shown on a logarithmic scale. Values are means (n=4 for 0.125x and 1x treatments; n=3 for 2x treatment). Error bars represent one standard error from the mean.

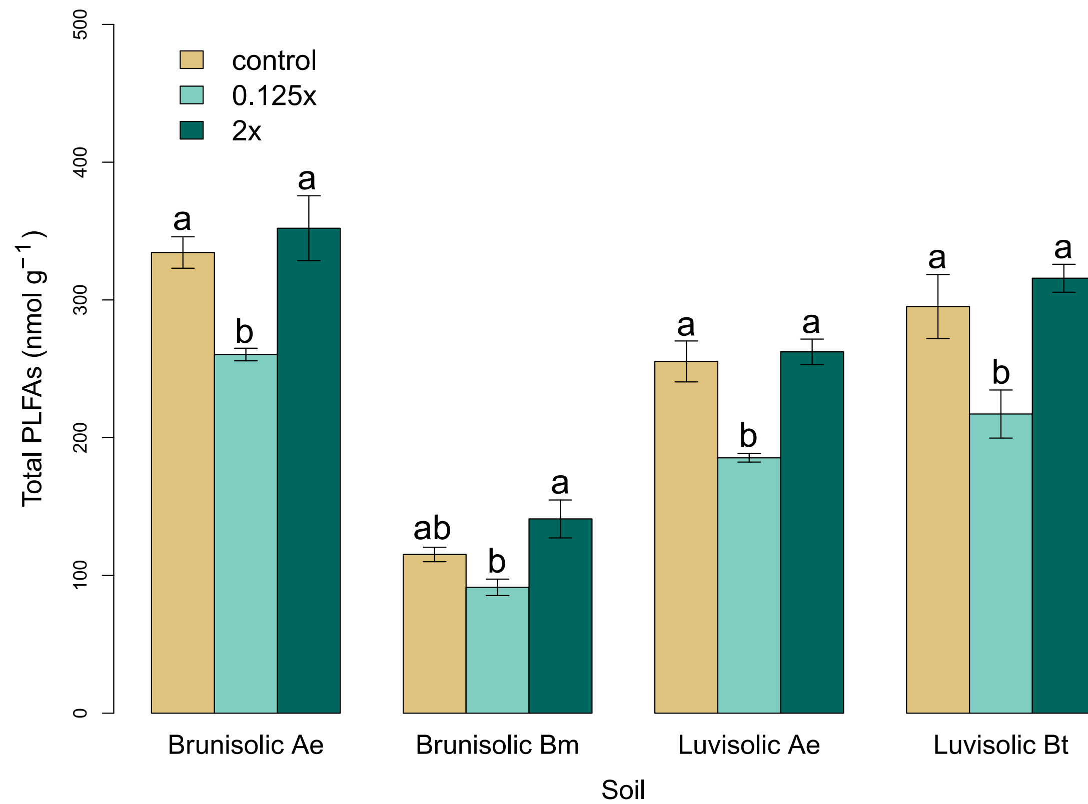

Figure 3.4. Mean total PLFAs (nmol g^{-1}) for each soil and glucose treatment. Error bars represent one standard error. Different lowercase letters represent significant differences ($p<0.05$, Tukey's test) within each soil.

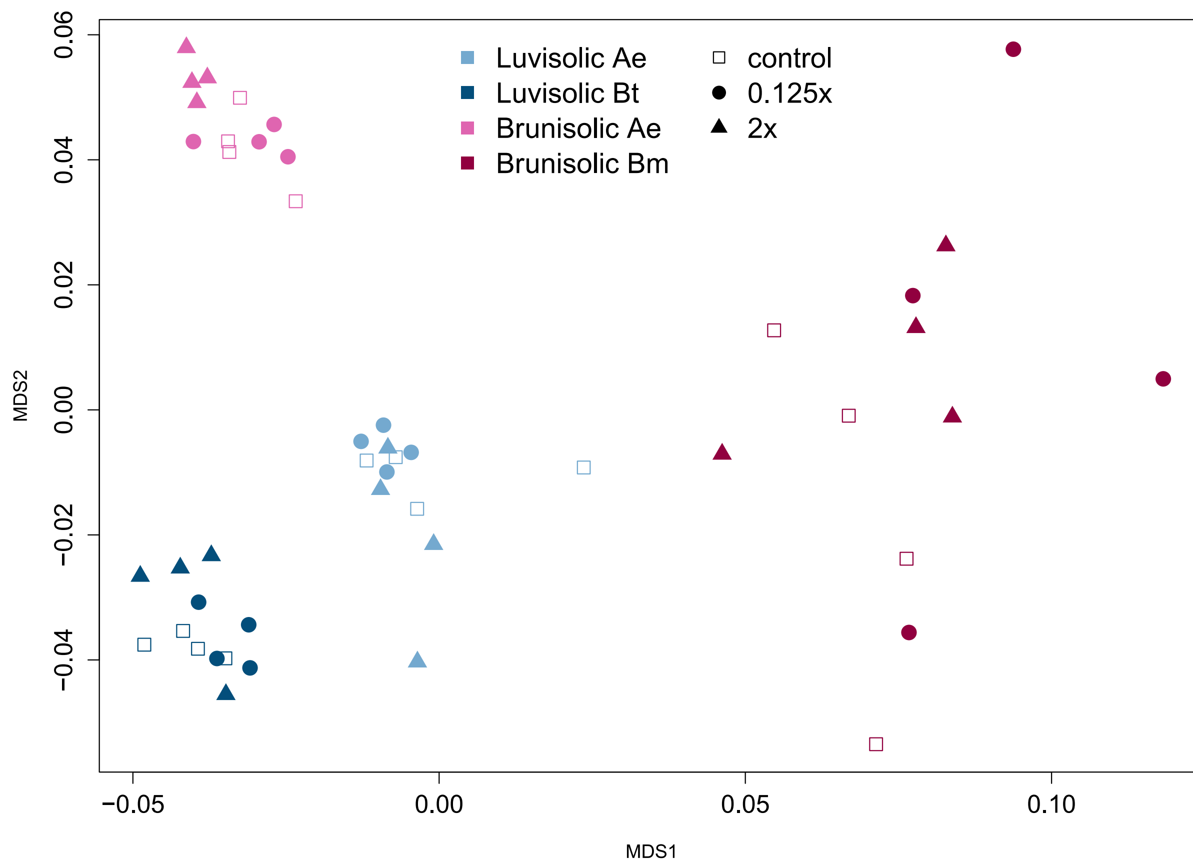

Figure 3.5. MDS ordination of PLFAs (mol %) from the different soils (Luvisolic Ae, Luvisolic Bt, Brunisolic Ae, and Brunisolic Bm) and glucose treatments (control, 0.125x, and 2x) used in PLFA analysis. A two-dimensional ordination with a stress of 10.1% was achieved after 20 iterations.

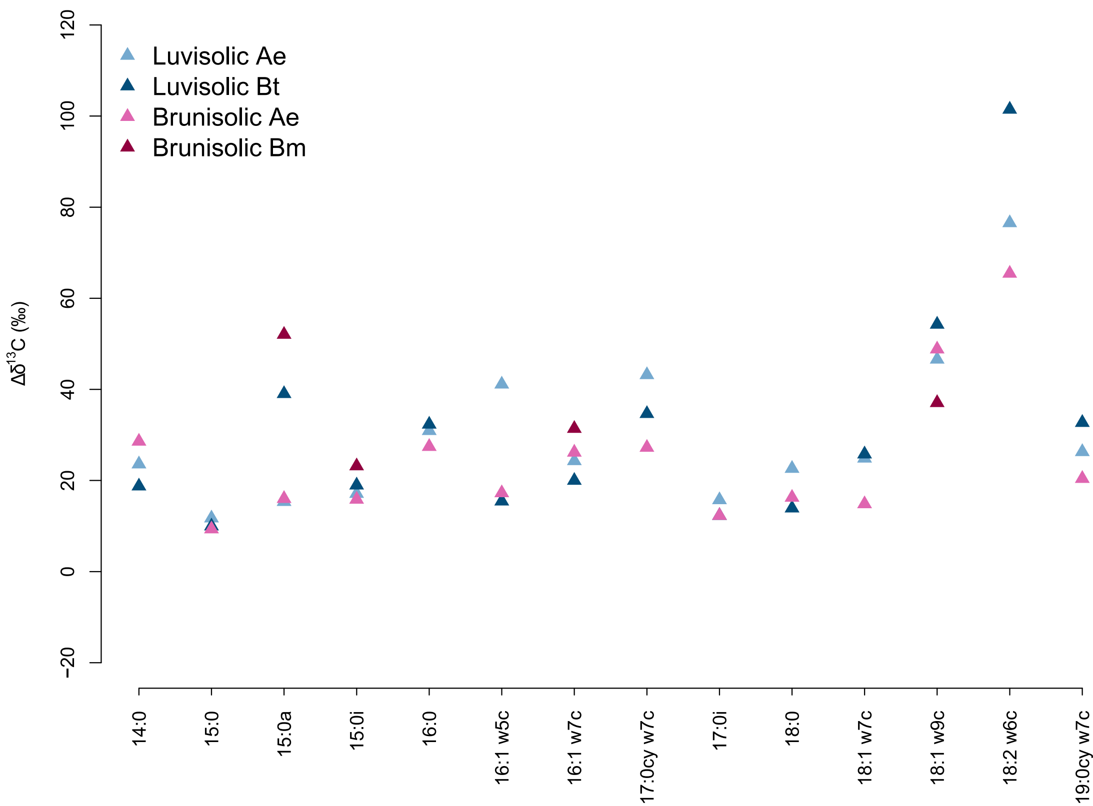

Figure 3.6. Mean $\Delta\delta^{13}C$ (‰) enrichment of individual PLFAs from the four different soils (Luvisolic Ae and Bt, Brunisolic Ae and Bm) for the 2x glucose treatment relative to the controls.

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Chapter 4 – CONCLUSIONS

In this thesis I worked to answer two overarching research questions:

1. How could rhizosphere microorganisms be affected by climate change in Alberta, and what could this mean for boreal carbon fluxes?
2. Can we observe differences in rhizosphere and bulk soil of forest floor material from the boreal forest, and if differences are observed, what are the implications?

In this chapter I summarize the findings and assess the answers to the two main research questions.

4.1. Summary and assessment of the first research question

I investigated two aspects of climate change in Alberta: vegetation shifts and changes in root exudation with expected increases in atmospheric CO_2. The boreal forest covers over 57% of Alberta's area (Alberta Wilderness Association, 2015). The boreal forest is the largest terrestrial sink of carbon on Earth (Watson et al., 2000), with global estimates of carbon stocks averaging 1095 Pg (Bradshaw and Warkentin, 2015). Clearly, the boreal forest is an important ecosystem for not only Alberta, but the entire globe. Vegetation shifts in the boreal forest of western Canada are expected to result in a shift from coniferous to deciduous dominated stands (Bradshaw et al., 2009). Aspen regeneration in former spruce stands resulted in microbial communities more comparable to aspen stands in less than two decades, with these changes occurring more quickly in the rhizosphere compared to bulk soil. Aspen trees were found to influence their rhizosphere to a greater degree than spruce. This research indicates that vegetation shifts have the potential to cause immediate and profound changes in the rhizosphere.

I performed an incubation experiment to assess the impact of glucose (used as a model root exudate) on soil organic matter mineralization and microbial communities in two soil types typical to Alberta's boreal forest. Priming, an increase in SOM mineralization resulting from labile C addition, has been found to occur in grassland and forest soils previously (for example, Karhu et al., 2016; Fan et al., 2013; Blagodatskaya et al., 2007; Ohm et al., 2007). Significant priming did not occur by the end of the incubation, indicating that increases in root exudation and root biomass with climate change may not significantly increase SOM mineralization in the soils studied. Primed C by the end of the incubation did not differ depending on soil texture, soil depth, or glucose addition rate, which contrasted the findings of other researchers (Karhu et al., 2016; Blagodatskaya and Kuzyakov, 2008). For the boreal, our results indicate that changes in rhizodeposition with climate change may not result in a significant loss of carbon from this ecosystem. Other research contradicts our findings though (Karhu et al., 2016; Lindén et al., 2014; Fan et al., 2013), and our experiment indicated that priming effects can be highly dependent on time of measurement and timing of labile C addition. For example, early in the incubation positive priming was observed in subsoil horizons and negative priming was observed in the topsoil. Priming observed early in the incubation led us to conclude that if labile C is added regularly to these soils, priming may occur in subsoil horizons but SOM may be protected in topsoil horizons; if labile C is added periodically (once or twice per month), substrate-C appears to have little effect on SOM mineralization. In terms of the microbial community, no shift in composition was observed after the two-month incubation. Fungi were found to be important in the uptake of labile C, in accordance with findings in previous studies (Nottingham et al., 2009; Paterson et al., 2007; Treonis et al. 2004; Butler et al. 2003).

Rhizosphere microorganisms may be more affected by vegetation shifts than by changes in rhizodeposition alone, at least in terms of microbial community composition. In the EMEND study, microbial community composition and function changed as a result of aspen regeneration in former spruce stands. In the incubation, microbial community composition did not change as a result of labile C addition by the end of two months. However, it is possible that changes occurred earlier in the incubation, which our analysis did not capture. Labile C addition may have played a role in microbial carbon use efficiency, as soils amended with glucose at the 0.125x MB-C rate respired less of the initial glucose C compared the 1x and 2x MB-C treatments.

It is not clear from this research how climate change will affect soil carbon fluxes. I assessed two very different soil materials: forest floor and mineral soil. The forest floor is the site of highest microbial activity in soil (Foster and Bhatti, 2006; Coleman et al., 2004) and in Canada's boreal it stores about a quarter of the ecosystem carbon (Kurz et al., 2013). While upper soil horizons are generally more carbon rich, subsoil could play an important role in the soil carbon balance with climate change. In the boreal forest, the top one metre of soil stores 112 Pg of soil organic carbon (Jobbágy and Jackson, 2000) and in Canada's boreal forest SOM in the mineral soil, to a one metre depth, stores approximately 40% of the ecosystem carbon (Kurz et al., 2013). At EMEND, the aspen rhizosphere was found to exhibit the greatest basal respiration, indicating that climate change induced vegetation shifts could increase soil carbon flux. This could make vegetation shifts a positive feedback for climate change. The incubation showed no considerable increase in SOM decomposition with added labile C at the end of the incubation, but in the early stages positive priming was observed in B horizons and negative priming in A horizons. The results of the incubation indicate that the effect of substrate addition on primed C may depend heavily on the nature of rhizodeposition itself and how climate change impacts patterns of rhizodeposition (for

example, as a result of climate change induced increases in root exudation and biomass, does rhizodeposition become a more continuous process?).

4.2. Summary and assessment of the second research question

Even in densely rooted forest floor material clear differences were observed in microbial communities between rhizosphere and bulk soil. Microbial community composition (determined via PLFA analysis) differed between rhizosphere and bulk soil, and microbial community function (determined via MSIR) differed in the aspen stands. The rhizosphere had a significantly higher proportion of fungi and a higher gram negative to gram positive bacteria ratio compared to bulk soil. Natural abundance ^{13}C PLFA analysis was used to investigate differences in carbon source acquisition between rhizosphere and bulk soil. Fungi and gram-negative bacteria biomarkers in the rhizosphere showed ^{13}C depletion compared to bulk forest floor, indicating that these rhizosphere microbes were accessing more recently fixed carbon than in bulk soil. This research demonstrates that the rhizosphere has significant influence over its microbial community, especially when looking at aspen dominated stands. It also highlights the usefulness of natural abundance PLFA stable isotope probing in assessing microbial carbon use.

While the incubation experiment did not look specifically at differences between rhizosphere and bulk soil, it revealed that fungi are a key microbial group in utilizing added labile C, whether feeding directly on the glucose or indirectly on dead bacterial biomass that had incorporated the ^{13}C label. Other researchers have found that labile C addition increased the activity of r-strategists but not K-strategists such as fungi (Blagodatskaya et al., 2007; Hamer and Marschner, 2005; Fontaine et al., 2003), while in the current study percent fungi increased in the A horizons by the end of the incubation, paired with high ^{13}C enrichment of fungal PLFA biomarkers. The results of the incubation are supported by other research that has found that fungi tend to uptake added ^{13}C

labelled substrates, becoming strongly enriched (Nottingham et al., 2009; Paterson et al., 2007; Treonis et al., 2004; Butler et al., 2003). In the EMEND study, rhizosphere soil had a higher proportion of fungi and natural abundance ^{13}C analysis revealed that fungi appeared to be utilizing more recently fixed C over microbially processed C, further indicating their importance in the rhizosphere. Both the EMEND study and incubation demonstrate that fungi are important rhizosphere microorganisms responsible for uptake and processing of labile C in forest floor and mineral soils.

4.3. Implications for future research

The EMEND field study looked at microbial communities and carbon in forest floor material while the incubation assessed mineral soil. Given the very different composition of these materials (one being organic soil, the other mineral), and differences observed with depth in the incubation, future studies of carbon dynamics in the boreal should assess both materials as important parts of the boreal ecosystem. Researchers tend to focus on either one soil material type or the other, but by analyzing both forest floor and mineral soil we can gain a deeper understanding of boreal carbon dynamics. It is important to tease apart whether the forest floor may be affected by climate change induced vegetation shifts and changes in root exudation differently than mineral soil, and what this could mean for carbon storage. It would be valuable to assess priming in forest floor material at EMEND and investigate the effect of vegetation shifts on mineral soil with depth at the same sites. Additionally, I focused on two effects of climate change in this thesis but, there are numerous ways in which climate change could affect soil carbon dynamics and microbial communities such as changes in temperature, precipitation, and freeze-thaw cycles (Price et al., 2013). Soil scientists need to consider all aspects in which climate change may alter soil dynamics.

If we want to better understand soil carbon dynamics, soil sampling should include separation of rhizosphere and bulk soil. Traditional soil sampling does not capture the role that the rhizosphere plays in soil microbial communities. The rhizosphere will come to play an increasingly important role in soil science as climate change has the potential to alter the rhizosphere through its intimate connection with aboveground vegetation. If we want to understand how the rhizosphere will be affected by climate change in years to come and how it affects processing of soil carbon, future research must analyze the rhizosphere separately from bulk soil and make it a key factor in soil science research.

Given the important role that the boreal plays in global carbon storage (Bradshaw and Warkentin, 2015; Watson et al., 2000), more research on priming should be conducted in soils common to the boreal. While there are papers investigating priming in the boreal (Karhu et al, 2016; Linkosalmi et al., 2015; Lindén et al., 2014; Fan et al., 2013; Chigineva et al., 2009), most studies have focused on grassland and agricultural systems (for example, Shahzad et al., 2018; Paterson and Sim, 2013; Bird et al., 2011; Blagodatskaya et al., 2007; Fontaine et al., 2007). There are also contrasting findings concerning whether positive priming occurs in boreal soils and whether priming has the potential to cause considerable SOM destabilization. Fan et al. (2013), Lindén et al. (2014), and Karhu et al. (2016) observed positive priming while Chigineva et al. (2009) and Linkosalmi et al. (2015) found negative or no priming. While some have proposed that the magnitude and direction of priming depends on labile C addition relative to microbial biomass (Blagodatskaya and Kuzyakov, 2008), others have found results that do not adhere to these models (Karhu et al., 2016). Additionally, while many mechanisms for priming have been conceived (Kuzyakov, 2002; Blagodatskaya and Kuzyakov, 2008), research on priming generally considers these mechanisms after the experiment, without designing experiments to explicitly test which

mechanisms are at work. Improved knowledge of the mechanisms at play during positive, negative, and neutral priming would allow us to better understand what is happening to soil microbial communities. Further research explicitly looking at how the timing of labile C addition affects priming would enable scientists to apply these priming experiments to predictions about soil carbon flux given changes in plant exudation.

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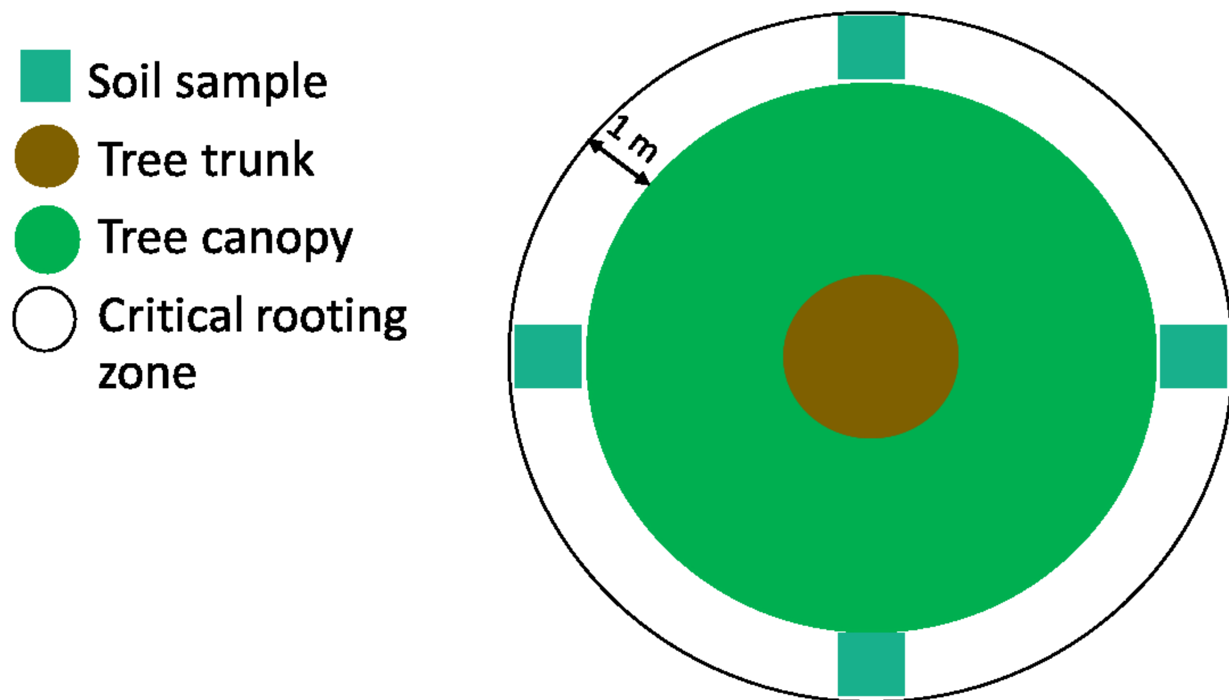
Appendix 1. Soil sampling diagram.



At each sampling site, a representative tree was selected and soil samples taken within the tree's critical rooting zone, in each cardinal direction. At each square marked "Soil sample" in the diagram, a rhizosphere and bulk soil sample were collected separately. Four rhizosphere samples and four bulk soil samples were collected at each sampling site, and composited into one rhizosphere sample and one bulk soil sample.

Appendix 2. EMEND raw permMANOVA outputs.

Raw permMANOVA output (using Adonis function in R) for EMEND PLFA microbial community composition analysis with site type (mature spruce, clear-cut spruce, mature aspen, clear-cut aspen) and sample type (rhizosphere and bulk soil).

```
Call:
adonis(formula = plfa ~ site_type * sample_type, distance = "bray")

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                      Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)    
site_type              3   0.18492 0.061640 21.2524 0.49310  0.001 ***
sample_type            1   0.02652 0.026524  9.1451 0.07073  0.001 ***
site_type:sample_type  3   0.00985 0.003283  1.1321 0.02627  0.304    
Residuals             53   0.15372 0.002900         0.40990           
Total                 60   0.37501                  1.00000           
```

Raw permMANOVA output (using Adonis function in R) for EMEND MSIR microbial community function analysis with site type (mature spruce, clear-cut spruce, mature aspen, clear-cut aspen) and sample type (rhizosphere and bulk soil).

```
Call:
adonis(formula = msir ~ site_type * sample_type, distance = "bray")

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

                      Df SumsOfSqs   MeanSqs F.Model      R2 Pr(>F)   
site_type              1  0.029726 0.0297260  5.5625 0.13327  0.002 **
sample_type            1  0.006076 0.0060758  1.1369 0.02724  0.352   
site_type:sample_type  1  0.016245 0.0162450  3.0399 0.07283  0.024 * 
Residuals             32  0.171008 0.0053440         0.76666          
Total                 35  0.223055                   1.00000          
```

Appendix 3. EMEND natural abundance $\delta^{13}C$ (‰) PLFA raw values.

Natural abundance $\delta^{13}C$ (‰) raw data values for individual PLFAs. Each sample type has nine replicates. Peak intensity of PLFAs on the GC-IRMS were considered in analysis, as low peak intensity indicates highly variable results. PLFAs with a peak intensity <100 mV were rejected, between 100 to 300 mV were included in the ^{13}C PLFA analysis but should be interpreted with caution: and a peak intensity >300 mV is considered reliable. Interpret with caution: 14:0i, 14:0, 15:0, 15:1i ω6c, 16:0i, 16:1 ω5c, 17:0a, 17:0cy ω7c, 18:0, 19:0cy ω7c, and 19:0. Reliable: 15:0i, 15:0a, 16:0, 18:2 ω6c, 18:1 ω9c, and 18:1 ω7c.

Site	Sample type	14:0	14:0i	15:0	15:0a	15:0i	15:1i w6c	16:0	16:0i	16:1 w5c	17:0a	17:0cy w7c	18:0	18:1 w7c	18:1 w9c	18:2 w6c	19:0	19:0cy w7c
11C	Spruce-Rhizo	-31.39		-29.75	-24.85	-24.55	-25.77	-28.67	-25.20	-26.07	-27.56	-26.20	-27.79	-27.72	-26.90	-29.51	-30.11	-27.90
12C	Spruce-Rhizo	-31.50		-30.70	-25.93	-26.04		-29.18	-26.08	-25.51	-28.27	-27.48	-28.02	-28.76	-27.29	-30.37	-29.81	-28.41
11A	Spruce-Rhizo	-29.03		-30.26	-24.84	-24.45		-28.84	-24.69	-25.47	-28.00	-26.87	-27.86	-28.57	-26.63	-30.28	-30.15	-28.05
12B	Spruce-Rhizo	-30.29		-31.15	-25.77	-25.31		-29.19	-27.23	-23.14	-28.98	-27.56	-28.20	-28.73	-26.63	-31.15	-30.54	-29.09
10B	Spruce-Rhizo	-31.25			-25.99	-26.00	-29.76	-30.65	-27.47	-27.76	-30.10	-28.02	-29.43	-28.89	-28.80	-31.60	-30.82	-30.37
11B	Spruce-Rhizo	-31.38			-25.42	-25.28		-29.11	-25.90	-25.39	-28.12	-27.20	-28.78	-29.27	-27.62	-31.02	-30.36	-28.67
12A	Spruce-Rhizo	-28.79		-30.12	-24.65	-24.66		-28.93	-24.71	-25.47	-28.73	-26.73	-28.87	-28.41	-26.41	-30.51	-30.70	-28.26
10A	Spruce-Rhizo	-30.32		-30.78	-25.85	-25.30	-25.76	-29.87	-26.98	-26.62	-28.26	-27.01	-28.54	-29.80	-28.15	-31.42	-29.74	-29.50
10C	Spruce-Rhizo	-31.61	-25.60		-26.07	-25.82	-27.47	-29.92	-26.14	-26.87	-29.16	-28.10	-28.35	-29.45	-29.21	-30.80	-30.08	-29.13
12C	Spruce-Bulk	-32.13	-26.80	-30.13	-25.26	-25.59	-34.53	-27.99	-27.03	-25.51	-26.67	-27.17	-27.22	-28.24	-26.49	-29.54	-30.07	-29.03
10C	Spruce-Bulk	-32.38			-26.09	-26.34	-29.29	-29.30	-26.67	-26.32	-28.66	-28.32	-27.39	-28.76	-28.53	-30.16	-30.48	-29.56
12A	Spruce-Bulk	-29.10	-25.17	-29.55	-24.29	-24.51	-25.22	-28.08	-25.17	-24.71	-26.55	-26.35	-26.69	-27.39	-26.00	-28.96	-29.67	-27.83
10A	Spruce-Bulk	-30.46	-26.68	-31.91	-25.78	-25.62	-26.98	-29.14	-26.64	-26.65	-27.79	-27.80	-27.42	-29.32	-27.15	-30.95	-30.07	-29.44
11B	Spruce-Bulk	-29.79			-25.05	-24.85		-28.51	-25.88	-25.70	-27.05	-26.20	-28.12	-28.83	-26.84	-30.51	-30.30	-28.44
12B	Spruce-Bulk	-29.62			-24.77	-24.48		-27.46	-26.22	-22.26	-27.49	-27.21	-26.83	-28.29	-25.90	-30.23	-30.33	-28.59
10B	Spruce-Bulk	-32.39	-25.43		-25.93	-25.59	-28.97	-29.25	-27.20	-26.12	-28.01	-27.28	-27.66	-28.63	-28.22	-30.96	-31.28	-28.91
11C	Spruce-Bulk	-29.90		-29.98	-23.66	-23.42	-23.90	-27.68	-23.38	-25.35	-27.07	-26.02	-26.44	-27.38	-26.46	-29.22	-30.32	-27.75
11A	Spruce-Bulk	-29.02		-29.30	-24.23	-23.76		-27.24	-28.02	-24.19	-26.23	-26.29	-27.02	-27.89	-25.34	-28.42	-30.30	-28.40
7B	Aspen-Rhizo	-29.60	-24.95	-32.22	-25.19	-26.06	-26.27	-30.77	-26.07	-25.67	-24.58	-25.83	-28.81	-28.45	-28.38	-30.89	-29.43	-36.07
9C	Aspen-Rhizo	-30.87	-27.95	-32.01	-26.71	-27.40	-27.80	-32.36	-27.95	-28.36	-27.98	-29.88	-30.47	-30.29	-30.46	-32.78	-30.31	-37.19
9A	Aspen-Rhizo	-28.34	-26.65	-31.34	-25.96	-26.63	-27.46	-31.26	-27.48	-27.97	-24.58	-27.55	-28.92	-30.14	-29.05	-32.16	-29.56	-36.00
8A	Aspen-Rhizo	-28.97	-26.10	-32.53	-25.91	-26.08	-26.85	-31.69	-27.37	-27.20	-26.73	-28.65	-30.46	-30.79	-29.47	-32.84	-30.20	-30.06
7A	Aspen-Rhizo	-29.51	-27.00	-32.44	-25.99	-26.67	-29.16	-31.83	-27.16	-27.80	-26.60	-28.85	-30.91	-30.87	-30.31	-32.17	-30.62	-36.42
8B	Aspen-Rhizo	-30.82	-27.64	-31.99	-26.45	-27.02	-29.44	-32.46	-27.64	-28.45	-25.80	-28.88	-31.24	-31.34	-31.38	-32.86	-30.31	-36.66
7C	Aspen-Rhizo	-30.00	-26.04	-32.01	-25.59	-26.31	-29.36	-32.25	-27.17	-27.16	-26.15	-28.55	-29.79	-30.49	-30.39	-32.91	-31.00	-37.23
9B	Aspen-Rhizo	-28.38	-26.38	-31.07	-25.83	-25.96	-27.25	-30.76	-27.30	-27.54	-26.55	-28.45	-29.36	-30.45	-29.01	-31.29	-30.21	-29.78
8C	Aspen-Rhizo	-30.29	-27.02	-31.98	-26.42	-26.83	-28.39	-32.48	-27.71	-29.75	-26.21	-28.67	-31.72	-31.54	-30.85	-32.54	-30.80	-39.17
8B	Aspen-Bulk	-30.00	-26.00		-25.78	-26.97	-29.12	-31.34	-27.32	-26.96	-26.26	-28.08	-29.26	-29.28	-28.82	-30.78	-29.93	-36.66
9B	Aspen-Bulk	-27.74	-25.93	-29.93	-25.21	-26.00	-27.31	-29.85	-26.32	-26.15	-24.73	-27.11	-28.22	-28.30	-27.23	-29.97	-29.77	-36.85
9A	Aspen-Bulk	-28.16	-26.14	-30.30	-25.34	-26.28	-26.36	-30.05	-26.71	-27.00	-25.56	-28.22	-28.74	-28.96	-27.93	-31.61	-30.50	-30.28
7A	Aspen-Bulk	-30.37	-26.63	-31.34	-25.89	-26.79	-31.16	-31.87	-27.48	-27.86	-26.03	-28.90	-29.87	-29.97	-29.65	-32.25	-30.06	-35.34
7C	Aspen-Bulk	-29.99	-25.90		-25.83	-26.66	-30.22	-32.05	-26.99	-26.48	-25.34	-27.79	-30.15	-29.82	-29.12	-31.39	-30.71	-35.82
9C	Aspen-Bulk	-29.76	-27.46	-31.65	-26.53	-26.67	-28.34	-31.64	-27.56	-27.49	-26.11	-28.33	-30.96	-29.95	-29.24	-32.61	-30.16	-36.79
7B	Aspen-Bulk	-29.91	-26.77	-32.51	-26.00	-26.47	-29.48	-31.76	-26.85	-26.60	-26.77	-28.93	-29.86	-30.42	-29.49	-31.89	-30.36	-36.45
8C	Aspen-Bulk	-29.76	-26.34	-31.55	-26.02	-26.68	-28.15	-32.20	-27.47	-27.78	-26.32	-28.93	-30.96	-31.13	-30.33	-32.93	-30.56	-38.01
8A	Aspen-Bulk	-28.41	-26.04	-31.01	-25.32	-25.42	-29.11	-30.53	-26.60	-26.36	-25.74	-28.09	-30.03	-29.44	-28.61	-31.43	-29.89	-29.89

Appendix 4. EMEND raw total PLFAs (nmol g^{-1}) data.

Raw total PLFAs (nmol g^{-1}) data for EMEND for the four site types (mature spruce, clear-cut spruce, mature aspen, clear-cut aspen) and two sample types (rhizosphere and bulk soil). Further information on EMEND compartments can be found on The EMEND Project website (http://www.emendproject.org).

Site type	Site	GPS coordinates	EMEND compartment	Sample type	Total PLFAs (nmol g^{-1})
Mature spruce	10A	N: 56.74874 W: 118.41718	D:889	Bulk	19837.6
	10A	N: 56.74874 W: 118.41718	D:889	Rhizosphere	18421.2
	10B	N: 56.44563 W: 118.25080	D:889	Bulk	9326.3
	10B	N: 56.44563 W: 118.25080	D:889	Rhizosphere	13963.4
	10C	N: 56.45015 W: 118.25061	D:889	Rhizosphere	10941.8
	10C	N: 56.45015 W: 118.25061	D:889	Bulk	12404.4
	11A	N: 56.79063 W: 118.36034	G:918	Bulk	5222.3
	11A	N: 56.79063 W: 118.36034	G:918	Rhizosphere	7794.7
	11B	N: 56.79202 W: 118.36230	G:918	Bulk	4251.0
	11B	N: 56.79202 W: 118.36230	G:918	Rhizosphere	5472.7
	11C	N: 56.47336 W: 118.21552	G:918	Rhizosphere	6930.4
	11C	N: 56.47336 W: 118.21552	G:918	Bulk	6038.1
	12A	N: 56.78694 W: 118.36583	G:915	Rhizosphere	6279.4
	12A	N: 56.78694 W: 118.36583	G:915	Bulk	5193.6
	12B	N: 56.47127 W: 118.22017	G:915	Bulk	4467.2
	12B	N: 56.47127 W: 118.22017	G:915	Rhizosphere	5210.9
	12C	N: 56.78598 W: 118.36977	G:915	Bulk	3531.8
	12C	N: 56.78598 W: 118.36977	G:915	Rhizosphere	4806.9
Clear-cut spruce	4A	N: 56.44598 W: 118.23587	C:892	Rhizosphere	6205.6
	4B	N: 56.74940 W: 118.40495	C:892	Rhizosphere	9851.5
	4C	N: 56.44592 W: 118.24295	C:892	Bulk	6250.7
	4C	N: 56.44592 W: 118.24295	C:892	Rhizosphere	8466.7
	5A	N: 56.44563 W: 118.24270	C:892	Rhizosphere	7993.6
	5B	N: 56.44596 W: 118.24041	C:892	Bulk	6370.0
	5B	N: 56.44596 W: 118.24041	C:892	Rhizosphere	7439.6
	5C	N: 56.75036 W: 118.40070	C:892	Rhizosphere	8088.8
	5C	N: 56.75036 W: 118.40070	C:892	Bulk	6635.3
	6A	N: 56.75058 W: 118.39248	C:880	Rhizosphere	7546.2
	6B	N: 56.75029 W: 118.39866	C:892	Rhizosphere	8605.0
	6C	N: 56.74999 W: 118.39799	C:892	Rhizosphere	8321.9
	6C	N: 56.74999 W: 118.39799	C:892	Bulk	7217.2
Mature aspen	7A	N: 56.75147 W: 118.32588	A:852	Rhizosphere	6476.2
	7A	N: 56.75147 W: 118.32588	A:852	Bulk	5596.2
	7B	N: 56.75185 W: 118.32731	A:852	Rhizosphere	8037.6
	7B	N: 56.75185 W: 118.32731	A:852	Bulk	6979.0

	7C	N: 56.75177 W: 118.32803	A:852	Bulk	6745.0
	7C	N: 56.75177 W: 118.32803	A:852	Rhizosphere	9074.0
	8A	N: 56.74729 W: 118.36064	B:862	Bulk	8179.4
	8A	N: 56.74729 W: 118.36064	B:862	Rhizosphere	8535.7
	8B	N: 56.74726 W: 118.36147	B:862	Bulk	6513.6
	8B	N: 56.74726 W: 118.36147	B:862	Rhizosphere	8088.5
	8C	N: 56.74589 W: 118.36186	B:862	Rhizosphere	8275.8
	8C	N: 56.74589 W: 118.36186	B:862	Bulk	6529.6
	9A	N: 56.49076 W: 118.21325	I:940	Bulk	35700.7
	9A	N: 56.49076 W: 118.21325	I:940	Rhizosphere	21650.7
	9B	N: 56.49071 W: 118.21467	I:940	Rhizosphere	8250.5
	9B	N: 56.49071 W: 118.21467	I:940	Bulk	14477.2
	9C	N: 56.81685 W: 118.37012	I:940	Bulk	16137.3
	9C	N: 56.81685 W: 118.37012	I:940	Rhizosphere	21038.0
Clear-cut aspen	1A	N: 56.44497 W: 118.19149	A:850	Rhizosphere	9151.2
	1B	N: 56.44529 W: 118.19132	A:850	Rhizosphere	5827.6
	1C	N: 56.44598 W: 118.19266	A:850	Rhizosphere	8579.3
	1C	N: 56.44598 W: 118.19266	A:850	Bulk	7823.5
	2A	N: 56.75176 W: 118. 36149	B:864	Rhizosphere	5644.3
	2B	N: 56. 75084 W: 118.36208	B:864	Rhizosphere	6391.4
	2C	N: 56.74856 W: 118.36148	B:864	Rhizosphere	5859.3
	2C	N: 56.74856 W: 118.36148	B:864	Bulk	5618.5
	3A	N: 56.81749 W: 118.37160	I:941	Rhizosphere	9575.7
	3B	N: 56.81915 W: 118.36877	I:941	Rhizosphere	7695.8
	3C	N: 56.49139 W: 118.22076	I:941	Rhizosphere	8934.6
	3C	N: 56.49139 W: 118.22076	I:941	Bulk	6811.5

Appendix 5. Soil description forms for the Woodbend and Cooking Lake sites.

Woodbend (Eluviated Dystric Brunisol)

Horizon	Depth (cm)	Munsell color (moist)	Mottles (pattern* & color)	Structure (grade, size, kind)	Texture
LFH	5-0	-	-	-	-
Ah	0-7	10YR 3/2	-	Fine, granular, weak	Loamy sand
Ae	7-22	10YR 5/3	-	Single grain, weak	Loamy sand
Bm	22-96	10YR 5/4	-	Single grain, weak	Sand

Horizon	Consistence (moist)	Coarse frags. (%vol, kind)	Roots (abundance, size, orientation)	Effervescence. (degree)	Horizon boundary (distinctness, form)
LFH	-	-	-	-	Smooth, clear
Ah	Loose	-	Plentiful, fine, horizontal	-	Wavy, clear
Ae	Loose	-	Few, fine-medium, oblique	-	Wavy, clear
Bm	Loose	-	Few, medium-coarse, horizontal and vertical	-	-

Cooking Lake (Gleyed Gray Luvisol)

Horizon	Depth (cm)	Munsell color (moist)	Mottles (pattern* & color)	Structure (grade, size, kind)	Texture
LFH	7-0	-	-	-	-
Ahe	0-4.5	10YR 3/2	-	Platy, medium, weak-moderate	Sandy clay loam
Ae	4.5-13	10YR 5/2	-	Platy, medium, moderate	Loam
Btgj	13-92	2.5YR 4/4	7.5YR 4/4 many, medium, distinct	Subangular blocky, fine-medium, medium-strong	Clay Loam
Cgj	92-100+	10YR 3/1	7.5YR 4/4 many, medium, distinct	Subangular blocky, coarse, strong	Clay Loam

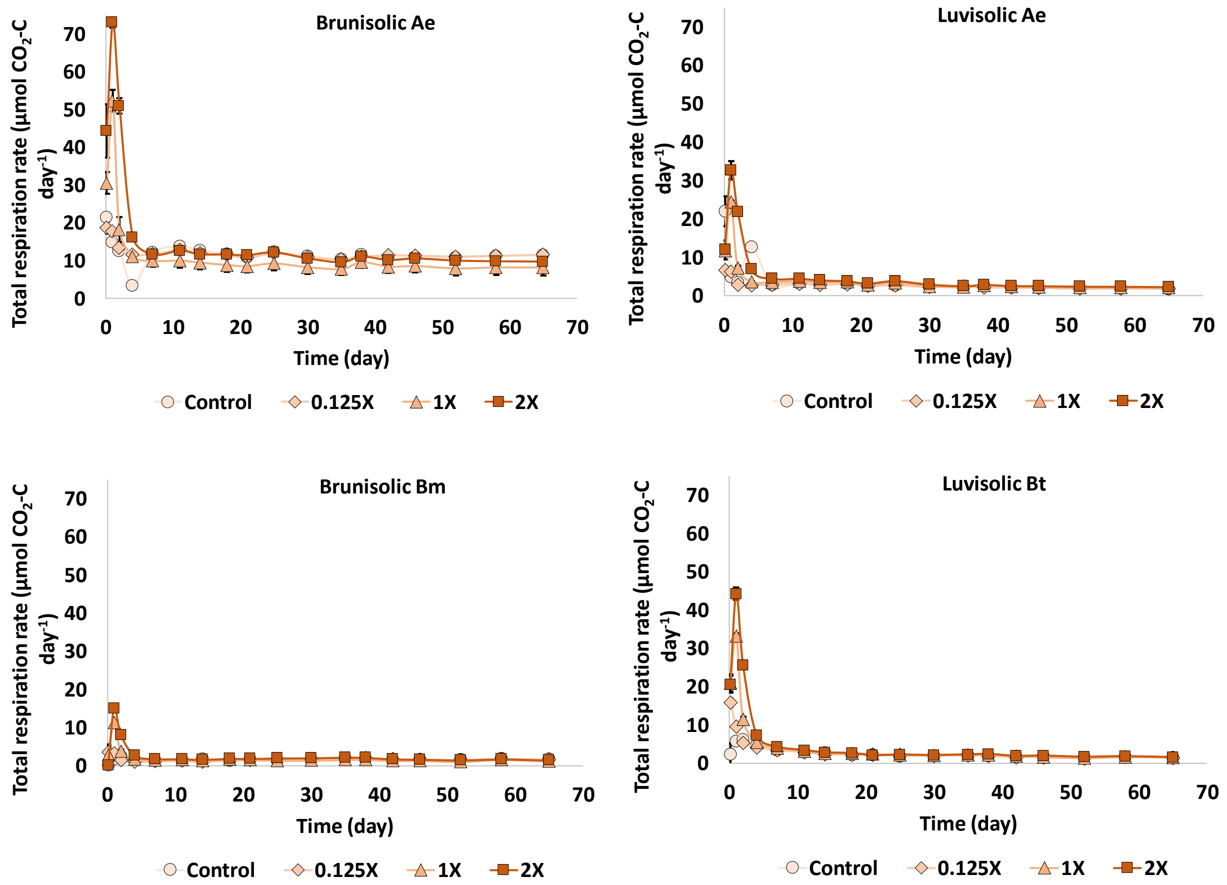
Horizon	Consistence (moist)	Coarse frags. (%vol, kind)	Roots (abundance, size, orientation)	Effervescence. (degree)	Horizon boundary (distinctness, form)
LFH	-	-	-	-	Abrupt, wavy
Ahe	Friable	-	Few, fine-medium, oblique	-	Clear, wavy
Ae	Friable	-	Few, fine-medium, horizontal	-	Clear, wavy
Btgj	Firm	-	Few, fine-medium, horizontal	-	Diffuse, wavy
Cgj	Firm	-	-	yes	-

Appendix 6. Incubation δ^{13}C (‰) PLFA raw values.

Raw δ^{13}C (‰) values of individual PLFAs in each PLFA sample used in the experiment where Sample Type refers to soil horizon (CL-Ae, CL-Bt, and WB-Ae) and glucose treatment (control, 0.125x MB-C, 1x MB-C, and 2x MB-C).

PLFA ID	Sample Type	14:0	15:0	15:0a	15:0i	16:0	16:1 ω5c	16:1 ω7c	17:0cy ω7c	17:0i	18:0	18:1 ω7c	18:1 ω9c	18:2 ω6c	19:0cy ω7c
S18	CL-Ae-0.125X-1	-25.06	-29.20	-21.93	-24.01	-23.24	-20.67	-23.20	-22.48	-25.60	-25.17	-27.39	-22.06	-20.44	-23.97
S22	CL-Ae-0.125X-2	-23.80	-29.09	-22.09	-24.26	-23.65	-22.19	-24.49	-23.42	-26.26	-24.81	-28.02	-23.17	-21.43	-27.32
S25	CL-Ae-0.125X-3	-25.54		-21.99	-24.51	-24.48	-22.03	-24.18	-23.10	-25.62	-24.96	-28.09	-23.13	-22.88	-25.14
S29	CL-Ae-0.125X-4	-25.31		-21.96	-24.34	-24.28	-22.88	-24.55	-22.73	-24.79	-26.18	-27.93	-22.43		-24.50
S39	CL-Ae-2X-1	-4.47	-21.33	-11.75	-10.52	5.14	17.81	-3.71	9.58	-11.04	-3.62	-7.39	19.27	48.21	-7.94
S48	CL-Ae-2X-2	-1.21	-17.19	-9.39	-7.28	8.01	24.19	0.10	14.99	-11.03	-5.55	-5.10	19.66	45.31	4.06
S42	CL-Ae-2X-3	-3.20		-10.72	-8.94	5.75	21.10	-1.94	15.05	-11.88	-6.19	-4.63	18.10	41.46	-6.89
S47	CL-Ae-2X-4	-2.76	-16.79	-5.25	-6.71	-4.89	13.02	-0.02	24.13	-9.63	-2.16	-4.61	23.10	54.03	-2.45
S2	CL-Ae-control-1	-27.54	-30.89	-24.77	-25.79		-26.35	-27.50	-27.55	-26.79	-26.40	-30.46	-27.46	-28.01	-30.78
S6	CL-Ae-control-2	-26.01	-30.59	-24.38	-25.36	-24.62	-21.09	-25.36	-26.90	-26.18	-26.99		-27.12	-28.61	-28.51
S11	CL-Ae-control-3			-24.87	-25.84	-30.26	-19.95	-24.72	-27.32	-26.51	-27.08				-31.38
S14	CL-Ae-control-4	-26.99	-30.72	-24.67	-25.74	-27.40	-23.40	-27.46	-28.90	-27.30	-26.71	-30.43	-27.96	-30.10	-30.23
S19	CL-Bt-0.125X-1	-26.09	-28.57	-18.45	-23.95	-23.81	-18.72	-23.29	-22.29	-26.05	-24.62	-26.33	-22.73	-18.34	-21.80
S24	CL-Bt-0.125X-2			-18.00	-23.65	-23.59		-22.57	-21.71	-24.98	-24.52	-26.89	-22.55	-16.69	-26.53
S28	CL-Bt-0.125X-3	-25.13	-29.46	-17.03	-24.05	-24.69	-23.66	-25.11	-23.57	-25.36	-24.79	-27.04	-23.41	-20.09	-23.22
S32	CL-Bt-0.125X-4			-17.29	-23.65	-24.12	-21.01	-24.57	-22.48	-26.19	-25.20	-26.71	-23.45	-19.29	-23.14
S37	CL-Bt-2X-1	-8.02	-17.44	21.14	-4.87	6.05	-7.48	-6.24	10.87	-15.88	-12.88	-5.47	30.44	78.63	-2.78
S43	CL-Bt-2X-2	-9.00	-22.87	10.98	-9.09	1.24	-7.90	-7.52	4.25	-17.69	-15.80	-7.30	22.84	66.19	-0.68
S45	CL-Bt-2X-3	-5.67	-19.17	15.79	-6.94	2.61	-9.37	-6.18		-9.41	-9.38	-6.02	23.80	66.45	-3.31
S40	CL-Bt-2X-4	-9.96	-22.20	11.40	-5.83	3.46	-3.80	-5.30	7.22	-17.47	-13.51	-6.46	27.64	79.44	2.96
S4	CL-Bt-control-1	-26.84	-30.41	-24.41	-25.61	-27.27	-19.44	-25.28	-27.41	-27.02	-27.04	-31.90	-28.27	-28.97	-34.45
S8	CL-Bt-control-2	-27.46	-30.36	-24.18	-25.78	-27.11	-20.06	-25.22	-27.71	-27.62	-27.24	-32.86	-27.95	-28.46	-33.23
S12	CL-Bt-control-3	-26.35	-30.29	-24.43	-25.87	-33.47	-29.31	-28.70	-27.16	-27.78	-26.74	-31.54	-28.11	-29.43	-33.35
S16	CL-Bt-control-4	-27.01	-30.51	-23.82	-25.36	-28.11	-21.57	-26.01	-26.56	-27.19	-26.24		-28.03	-28.32	-33.61
S17	WB-Ae-0.125X-1	-23.56	-31.23	-22.71	-24.03	-24.51	-24.78	-23.88	-25.94	-27.28	-26.48	-31.54	-22.08	-23.84	-28.28
S30	WB-Ae-0.125X-2	-22.74	-29.48	-22.23	-24.25	-24.30	-22.11	-23.46	-24.64	-25.97	-25.26	-31.13	-21.79	-22.59	-28.04
S21	WB-Ae-0.125X-3	-23.18	-30.37	-22.24	-23.91	-25.33	-29.64	-25.67	-24.99	-27.44	-26.17	-31.51	-21.91	-23.59	-27.32
S26	WB-Ae-0.125X-4	-23.05	-30.45	-22.63	-24.09	-26.06	-31.10	-26.67	-25.83	-27.74	-25.94	-31.47	-22.15	-23.78	-27.99
S38	WB-Ae-2X-1	0.87	-20.99	-9.34	-10.27	-2.01	-11.42	-0.55	-3.82	-17.10	-13.20	-18.48	20.85	34.41	-11.22
S41	WB-Ae-2X-2	5.46	-22.00	-8.96	-8.73	1.53	-7.72	0.61	0.04	-15.50	-10.02	-17.62	23.16	34.64	-9.24
S44	WB-Ae-2X-3	0.64	-20.69	-9.70	-9.28	1.63	-8.56	-0.83	1.39	-15.47					
S46	WB-Ae-2X-4	2.59	-19.89	-8.93	-9.32	0.15	-8.99	-0.40	0.14	-14.88	-10.11	-17.34	21.61	36.77	-9.16
S1	WB-Ae-control-1	-26.09	-29.09	-25.03	-25.08	-27.19	-25.13	-25.68	-27.52	-27.30	-27.19	-32.25	-26.83	-29.23	-30.43
S5	WB-Ae-control-2	-26.22	-30.50	-25.33	-25.29	-27.44	-26.08	-26.46	-28.05	-27.93	-27.65	-32.98	-26.95	-30.48	-30.22
S9	WB-Ae-control-3	-26.32	-30.93	-25.46	-25.41	-28.23	-29.04	-27.68	-28.18	-28.55	-27.60	-32.94	-26.95	-30.33	-30.59
S13	WB-Ae-control-4	-26.18	-30.38	-25.19	-25.21	-27.34	-25.52	-26.07	-27.48	-28.37	-27.14	-32.47	-27.10	-30.71	-29.74
S23	WB-B-0.125X-1			-15.85	-24.29	-25.27		-24.42	-23.14			-27.35	-24.20		-24.72
S27	WB-B-0.125X-2			-13.94	-23.53	-24.13	-20.12	-23.71	-22.57	-24.37	-25.48		-23.15		-24.04
S31	WB-B-0.125X-3			-12.34	-23.66	-24.07		-23.10	-21.56				-24.21		-23.72
S20	WB-B-0.125X-4			-15.48	-24.27	-24.60		-24.69	-24.71			-27.18	-23.27		-23.37
S33	WB-B-2X-1			23.02	-4.43	0.92		3.32							
S34	WB-B-2X-2			35.90	-1.11			7.77							
S35	WB-B-2X-3			22.02	-4.82	0.28		1.55					11.64		
S36	WB-B-2X-4			28.05	-2.42	3.12		3.94					9.98		
S3	WB-B-control-1			-24.87	-26.11		-22.85	-27.54	-28.23	-27.76	-27.82				
S10	WB-B-control-2			-25.44	-26.83		-22.43	-26.79	-27.46	-28.83	-26.54				
S7	WB-B-control-3			-24.48	-26.58		-23.52	-27.59	-26.47	-26.63	-25.78		-26.75		
S15	WB-B-control-4			-24.44	-25.99			-27.08	-24.29				-25.76		

Appendix 7. Incubation total CO_2 efflux rates (µmol CO_2-C g^{-1} day^{-1}).



Total CO_2 efflux rate (µmol CO_2-C g^{-1} day^{-1}) of the Brunisolic Ae and Bm and Luvisolic Ae and Bt horizons amended with glucose at three different rates (0.125x MB-C, 1x MB-C, and 2x MB-C) and of control soils with only water added. Error bars represent one standard error from the mean (n=4 for control, 0.125x, and 1x treatments; n=3 for 2x treatment).

Appendix 8. Incubation total PLFAs (nmol g^{-1}) and proportion of PLFA microbial groups (mol %).

Total PLFAs (nmol g^{-1}) and proportion of PLFA microbial groups (mol %) in the different soil horizons and glucose treatments used in PLFA analysis. Values are means (n=4) with standard deviation in parentheses. Different lowercase letters indicate significant differences between treatments within each soil /horizon combination (p values < 0.1; Tukey's test).

Site	Horizon	Treatment	Total PLFAs (nmol g^{-1})	Fungi (%)	Gram negative bacteria (%)	Gram positive bacteria (%)	Actinomycetes (%)	Protists (%)
Cooking Lake	A	Control	255.34 (29.73)a	7.15 (0.12)b	14.72 (0.56)a	24.15 (0.56)a	9.97 (0.14)a	0.00 (0.00)a
	A	0.125x	185.41 (6.19)b	7.72 (0.24)a	14.62 (0.12)a	23.14 (0.12)b	9.66 (0.26)ab	0.00 (0.00)a
	A	2x	262.37 (18.51)a	7.81 (0.04)a	14.29 (0.27)a	23.70 (0.71)ab	9.38 (0.39)a	0.07 (0.14)a
	B	Control	295.22 (46.53)a	7.91 (0.74)ab	14.14 (0.99)a	23.34 (0.36)a	10.08 (0.85)a	0.07 (0.14)b
	B	0.125x	217.19 (34.89)b	9.12 (0.91)a	14.32 (2.41)a	22.79 (1.14)a	9.64 (1.80)a	0.58 (0.09)a
	B	2x	315.76 (20.31)a	6.84 (0.63)b	15.66 (0.64)a	22.04 (2.09)a	11.47 (0.50)a	0.58 (0.09)a
Woodbend	A	Control	334.45 (22.78)a	7.85 (0.86)b	13.90 (1.14)a	23.49 (0.91)a	10.43 (0.87)a	0.00 (0.00)a
	A	0.125x	260.40 (9.16)b	9.28 (0.29)a	14.07 (2.45)a	23.20 (1.45)a	9.59 (1.68)a	0.49 (0.57)a
	A	2x	352.08 (47.09)a	7.00 (0.55)b	15.58 (1.05)a	22.85 (1.53)a	11.04 (0.46)a	0.55 (0.13)a
	B	Control	115.22 (10.53)ab	6.55 (0.36)a	15.81 (0.88)a	23.43 (1.09)a	11.06 (0.39)a	0.33 (0.23)a
	B	0.125x	91.38 (11.94)b	7.27 (0.55)a	15.46 (0.87)a	20.69 (0.68)b	11.36 (0.64)a	0.48 (0.32)a
	B	2x	141.02 (27.53)a	7.17 (0.35)a	14.89 (0.25)a	23.93 (0.49)a	11.11 (0.33)a	0.61 (0.23)a

Appendix 9. Estimation of initial glucose-C remaining in the soil and microbial biomass after the 65 day incubation.

Estimation of the fraction of initial glucose-C, for the 2x microbial biomass carbon glucose treatment, remaining in the soil and in the microbial biomass after the 65 day incubation based on gas measurements, final bulk soil isotopic signature, and final isotopic enrichment of PLFAs.

Fraction of glucose	Calculations based on	Luvisol		Brunisol	
		Ae	Bt	Ae	Bm
Remaining in the soil (%)	Gas measurements	41.2	50.2	40.5	37.0
	Bulk soil isotopic signature	40.1	49.6	37.9	35.2
Incorporated into microbial biomass (%)	PLFA ^{13}C enrichment	0.97	1.24	0.96	1.30