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Soil organic matter cycling in novel and natural boreal forest ecosystems

by

Charlotte Emma Norris

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Abstract

The western boreal forest of Canada, where the upland regions are dominated by stands of aspen (*Populus tremuloides* Michx.) and spruce (*Picea glauca* (Moench) Voss), is now home to novel ecosystems, i.e.; ecosystems composed of reclaimed stands formed from trees planted on constructed anthropogenic soils. This study set out to determine if soils from these natural and novel ecosystems differed in terms of their biogeochemical functioning. Using a multi-faceted approach this study examined several ecosystem function variables linked to soil organic matter composition, microbial communities and nitrogen fluxes. A survey of 42 sites showed that soil *n*-alkanes, biomarkers of vegetation inputs, were more concentrated and had distinct signatures in natural compared to novel ecosystems. Mineral soils from reclaimed stands, natural aspen and spruce stands showed a distinct microbial community structure as was demonstrated using phospholipid fatty acids (PLFAs) as microbial biomarkers following addition of ^{13}C-glucose in a laboratory incubation. Further probing by compound specific analysis (CSA) of the ^{13}C-enriched PLFAs determined that microbial incorporation of ^{13}C-glucose was different among soils. Solid-state nuclear magnetic resonance characterization of double-labeled (^{13}C, ^{15}N) aspen leaves and roots generated for tracer studies confirmed that isotopic enrichment across biopolymers and tissues was time dependent. In a subsequent field incubation, where the labeled aspen leaf litter was added to the forest floors of aspen and spruce stands, soil microorganisms maintained an active nitrogen cycle between fresh litter and live vegetation at both stands, yet remained structurally distinct. However, CSA indicated overlap in the ^{13}C enrichment of some PLFA biomarkers between

stands. Finally, the addition of ^{15}N labelled aspen leaf litter to reclaimed and natural forest stands demonstrated the importance of vegetation inputs not only as a source of nitrogen for growing vegetation but also as a way to improve soil moisture and soil microbial biomass on all sites. Cumulatively, these results not only enhance our understanding of organic matter cycling in natural and novel boreal forest ecosystems but, more importantly, they also provide results on conceptual ideas to guide future research.

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List of Abbreviations

AOSR	Athabasca oil sands region
BD	Bloch decay
CP	cross polarization
CSA	compound specific analysis
DOC	dissolved organic carbon
DP	direct pulse
GC	gas chromatograph
GC-C-IRMS	gas chromatograph combustion isotope ratio mass spectrometer
IRMS	isotope ratio mass spectrometer
LTMP	long term monitoring plot
MRPP	multi-response permutation procedure
MRT	multivariate regression tree
NMR	nuclear magnetic resonance
NMS or NMDS	nonmetric multidimensional scaling
OM	organic matter
PCA	principle component analysis
PLFA	phospholipid fatty acid
SOC	soil organic carbon
SOM	soil organic matter

Chapter 1. Introduction

1.1. Soil

Soil formation, which is well described by Jenny (1994), arises from the interacting influences of climate, organisms, relief, parent material, and time. These five factors combine to form a mosaic of soils across the globe with 10 Soil Orders (the broadest level of classification) recognized in Canada (Soil Classification Working Group 1998). Soil is a critical resource which delivers several key ecosystem services, and restoring soil organic matter directly improves water retention and soil fertility for plant growth (Banwart 2011; Lal 2004). Soil organic matter is therefore recognized as a key component of soil (Carter 2002), which when removed decreases porosity (Powers et al. 2005) and productivity (Paterson et al. 2011). However, decreases in soil quality have been shown to be reversed with increased organic matter inputs in agricultural (Lal et al. 2007) and reclamation settings (Larney and Angers 2012). Landscape level reclamation is currently happening in the Athabasca Oil Sands Region (AOSR) of Alberta, Canada, and soil, and associated organic matter, will play a key role in its possible success.

1.2. Organic Matter Inputs

Soil organic matter accumulates from above and below-ground inputs such as leaf litter fall or roots. The macromolecular chemistry of litter input to soil differs in types and proportions of biopolymers depending on the tissue type (e.g., needle vs. twig; Preston et al. 2006), plant type (e.g., lichen vs. jack pine; Norris et al. 2011) and environmental conditions (Donaldson et al. 2006). Common biopolymers in vegetation include: carbohydrates, lignins, tannins and waxes (Kögel-Knabner 2002). Generally proteins and soluble sugars are readily consumed by the soil microbial community followed by carbohydrates and more complex compounds (Baldock et al. 1997). An increase in macromolecular structure or complexity (e.g., lignin) has been linked to increased stability, or residence time, of the organic carbon contained in the soil (Preston et al. 2000). With addition of such specific litter to the soil surface, both the forest floor and

microbial community have been shown to reflect the source of organic matter inputs (Hannam et al. 2006; 2004). However, increased decomposition of soil organic matter does appear to lead to increasing macromolecular homogeneity in the soil organic matter (Mahieu et al. 1999).

1.3. Soil Nutrient Cycling

The soil microbial community mediates the nitrogen cycle in its search for available carbon. Hence the two cycles are entwined- the movement of nitrogen within a system is inexorably tied to the type of carbon present. Key pools and fluxes of soil carbon and nitrogen include litter inputs, soil microbes, dissolved organic matter, and organic matter associated with the mineral matrix; these fluxes are mediated by abiotic conditions such as soil texture, temperature and moisture. In addition, movement, or fluxes, of organic carbon and nitrogen are linked to the macromolecular chemistry of the litter and the soil organic matter (i.e., its molecular complexity), and to the microbial community present and the preference it may have for specific macromolecules.

The driving force for modification to the macromolecular structure of organic matter inputs to soil is the microbial community as microbes cleave biopolymers in their search for carbon; often with the byproduct of complex repolymerization of organic matter. The soil microbial community is large, diverse and neither fully characterized nor understood (Harris 2009; Kirk et al. 2004). Generally when discussing soil microbial biomass it is bacteria and fungi which are being considered; however soil microfauna such as protozoa are also recognized as an important component of nutrient cycling (Swallow et al. 2013). When describing the microbial community in terms of ecosystem processes the community may be grouped based on its growth environment (e.g., aerobic vs anaerobic), function (e.g., enzyme activity), or identification technique (e.g., Gram positive or negative); however there is an increasing use of novel molecular methodology (e.g., metagenomics; Torsvik and Øvreås 2002). These microbial communities have been found to be influenced by the above-ground vegetation (Grayston and Prescott 2005; Priha et al. 2001), with different community

structures existing, for example, under aspen and white spruce dominated stands (Hannam et al. 2006).

Soil organic matter inputs are initially scavenged for easily decomposable carbohydrates while macromolecules such as lipids are bypassed and may accumulate in soils together with microbial byproducts. The byproducts may re-polymerize to form complex large molecular weight amorphous structures or they may bind to mineral soil forming aggragates; both of which have long turnover times (Baldock et al. 2004). Organic matter which binds to mineral edges and coats silt and clay particles also has a long turnover time. Another pool of organic matter within the soil, the least modified organic matter (light fraction) which most resembles the litter input, is larger in size and found with sand sized particles (50 – 2000 μm) (Christensen 2001; Gregorich et al. 2006). Organic matter turnover time is influenced not only by macromolecular composition, biochemical, chemical and/or physical stabilization but also by the abiotic conditions of moisture and, especially, temperature as these environmental factors are coupled to microbial activity (Craine et al. 2010). By investigating organic matter pools and monitoring the larger scale key response variables of nutrient supply, microbial community and organic matter, we can increase our understanding of the biogeochemical cycles of both carbon and nitrogen.

1.4. Boreal Forest & Athabasca Oil Sands Region

The AOSR lies north of Fort McMurray, Alberta, within the western boreal forest. The boreal forest is the second largest forest biome on Earth with an area of 1.37×10^9 ha covering the globe between the steppes and the arctic (Watson et al. 2000). Stand dynamics in the boreal forest are driven by large scale disturbances, including wind and snow in the maritime boreal, and fire and insects in the continental boreal forest (Engelmark 1999). Yet, despite the importance of the western boreal forest as part of the larger global biome, very little is known on how disturbances affect both the carbon and nitrogen cycles in these ecosystems.

The AOSR is currently being mined for the Athabasca oil sands deposit. The deposit holds 169 billion barrels of crude oil over an area of 142 000 km^2 and

3 % of this area may be surface mined (Stringham 2012). Oil sands mining in the boreal forest has currently disturbed an area of 715 km^2 with 1.04 km^2 officially reclaimed to date (Government of Alberta 2011). Reclamation by the mining companies is a legislated requirement where the disturbed land must be returned to an equivalent pre-mining land capability (Government of Alberta 1993).

1.5. AOSR Soil Construction

Mine closure plans include a heterogeneous landscape (Devito et al. 2012). However, this new landscape has a greater proportion of upland regions than the pre-disturbance ecosystem. This discrepancy is due to a bulking factor of about 1.4 (Mikula 2012). Therefore upland forest regions will comprise a large proportion of the final new landscape. Constructing these forest regions begins with a variety of salvaged material including overburden, mineral soils and tailing sands, a process by-product (Fung and Macyk 2000; Macdonald et al. 2012). Suitable combinations of these materials are then used to build a foundation for reclamation. A layer of an organic matter mineral mixture 20 – 50 cm thick is placed on top of this base (Macdonald et al. 2012; Turcotte et al. 2009).

Combinations of two main soil organic matter amendments, peat and forest floor material, are generally used in novel upland forest ecosystems in the AOSR. Forest floor material was found to be beneficial for reclamation through its inclusion of plant propagules from the native seed bank (Mackenzie and Naeth 2010). Peat material is used for its ability to decrease bulk density and increase both the moisture holding capacity and organic nutrient bank of the constructed soils (Fung and Macyk 2000). While peat is plentiful in the oil sands region, making it a preferred substrate, it may have restrictions when used to restore upland forest soils. Nitrogen is often the limiting nutrient in natural boreal forest soils (Kaye and Hart 1997) and, as peat is low in organic nitrogen, the use of peat mineral mixtures with low available nitrogen is of concern. Furthermore, peat has few complex carbon compounds making it a relatively uniform substrate of simple macromolecules, mainly O-alkyl C carbohydrates (Barkovskii et al. 2009).

How this peat substrate from a lowland environment will behave in the fundamentally different environment of upland soils is currently of concern.

Aspen (*Populus tremuloides* Michx.) stands dominate natural upland regions throughout the boreal forest in North America (Johnson et al. 1995). Aspen productivity has been shown to be impacted by forest floor organic matter quality, decomposition and nutrient release (Flanagan and Van Cleve 1983). The stands are one of the most productive forest types in the western boreal with high annual above-ground biomass (Van Cleve et al. 1983) and litterfall biomass twice that of conifers in the same region (Gower et al. 2000). The high productivity of aspen trees leads to a much higher demand for nitrogen than in conifers (Gower et al. 2000; Van Cleve et al. 1983). Very little nutrient mineral nitrogen is found in aspen stands (Huang and Schoenau 1996). Instead nitrogen accumulates in organic form within the forest floor, which is favored under low mean annual temperatures restricting decomposition. As aspen forests are productive and widely distributed throughout the western boreal forest, they are one of the desired objectives of forest reclamation practices in the AOSR. However, to achieve a mature stand on a peat mineral mixture substrate that may be lacking in nitrogen, the vegetation must develop the ability to recycle nutrients from its fresh and decomposing litter; i.e., it needs to become self sustaining.

The nutrient status of the forest floor and soil organic matter will be critical in achieving the objective of aspen dominated stands, as it has been determined that the majority of nitrogen in a mature natural aspen stand in Quebec was held within these two organic matter pools (Ste-Marie et al. 2007). Similar nitrogen stocks were reported for a natural aspen dominated soil in north central Alberta (Kishchuk 2004). While aspen litter, like peat, has a high carbon to nitrogen ratio (70:1) the litter is composed of more complex compounds, particularly dominated by alkyl carbons or straight chain carbon from waxes or lipids (Preston et al. 2000). As the retention of organic nutrients, such as nitrogen, is primarily controlled by microbial processes (e.g., mineralization, immobilization), it is important to understand if the shift in organic matter inputs from simple peat to complex tree litter compounds will also cause a subsequent

shift in the microbial community. A change in the microbial community is required in order for the organic matter nutrients to be efficiently cycled; and this efficiency will be crucial for possible reclamation success where the growing vegetation will no longer depend on external nutrient sources.

Due to construction practices and organic matter additions to cap soils in the AOSR, the total soil nitrogen content on disturbed sites was determined to be at or above the natural range (Hemstock et al. 2010; McMillan et al. 2007; Rowland et al. 2009). McMillan et al. (2007) noted that the soil microbial biomass on disturbed sites was significantly less compared to an aspen dominated natural site and yet their results indicated that community on both the natural and disturbed sites had the same potential to mineralize organic nitrogen to ammonium. The authors speculated that differences were due in part to the composition of the organic matter. Results also indicated that the addition of forest floor material stimulated microbial activity compared to peat alone (McMillan et al. 2007). Futhermore, net mineralization rates from peat mineral mixes were at the low end of the range of reported rates from mature upland forests in the scientific literature (Hemstock et al. 2010). Additional work in the AOSR has reported differences in forest floor organic matter chemistry (Turcotte et al. 2009), lower rate of organic matter decomposition (Rowland et al. 2009) and decreased enzyme activity (Dimitriu et al. 2010) in reclaimed stands compared to natural sites. When comparing among reclaimed stands, the sampling time (MacKenzie and Quideau 2010), percent canopy cover (Sorenson et al. 2011) and type of organic matter amendments used in soil reconstruction (Hahn and Quideau 2012) were all found to affect the soil microbial community. It is this community which drives nutrient availability and therefore soil quality.

1.6. Methods of Investigating Organic Matter Cycling

1.6.1. *n*-Alkanes

Lipids, or waxes, include some of the common biopolymers present in organic matter inputs (Kögel-Knabner 2002). In soil, lipids form a minor component of soil carbon (e.g. 4 – 8 % SOC). Yet, lipids are useful tracers of both

the source and extent of degradation of organic matter inputs. Lipids vary in structure, and their molecular arrangement has been linked to specific organic matter inputs; further, their structural diversity gives rise to variable rates of degradation/transformation that may be used to investigate soil processes (Bull et al. 2000). Vascular plants have distinctive distributions of *n*-alkanes from epicuticular wax ($\geq C_{21}$) with a strong odd/even predominance (Eglinton and Hamilton 1967), which are considered to be resistant to degradation (Derenne and Largeau 2001). Local vegetation provides the greatest source of soil lipids (van Bergen et al. 1997); therefore, the dominance of particular *n*-alkanes in surface soil horizons can serve as a biomarker to characterize and quantify local plant inputs (Jansen et al. 2006). Monocots are typically dominated by C_{29} and C_{31} *n*-alkanes in their epicuticular wax (Maffei 1996), while C_{27} is characteristic of woody species (Lockheart et al. 1995) and C_{23} and C_{25} of *Sphagnum* spp. (Baas et al. 2000). Consequently, soils can be characterized based on the corresponding proportions of vegetation biomarkers for grassland soils (Jansen et al. 2006; van Bergen et al. 1997), forest soils (Marseille et al. 1999; Trendel et al. 2010), and *Sphagnum* spp. peat (Bingham et al. 2010), respectively. So far, *n*-alkanes have been used as biomarkers to quantify the contributions of different sources of terrestrial OM in contemporary soil studies (Bull et al. 2000; Jandl et al. 2012), oceanic and lacustrine sediment research (Eglinton and Eglinton 2008) and palaeoclimate investigations (Bingham et al. 2010; Nott et al. 2000).

1.6.2. Isotopes

Carbon and nitrogen atoms are found in the environment in different forms. Carbon atoms are most often found in the environment in the abundant form ^{12}C with ^{13}C at 1.1% and ^{14}C in trace amounts; N atoms may occur more commonly as ^{14}N or ^{15}N (0.4 %). Differences in the number of neutrons present in different atoms of the same element are the cause of elemental isotopes; i.e., ^{13}C has one more neutron than ^{12}C. All organic matter compounds contain various amounts of the different isotopes with amounts reported as atom % (a ratio of isotope atoms to total number of atoms present) or delta values ($\delta^{13}C$ or δ

^{15}N represent the amount of the isotope present with respect to a given standard) (Fry 2006). Different isotopes undergo similar chemical reactions; however, reaction rates vary among isotopes, and isotopic ratios follow general trends throughout the environment and specific photosynthetic and microbial pathways as the lighter ^{12}C is preferentially utilized compared to ^{13}C. In addition, plants are known to prioritize their use of recent photosynthates with allocation first to new foliage and buds and lastly to protective secondary chemicals (Oliver and Larson 1996). Growing plants under an atmosphere of $^{13}CO_2$ gas causes a rapid ^{13}C enrichment within the plant tissues, hence allowing generation of an isotopic tracer. Utilizing a variety of analytical techniques in conjunction with this isotopic tracer, the movement of carbon can be followed from the plant to the soil, including incorporation into the microbial community biomass, and subsequent microbial respiration or stabilization with the soil mineral matrix.

A variety of plant materials has been labeled with isotopes to trace carbon and nitrogen biogeochemical cycles. Douglas fir and paper birch seedlings were pulse labeled with $^{13}CO_2$ and, using multiple chase times, Simard et al. (1997) determined that the distribution of ^{13}C decreased in foliage but increased in roots with time. A soil decomposition experiment used Ponderosa pine needles that were doubly labeled to $\delta^{13}C$ 2487 ‰ and 5.5 atom % ^{15}N (Bird et al. 2008; Bird and Torn 2006). Hybrids of *Populus balsamifera* were labeled with ^{13}C to determine recent photosynthate movement in different environmental conditions (Arevalo et al. 2010). After 9 weeks of ^{13}C labeling Douglas fir seedling needles were enriched to 1.56 atom % where the allocation of ^{13}C varied by biopolymer: $\delta^{13}C$ cellulose 244 ‰, hemicellulose 262 ‰, and lignin 356 ‰ (Moore-Kucera and Dick 2008).

1.6.3. NMR

Solid state nuclear magnetic resonance (NMR) may be used as a non-destructive analytical method to examine the macromolecular structure of organic matter (Simpson and Preston 2007). The technique relies on the presence of nuclei which are spin active and have a magnetic moment, e.g., ^{13}C and ^{15}N. This section

is meant as an introduction on the use of NMR as a tool to investigate organic matter composition; for an introduction to NMR theory there are a multitude of sources from primers to books which eloquently describe the background concepts of spectroscopy (e.g., Hore 1995; Pavia et al. 2001) to more advanced NMR theory (e.g., Axelson 2012; Harris 1986). Early work characterizing organic matter with NMR was based on the material of interest having to be in solution but a breakthrough came from Wilson et al. (1981) when the authors reported on the analysis of organic matter in whole soils (i.e.; solid samples) by using cross polarization (CP) magic angle spinning solid state NMR. Since then this unparalleled technique has been widely utilized to characterize the biopolymers in organic matter (e.g., Baldock et al. 1992; Kögel-Knabner 1997; Kögel-Knabner 2002; Preston et al. 2000; Preston et al. 1997; Wilson 1987). Characterization of organic matter has been thoroughly discussed in the broader context of NMR and environmental research (Simpson et al. 2011; Simpson and Simpson 2013) with a particular emphasis on the forest ecology and soils described by Quideau et al. (2013). Practical aspects of using solid state NMR were well discussed by Bryce et al. (2001) with Preston (2001) and Simpson and Preston (2007) providing exceptional reviews for effectively using the technique in soil science research.

Wilson et al. (1981) utilized the CP pulse sequence for its benefits in terms of enhanced signal to noise and shorter acquisition time making it a powerful pulse sequence in organic matter studies as it can broadly characterize the structures present and, to some extent, quantify their proportions (Baldock et al. 1992). The second most common pulse program used in characterizing biopolymers in organic matter is direct pulse (DP) which provides quantitative results but has longer acquisition times. This difference in acquisition time and signal enhancement has led to the preferential use of CP in the examination of organic matter in soil science. Researchers have therefore made modifications to the experimental program in an effort to optimize the quantitative, and minimize the semi-quantitative, nature of the CP program (Bryce et al. 2001; Conte et al. 2004; Smernik 2005). For ^{13}C solid state NMR of organic matter, the resulting spectrum provides details on alkyl, methoxyl, O-alkyl, di-O-alkyl, aromatic,

phenolic and carboxylic type carbons with their relative quantities determined by integrating the area within the respective regions (Simpson and Preston 2007). Investigations into organic matter with NMR led to the development of an index of decomposition, the alkyl/O-alkyl ratio, where the ratio derives from integration of the corresponding areas on the NMR spectrum (Baldock et al. 1997). The ratio has become widely used as it illustrates the preferential consumption by the microbial community of O-alkyl carbon (carbohydrates), before the decomposition of alkyl carbon, or straight chain carbon (i.e., waxes and lipids) (e.g., Baldock et al. 1997; Norris et al. 2009; Preston 2001; Sjögersten et al. 2003). More recently NMR analysis has been used to characterize the biopolymers of organic matter for assessments in terms of deep boreal carbon stability (Norris et al. 2009) and NMR results have significantly contributed to the discussion surrounding the composition and stability of black carbon within soil carbon stocks (Preston and Schmidt 2006; Soucémarianadin 2013).

1.6.4. PLFA

Soil microbial communities may be broadly characterized and quantified by the extraction and analysis of soil derived phospholipid fatty acids (PLFAs) (Frostegård et al. 2011). Microbial composition is determined through an indirect and culture independent method (White and Ringelberg 1998). PLFAs vary in carbon chain length, unsaturation and branching, and this variability can be used to determine the community composition (Frostegård et al. 2011). In addition, PLFAs readily break down on cell death making for an ideal method of quantification of the live component of soil microbial community (Glaser 2005; Maxfield and Evershed 2011). Using the PLFA method it has been demonstrated that soil microbial community structure is influenced by: 1) the type of above ground vegetation in mature undisturbed natural forest stands (Brockett et al. 2012; Grayston and Prescott 2005; Hannam et al. 2006; Priha et al. 2001); 2) the impact of disturbance from harvest (Mummey et al. 2010), fire (Williams et al. 2012), and surface mining (Dimitriu et al. 2010; Mummey et al. 2002b); and 3) time, as mine reclamation chronosequences have been reported to change in

microbial structure with time (Banning et al. 2011; Claassens et al. 2008; Hahn and Quideau 2012; Mummey et al. 2002a). While the microbial community structure has been shown to vary among differing ecosystems, there is still a lack of knowledge on the differences in soil organic matter processing by soil microorganisms, specifically in terms of nutrient cycling.

1.6.5. Soil Fractionation

Separation of the soil based upon density and particle size categories imposes some order on the soil highly heterogeneous nature; the C:N ratio is typically observed to decrease and there is an enrichment of ^{13}C from the light fraction to the sand, silt and clay fraction (Bird et al. 2002; Quideau et al. 2003). For instance, clay and silt-sized separates that are dominated by microbially altered, recalcitrant OC (Guggenberger et al. 1995), have reported mean residence times based on ^{14}C analysis of 800-1660 years for silt and 75-4400 years for clay (von Lützow et al. 2007). In contrast, with no means of stabilization, the sand-sized fraction encompasses a labile pool with rapid turnover, which shows little chemical alteration from fresh plant residues (Christensen 1992). A subcomponent of the sand-sized fraction, the low density, or light fraction, is even less degraded and more similar to original plant inputs (Gregorich et al. 1996; Six et al. 2002), although a gradient exists within the light fraction, with the lower density organic carbon chemically more identical to the plant inputs compared to heavier free organic carbon particles (Wagai et al. 2009). The labile light fraction pool, resembling plant inputs, has provided a means of monitoring relatively rapid changes in soil organic matter following variations in land use or management practices (Gregorich et al. 2006) or in decomposition of labeled organic matter (Bird et al. 2008; Kölbl et al. 2007). In all cases, from an experimental point of view, quantifying soil organic matter distribution among density and size fractions increases our insights into organic matter dynamics compared to examining soil organic matter as a single pool.

1.6.6. Compound Specific Stable Isotope Analysis

Techniques mentioned thus far generally describe or quantify the soil system and processes therefore must be inferred. Combining biomarkers with stable isotopes helps to solve some of this dilemma. Isotope tracer additions to the system and observed changes in the isotopic ratio of specific compounds can directly inform on specific soil organic matter processes (whether these involve formation or decomposition). Reviews on the subject of compound specific stable isotope analysis have been published for several different areas: agricultural research (Amelung et al. 2008), soil food webs (Ruess and Chamberlain 2010), soil microbial ecology (Maxfield and Evershed 2011), general soil science (Glaser 2005), and touched upon in terms of plant ecology (Dawson et al. 2002). The power of the analysis lies within the specificity of the biomarkers and from this uniqueness conclusions on specific turnover times or processes may be made. In early work with PLFAs, Boschker et al. (1998) determined that the sulphate-reducing bacteria, similar to a Gram-positive bacteria and not the more widely studied Gram-negative, consumed a uniformly labelled ^{13}C acetate substrate in a marine sediment environment by comparing the ^{13}C enrichment levels of different representative PLFAs. More recently the technique has been used to investigate soil microbial community response due to the addition of ^{13}C-glucose in terms of: recycling of the label (Ziegler et al. 2005), different soil types (Brant et al. 2006; Dungait et al. 2011), and priming (Garcia-Pausas and Paterson 2011). While the work done is fundamental in terms of refining the methodology and understanding specific processes there appears to be a paucity of studies utilizing complex organic matter substrates and moving the incubations from controlled laboratory conditions to the field scale. Until we understand how complex organic matter is processed by the diverse microbial community present within forest soils, we will not be able to precisely determine soil organic matter turnover times and accurately assess soil organic matter quality.

1.7. Objectives and Outline

The overall objective of this study was to determine whether organic matter cycling differed between novel and natural ecosystem soils. Within this

context the thesis addresses five specific objectives: 1. to determine if soil organic matter from novel ecosystems begins to resemble that of natural ecosystems with time since reclamation 2. to determine if a labile substrate, ^{13}C glucose, is decomposed via the same pathways in a peat mineral mixture (novel soils) as in natural soils, 3. to determine if the accumulation of labeled isotope (^{13}C or ^{15}N) varies both within the structure (leaves, stems, or roots) and within the macromolecular chemistry of an aspen seedling, 4. to verify if ^{15}N labelled leaf litter is utilized as a nutrient source for growing vegetation on constructed and natural soils, and 5. to establish if the addition of organic matter to the soil surface shifts the composition and function of the microbial community. The thesis is presented as seven chapters where, following this Introduction, the chapters address each of the specific objectives individually before the key results are summarized in a concluding chapter. Each of the chapters is laid out as an independent work with the relevant background literature introduced prior to discussion of the methodology and results followed by the references, tables and figures.

References

Amelung, W., Brodowski, S., Sandhage-Hofmann, A., Bol, R. 2008. Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter. Advances in Agronomy 100, 155-250.

Arevalo, C.B.M., Bhatti, J.S., Chang, S.X., Sidders, D. 2010. Distribution of recent photosynthates in saplings of two hybrid poplar clones. Comm. Soil Sci. Plant Anal. 41, 1004-1015.

Axelson, D.E. 2012. 'Solid state nuclear magnetic resonance: a practical introduction.' (Natural Resources Canada: Ottawa, CA).

Baas, M., Pancost, R., van Geel, B., Sinninghe Damsté, J.S. 2000. A comparative study of lipids in *Sphagnum* species. Org. Geochem. 31, 535-541.

Baldock, J.A., Masiello, C.A., Gélinas, Y., Hedges, J.I. 2004. Cycling and composition of organic matter in terrestrial and marine ecosystems. Mar. Chem. 92, 39-64.

Baldock, J.A., Oades, J.M., Nelson, P.N., Skene, T.M., Golchin, A., Clarke, P. 1997. Assessing the extent of decomposition of natural organic materials using solid-state ^{13}C NMR spectroscopy. Aust. J. Soil Res. 35, 1061 - 1083.

Baldock, J.A., Oades, J.M., Waters, A.G., Peng, X., Vassallo, A.M., Wilson, M.A. 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state ^{13}C NMR spectroscopy. Biogeochemistry 16, 1-42.

Banning, N.C., Gleeson, D.B., Grigg, A.H., Grant, C.D., Andersen, G.L., Brodie, E.L., Murphy, D.V. 2011. Soil microbial community successional patterns during forest ecosystem restoration. Appl. Environ. Microbiol. 77, 6158-6164.

Banwart, S. 2011. Save our soils. Nature 474, 151-152.

Barkovskii, A.L., Fukui, H., Leisen, J., Kim, S.-H., Marsh, T.L., Khijniak, A.I. 2009. Rearrangement of bacterial community structure during peat diagenesis. Soil Biol. Biochem. 41, 135-143.

Bingham, E.M., McClymont, E.L., Väliranta, M., Mauquoy, D., Roberts, Z., Chambers, F.M., Pancost, R.D., Evershed, R.P. 2010. Conservative composition of *n*-alkane biomarkers in *Sphagnum* species: Implications for palaeoclimate reconstruction in ombrotrophic peat bogs. Org. Geochem. 41, 214-220.

Bird, J.A., Kleber, M., Torn, M.S. 2008. ^{13}C and ^{15}N stabilization dynamics in soil organic matter fractions during needle and fine root decomposition. Org. Geochem. 39, 465-477.

Bird, J.A., Torn, M.S. 2006. Fine roots vs. needles: a comparison of ^{13}C and ^{15}N dynamics in a ponderosa pine forest soil. Biogeochemistry 79, 361-382.

Bird, M., Santrùcková, H., Lloyd, J., Lawson, E. 2002. The isotopic composition of soil organic carbon on a north-south transect in western Canada. Eur. J. Soil Sci. 53, 393-403.

Boschker, H.T.S., Nold, S.C., Wellsbury, P., Bos, D., de Graff, W., Pel, R., Parkes, R.J., Cappendberg, T.E. 1998. Direct linking of microbial populations to specific biogeochemical processes by ^{13}C-labelling of biomarkers. Nature 392, 801-805.

Brant, J.B., Sulzman, E.W., Myrold, D.D. 2006. Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. Soil Biol. Biochem. 38, 2219-2232.

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

Bryce, D.L., Bernard, G.M., Gee, M., Lumsden, M.D., Eichele, K., Wasylishen, R.E. 2001. Practical aspects of modern routine solid-state multinuclear magnetic resonance spectroscopy: one-dimensional experiments. Can. J. Anal. Sci. Spectros. 46, 46-82.

Bull, I.D., van Bergen, P.F., Nott, C.J., Poulton, P.R., Evershed, R.P. 2000. Organic geochemical studies of soils from the Rothamsted classical experiments-V. The fate of lipids in different long-term experiments. Org. Geochem. 31, 389-408.

Carter, M.R. 2002. Soil quality for sustainable land management: Organic matter and aggregation interactions that maintain soil functions. Agron. J. 94, 38-47.

Christensen, B.T. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates, in: Stewart, B.A. (Eds.) Advances in Soil Science. Springer-Verlag, New York. pp. 1-90.

Christensen, B.T. 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. Eur. J. Soil Sci. 52, 345-353.

Claassens, S., Jansen van Rensburg, P.J., Maboeta, M.S., van Rensburg, L. 2008. Soil microbial community function and structure in a post-mining chronosequence. Water Air Soil Pollut. 194, 315-329.

Conte, P., Spaccini, R., Piccolo, A. 2004. State of the art of CPMAS ^{13}C-NMR spectroscopy applied to natural organic matter. Progress in Nuclear Magnetic Resonance Spectroscopy 44, 215-223.

Craine, J.M., Fierer, N., McLauchlan, K.K. 2010. Widespread coupling between the rate and temperature sensitivity of organic matter decay. Nature Geoscience 3, 854-857.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., Tu, K.P. 2002. Stable isotopes in plant ecology. Annu. Rev. Ecol. Syst. 33, 507-559.

Derenne, S., Largeau, C. 2001. A review of some important families of refractory macromolecules: Composition, origin and fate in soils and sediments. Soil Sci. 166, 833-847.

Devito, K., Mendoza, C., Qualizza, C. 2012. Conceptualizing water movement in the Boreal Plains. Implications for watershed reconstruction. Canadian Oil Sands Network for Research and Development, Environmental and Reclamation Research Group. p. 164.

Dimitriu, P.A., Prescott, C.E., Quideau, S.A., Grayston, S.J. 2010. Impact of reclamation of surface-mined boreal forest soils on microbial community composition and function. Soil Biol. Biochem. 42, 2289-2297.

Donaldson, J.R., Kruger, E.L., Lindroth, R.L. 2006. Competition- and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (*Populus tremuloides*). New Phytol. 169, 561-570.

Dungait, J.A.J., Kemmitt, S.J., Michallon, L., Guo, S., Wen, Q., Brookes, P.C., Evershed, R.P. 2011. Variable responses of the soil microbial biomass to trace concentrations of ^{13}C-labelled glucose, using ^{13}C-PLFA analysis. Eur. J. Soil Sci. 62, 117-126.

Eglinton, G., Hamilton, R.J. 1967. Leaf epicuticular waxes. Science 156, 1322-1335.

Eglinton, T.I., Eglinton, G. 2008. Molecular proxies for paleoclimatology. Earth and Planetary Science Letters 275, 1-16.

Engelmark, O. 1999. Boreal forest disturbances, in: Walker, L.R. (Eds.) Ecosystems of disturbed ground. Elsevier Science, Amsterdam. pp. 161-186.

Flanagan, P.W., Van Cleve, K. 1983. Nutrient cycling in relation to decomposition and organic-matterr quality in taiga ecosystems. Can. J. For. Res. 13, 795-817.

Frostegård, Å., Tunlid, A., Bååth, E. 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43, 1621-1625.

Fry, B. 2006. 'Stable isotope ecology.' (Springer Science+Business Media: New York).

Fung, M.Y.P., Macyk, T.M. 2000. Reclamation of oil sands mining areas, in: Barnhisel, R.I., Darmody, R.G., Daniels, W.L. (Eds.) Reclamation of Drastically Disturbed Lands. American Society of Agronomy, Inc., Madison, WI. pp. 755-774.

Garcia-Pausas, J., Paterson, E. 2011. Microbial community abundance and structure are determinants of soil organic mineralisation in the presence of labile carbon. Soil Biol. Biochem. 43, 1705-1713.

Glaser, B. 2005. Compound-specific stable-isotope ($\delta^{13}C$) analysis in soil science. J. Plant Nutr. Soil Sci. 168, 633-648.

Government of Alberta 1993. Environmental Protection and Enhancement Act: Conservation and Reclamation Regulation.

Government of Alberta 2011. Alberta's oil sands: Protecting the environment. (Government of Alberta.

Gower, S.T., Hunter, A., Campbell, J., Vogel, J., Veldhuis, H., Harden, J., Trumbore, S., Norman, J.M., Kucharik, C.J. 2000. Nutrient dynamics of the southern and northern BOREAS boreal forests. Écoscience 7, 481-490.

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

Gregorich, E.G., Beare, M.H., McKim, U.F., Skjemstad, J.O. 2006. Chemical and biological characteristics of physically uncomplexed organic matter. Soil Sci. Soc. Am. J. 70, 975-985.

Gregorich, E.G., Monreal, C.M., Schnitzer, M., Schulten, H.-R. 1996. Transformation of plant tissues into soil organic matter: chemical characterization of plant tissue, isolated fractions, and whole soils. Soil Sci. 161, 680-693.

Guggenberger, G., Zech, W., Haumaier, L., Christensen, B.T. 1995. Land-use effects on the composition of organic matter in particle-size separates of soils: II. CPMAS and solution ^{13}C NMR analysis. Eur. J. Soil Sci. 46, 147-158.

Hahn, A.S., Quideau, S.A. 2012. Long-term effects of organic amendments on the recovery of plant and soil microbial communities following disturbance in the Canadian boreal forest. Plant Soil, 1-14.

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

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

Harris, J. 2009. Soil microbial communities and restoration ecology: facilitators or followers. Science 325, 573-574.

Harris, R.K. 1986. 'Nuclear magnetic resonance spectroscopy: a physicochemical view.' (Longman Scientific & Technical).

Hemstock, S.S., Quideau, S.A., Chanasyk, D.S. 2010. Nitrogen availability from peat amendments used in boreal oil sands reclamation. Can. J. Soil Sci. 90, 165-175.

Hore, P.J. 1995. 'Nuclear magnetic resonance.' (Oxford University Press: Oxford, UK).

Huang, W.Z., Schoenau, J.J. 1996. Forms, amounts and distribution of carbon, nitrogen, phosphorus and sulfur in a boreal aspen forest soil. Can. J. Soil Sci. 76, 373-385.

Jandl, G., Baum, C., Blumschein, A., Leinweber, P. 2012. The impact of short rotation coppice on the concentration of aliphatic soil lipids. Plant Soil 350, 163-177.

Jansen, B., Nierop, K.G.J., Hageman, J.A., Cleef, A.M., Verstraten, J.M. 2006. The straight-chain lipid biomarker composition of plant species responsible for the dominant biomass production along two altitudinal transects in the Ecuadorian Andes. Org. Geochem. 37, 1514-1536.

Jenny, H. 1994. 'Factors of soil formation: A system of quantitative pedology.' (Dover Publications, Inc: Mineola, NY).

Johnson, D., Kershaw, L., MacKinnon, A., Pojar, J. 1995. 'Plants of the Western Boreal Forest and Aspen Parkland.' (Lone Pine Publishing: Edmonton).

Kaye, J.P., Hart, S.C. 1997. Competition for nitrogen between plants and soil microorganisms. Trends in Ecology & Evolution 12, 139-143.

Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H., Trevors, J.T. 2004. Methods of studying soil microbial diversity. J. Microbiol. Methods 58, 169-188.

Kishchuk, B.E. 2004. Soils of the Ecosystem Management Emulating Natural Disturbance (EMEND) experimental area, northwestern Alberta. (Ed. Canadian Forest Service). (Natural Resources Canada, Government of Canada.

Kögel-Knabner, I. 1997. ^{13}C and ^{15}N spectroscopy as a tool in soil organic matter studies. Geoderma 80, 243-270.

Kögel-Knabner, I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol. Biochem. 34, 139-162.

Kölbl, A., Von Lützow, M., Rumpel, C., Munch, J.C., Kögel-Knaber, I. 2007. Dynamics of ^{13}C-labeled mustard litter (Sinapis alba) in particle-size and aggregate fractions in and agricultural cropland with high- and low-yield areas. J. Plant Nutr. Soil Sci. 170, 123-133.

Lal, R. 2004. Soil carbon sequestrtion impacts on global climate change and food security. Science 304, 1623-1627.

Lal, R., Follett, R.F., Stewart, B.A., Kimble, J.M. 2007. Soil carbon sequestration to mitigate climate change and advance food security. Soil Sci. 172, 943-956.

Larney, F.J., Angers, D.A. 2012. The role of organic amendments in soil reclamation: A review. Can. J. Soil Sci. 92, 19-38.

Lockheart, M.J., van Bergen, P.F., Evershed, R.P. 1995. Variations in the stable carbon isotope compositions of individual lipids from the leaves of modern angiosperms: Implications for the study of higher plant-derived sedimentary organic matter. Org. Geochem. 26, 137-153.

Macdonald, E., Quideau, S., Landhäusser, S. 2012. Rebuilding boreal forest ecosystems after industrial disturbance, in: Vitt, D., Bhatti, J. (Eds.) Restoration and Reclamation of Boreal Ecosystems. Cambridge University Press. pp. 123-160.

Mackenzie, D.D., Naeth, M.A. 2010. The role of the forest soil propagule bank in assisted natural recovery after oil sands mining. Restor. Ecol. 18, 418-427.

MacKenzie, M.D., Quideau, S.A. 2010. Microbial community structure and nutrient availability in oil sands reclaimed boreal soils. Appl. Soil Ecol. 44, 32-41.

Maffei, M. 1996. Chemotaxonomic significance of leaf wax alkanes in the Gramineae. Biochemical Systematics and Ecology 24, 53-64.

Mahieu, N., Powlson, D.S., Randall, E.W. 1999. Statistical analysis of published carbon-13 CPMAS NMR spectra of soil organic matter. Soil Sci. Soc. Am. J. 63, 307-319.

Marseille, F., Disnar, J.R., Guillet, B., Noack, Y. 1999. n-Alkanes and free fatty acids in humus and A1 horizons of soils under beech, spruce and grass in the Massif-Central (Mont-Lozère), France. Eur. J. Soil Sci. 50, 433-441.

Maxfield, P.J., Evershed, R.P. 2011. Phospholipid fatty acid stable isotope probing techniques in microbial ecology, in: Murrell, J.C., Whiteley, A.S. (Eds.) Stable isotope probing and related technologies. ASM Press, Washington, DC.

McMillan, R., Quideau, S.A., MacKenzie, M.D., Biryukova, O. 2007. Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. J. Environ. Qual. 36, 1470-1478.

Mikula, R. 2012. Advances in oil sands tailings handling: building the base for reclamation, in: Vitt, D., Bhatti, J.S. (Eds.) Restoration and reclamation of boreal ecosystems: attaining sustainable development. Cambridge University Press. pp. 103-122.

Moore-Kucera, J., Dick, R.P. 2008. A pulse-chase method to ^{13}carbon-label Douglas-fir seedlings for decomposition studies. Soil Sci. 173, 46-53.

Mummey, D.L., Clarke, J.T., Cole, C.A., O'Connor, B.G., Gannon, J.E., Ramsey, P.W. 2010. Spatial analysis reveals differences in soil microbial community interactions between adjacent coniferous forest and clearcut ecosystems. Soil Biol. Biochem. 42, 1138-1147.

Mummey, D.L., Stahl, P.D., Buyer, J.S. 2002a. Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation. Appl. Soil Ecol. 21, 251-259.

Mummey, D.L., Stahl, P.D., Buyer, J.S. 2002b. Soil microbiological properties 20 years after surface mine reclamation: spatial analysis of reclaimed and undisturbed sites. Soil Biol. Biochem. 34, 1717-1725.

Norris, C.E., Quideau, S.A., Bhatti, J.S., Wasylishen, R.E. 2011. Soil carbon stabilization in jack pine stands along the Boreal Forest Transect Case Study. Glob. Change Biol. 17, 480-494.

Norris, C.E., Quideau, S.A., Bhatti, J.S., Wasylishen, R.E., MacKenzie, M.D. 2009. Influence of fire and harvest on soil organic carbon in jack pine sites. Can. J. For. Res. 39, 642-654.

Nott, C.J., Xie, S., Avsejs, L.A., Maddy, D., Chambers, F.M., Evershed, R.P. 2000. *n*-Alkane distributions in ombrotrophic mires as indicators of vegetation change related to climatic variation. Org. Geochem. 31, 231-235.

Oliver, C.D., Larson, B.C. 1996. 'Forest stand dynamics.' (John Wiley & Sons, Inc.: New York).

Paterson, E., Sim, A., Osborne, S.M., Murray, P.J. 2011. Long-term exclusion of plant-inputs to soil reduces the functional capacity of microbial communities to mineralise recalcitrant root-derived carbon sources. Soil Biol. Biochem. 43, 1873-1880.

Pavia, D.L., Lampman, G.M., Kriz, G.S. 2001. 'Introduction to Spectroscopy.'

Powers, R.F., Scott, D.A., Sanchez, F.G., Voldseth, R.A., Page-Dumroese, D., Elioff, J.D., Stone, D.M. 2005. The North American long-term soil productivity experiment: Findings from the first decade of research. For. Ecol. Manage. 220, 31-50.

Preston, C.M. 2001. Carbon-13 solid-state NMR of soil organic matter - using the technique effectively. Can. J. Soil Sci. 81, 255-270.

Preston, C.M., Bhatti, J.S., Flanagan, L.B., Norris, C. 2006. Stocks, chemistry, and sensitivity to climate change of dead organic matter along the Canadian Boreal Forest Transect Case Study. Clim. Change 74, 223-251.

Preston, C.M., Schmidt, M.W.I. 2006. Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions. Biogeosciences 3, 397-420.

Preston, C.M., Trofymow, J.A., Canadian Intersite Decomposition Experiment Working Group 2000. Variability in litter quality and its relationship to litter decay in Canadian forests. Can. J. Bot. 78, 1269-1287.

Preston, C.M., Trofymow, J.A.T., Sayer, B.G., Niu, J. 1997. ^{13}C nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning investigation of the proximate-analysis fractions used to assess litter quality in decomposition studies. Can. J. Bot. 75, 1601-1613.

Priha, O., Grayston, S.J., Hiukka, R., Pennanen, T., Smolander, A. 2001. Microbial community structure and characteristics of the organic matter in soils

under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. Biol. Fert. Soils 33, 17-24.

Quideau, S.A., Graham, R.C., Feng, X., Chadwick, O.A. 2003. Natural isotopic distribution in soil surface horizons differentiated by vegetation. Soil Sci. Soc. Am. J. 67, 1544-1550.

Quideau, S.A., Norris, C.E., Soucémarianadin, L.N., Wasylishen, R.E. 2013. Forest ecology and soils, in: Simpson, M., Simpson, A. (Eds.) NMR Spectroscopy: A versatile tool for environmental research. Wiley. p. in press.

Rowland, S.M., Prescott, C.E., Grayston, S.J., Quideau, S.A., Bradfield, G.E. 2009. Recreating a functioning forest soil in reclaimed oil sands in Northern Alberta: An approach for measuring success in ecological restoration. J. Environ. Qual. 38, 1580-1590.

Ruess, L., Chamberlain, P.M. 2010. The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biol. Biochem. 42, 1898-1910.

Simard, S.W., Durall, D.M., Jones, M.D. 1997. Carbon allocation and carbon transfer between *Betula papyrifera* and *Pseudotsuga menziesii* seedlings using a ^{13}C pulse-labeling method. Plant Soil 191, 41-55.

Simpson, A.J., McNally, D.J., Simpson, M.J. 2011. NMR spectroscopy in environmental research: From molecular interactions to global processes. Progress in Nuclear Magnetic Resonance Spectroscopy 58, 97-175.

Simpson, M., Simpson, A. 2013. 'NMR Spectroscopy: A versatile tool for environmental research.' (Wiley).

Simpson, M.J., Preston, C.M. 2007. Soil organic matter analysis by solid-state ^{13}C nuclear magnetic resonance spectroscopy, in: Carter, M.R., Gregorich, E.G. (Eds.) Soil Sampling and Methods of Analysis. CRC Press, Boca Raton USA. pp. 681 - 692.

Six, J., Conant, R.T., Paul, E.A., Paustian, K. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil 241, 155-176.

Sjögersten, S., Turner, B.L., Mahieu, N., Condrons, L.M., Wookey, P.A. 2003. Soil organic matter biochemistry and potential susceptibility to climatic change across the forest-tundra ecotone in the Fennoscandian mountains. Glob. Change Biol. 9, 759-772.

Smernik, R.J. 2005. Solid-state 13C NMR spectroscopic studies of soil organic matter at two magnetic field strengths. Geoderma 125, 249-271.

Soil Classification Working Group 1998. The Canadian System of Soil Classification, in: Agriculture and Agri-Food Canada- Research Branch (Eds.). NRC Research Press. pp. 53 - 59.

Sorenson, P.T., Quideau, S.A., MacKenzie, M.D., Landhäusser, S.M., Oh, S.-W. 2011. Forest floor development and biochemical properties in reconstructed boreal forest soils. Appl. Soil Ecol. 49, 139-147.

Soucémarianadin, L.N. 2013. Black carbon in Québec boreal black spruce forests. University of Alberta.

Ste-Marie, C., Paré, D., Gagnon, D. 2007. The contrasting effects of aspen and jack pine on soil nutritional properties depend on parent material. Ecosystems 10, 1299-1310.

Stringham, G. 2012. Energy developments in Canada's oil sands, in: Percy, K.E. (Eds.) Alberta Oil Sands. Elsevier. pp. 19-34.

Swallow, M.J.B., Quideau, S.A., Norris, C.E. 2013. Ciliate dependent production of microbial anthranilic acid occurring within aspen litter. Soil Biol. Biochem. 60, 113-121.

Torsvik, V., Øvreås, L. 2002. Microbial diversity and function in soil: from genes to ecosystems. Current Opinion in Microbiology 5, 240-245.

Trendel, J.M., Schaeffer, P., Adam, P., Ertlen, D., Schwartz, D. 2010. Molecular characterisation of soil surface horizons with different vegetation in the Vosges Massif (France). Org. Geochem. 41, 1036-1039.

Turcotte, I., Quideau, S.A., Oh, S.-W. 2009. Organic matter quality in reclaimed boreal forest soils following oil sands mining. Org. Geochem. 40, 510-519.

van Bergen, P.F., Bull, I.D., Poulton, P.R., Evershed, R.P. 1997. Organic geochemical studies of soils from the Rothamsted Classical Experiments - I. Total lipid extracts, solvent insoluble residues and humic acids from Broadbalk Wilderness. Org. Geochem. 26, 117-135.

Van Cleve, K., Oliver, L., Schlentner, R., Viereck, L.A., Dyrness, C.T. 1983. Productivity and nutrient cycling in taiga forest ecosystems. Can. J. For. Res. 13, 747-766.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B. 2007. SOM fractionation methods: Relevance to

functional pools and to stabilization mechanisms. Soil Biol. Biochem. 39, 2183 - 2207.

Wagai, R., Mayer, L.M., Kitayama, K. 2009. Nature of the "occluded" low-density fraction in soil organic matter studies: A critical review. Soil Science and Plant Nutrition 55, 13-25.

Watson, R.T., Noble, I.R., Bolin, B., Ravindranath, N.H., Verardo, D.J., Bokken, D.J. 2000. 'IPCC Special Report: Land use, land-use change, and forestry.' (Intergovernmental Panel on Climate Change: Cambridge).

White, D.C., Ringelberg, D.B. 1998. Signature lipid biomarker analysis, in: Burlage, R.S., Atlas, R., Shahl, D., Geesey, G., Sayler, G. (Eds.) Techniques in microbial ecology. Oxford University Press, New York, NY.

Williams, R.J., Hallgren, S.W., Wilson, G.W.T. 2012. Frequency of prescribed burning in an upland oak forest determines soil and litter properties and alters soil microbial community. For. Ecol. Manage. 265, 241-247.

Wilson, M.A. 1987. Whole soil N.M.R., in: Wilson, M.A. (Eds.) N.M.R. techniques and applications in geochemistry and soil chemistry. Pergamon Press, Oxford.

Wilson, M.A., Pugmire, R.J., Zilm, K.W., Goh, K.M., Heng, S., Grant, D.M. 1981. Cross-polarization 13C-NMR spectroscopy with 'magic angle' spinning characterizes organic matter in whole soils. Nature 294, 648-650.

Ziegler, S.E., White, P.M., Wolf, D.C., Thoma, G.J. 2005. Tracking the fate and recycling of ^{13}C-labeled glucose in soil. Soil Sci. 170, 767-778.

Chapter 2. Biomarkers of novel ecosystem development in boreal forest soils

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2.1. Introduction

Surface mining is a landscape-scale disturbance that effectively returns entire ecosystems to early stage succession in terms of soil genesis (Macdonald et al. 2012). The success of post-mining reclamation, by reconstructing soil to support ecosystem development, depends on recreating a soil surface horizon with sufficient soil organic matter (SOM) to sustain productivity (Akala and Lal 2000). Thus, measurements of SOM stocks and other directly associated parameters, e.g. microbial activity, are used as indicators of the recovery of ecosystem processes that support the re-establishment of vegetation communities (e.g. Banning et al. 2008; Shrestha and Lal 2007). A wide range of natural, agricultural and industrial organic amendments are used to reconstruct soils (see recent review by Larney and Angers 2012). However, SOM dynamics differ between plant communities, leading Vetterlein and Hüttl (1999) to question whether applied organic amendments had the same quality, and thus the same function, as that in natural ecosystems. Reclamation guidelines require the re-establishment of vegetation within the natural range of ecotypes and wildlife capabilities similar to predisturbance conditions (Government of Alberta 1993). Therefore, measures to solely increase SOM stocks as a proxy for successful reclamation may not always relate to the success of ecosystem rehabilitation. A more robust indicator of the generation of a natural ecosystem is the development of SOM accumulated through litter production from new vegetation (Vetterlein and Hüttl 1999).

Reclamation practices following open pit mining in the Athabasca oil sands region (AOSR; Alberta, Canada) typically include reconstruction of soil-like profiles using a combination of native soil material, industrial by-products and fertilizer. Peat (derived from *Sphagnum* spp.) is in plentiful supply in the region

and is the major organic amendment used, specifically for its ability to decrease bulk density and increase moisture retention whilst increasing organic nutrient supply (Fung and Macyk 2000). The AOSR lies within the western boreal forest, a region characterized by a mosaic of upland pioneering *Populus tremuloides* Michx. (trembling aspen) and slower growing *Picea glauca* (Moench) Voss (white spruce), with *Pinus banksiana* Lamb. (jack pine) on the drier sites (Macdonald et al. 2012). A near-to-natural mixture of canopy cover by these three trees is the ultimate objective of the forest reclamation treatment. Novel ecosystems, as defined here, are those composed of reclaimed stands growing on anthropogenically reconstructed soils. Research in the AOSR has investigated the characteristics of SOM in a survey of reclamation and natural stands from long term soil and vegetation monitoring plots (LTMPs) and in a series of reclaimed stands that had been capped with peat (20 – 50 cm) and planted with the target tree species (Sorenson et al. 2011; Turcotte et al. 2009). Turcotte et al. (2009) compared changes in the characteristics of the light fraction of SOM in the LTMP using semi-quantitative solid-state nuclear magnetic resonance spectroscopy (NMR) as this fraction has been shown to be sensitive to cultivation and management practices (Six et al. 2002). NMR revealed that the novel sites had a significantly higher proportion of the whole spectral area in the O-alkyl C region (45 – 112 ppm), i.e. carbohydrate, whilst the natural sites displayed a significantly higher proportion in the alkyl region (30 ppm), i.e. aliphatic lipids. The authors suggested that the O-alkyl/alkyl ratio may be a good indicator of an overall system shift in the macromolecular chemistry of the organic matter (OM) between end -member conditions, i.e. novel and natural ecosystems. However, the source of the OM as either the organic amendment, i.e. peat, or novel input from the colonising trees could not be confirmed. Vegetation biomarkers have previously been used in the Canadian prairies to investigate land -use change (Schnitzer et al. 2006) and sources of OM in grassland and forest soils (Otto and Simpson 2006). To differentiate between OM sources using vegetation biomarkers could provide an important indicator of the development of true forest carbon dynamics.

Lipids are a minor component of soil (e.g. 4 – 8 % soil organic carbon (SOC)) but their functional diversity confers a variable rate of degradation/ transformation that may be exploited to obtain quantitative information about soil processes (Bull et al. 2000). The *n*-alkanes of the epicuticular wax of vascular plants have distinctive distributions ($\geq C_{21}$) with a strong odd/even predominance (Eglinton and Hamilton 1967), and are considered to be resistant to degradation (Derenne and Largeau 2001). The greatest proportion of soil lipids originates directly from the local vegetation (van Bergen et al. 1997); therefore, the dominance of particular *n*-alkanes in surface soil horizons can provide chemotaxonomic and quantitative information on local plant inputs (Jansen et al. 2006). Jansen et al. (2006) found that the extractable straight-chain lipid concentrations in roots were generally much lower than in leaves of the same species, and were in many cases less specific, and they recommended the use of leaf *n*-alkanes as the biomarkers of choice for plant input to soils. The dominance of specific *n*-alkanes in the leaf epicuticular waxes of major contributing plant species are typically C_{29} and C_{31} in monocots (Maffei 1996a), C_{27} in woody species (Lockheart et al. 1995) and C_{23} and C_{25} in *Sphagnum* spp. (Baas et al. 2000). Consequently, soils are typically characterized by corresponding high proportions of these vegetation biomarkers for grassland soils (Jansen et al. 2006; van Bergen et al. 1997), forest soils (Marseille et al. 1999; Trendel et al. 2010; Vancampenhout et al. 2009), and *Sphagnum* spp. peat (Bingham et al. 2010), respectively. Thus, *n*-alkanes have been used to quantify the contributions of different sources of terrestrial OM in contemporary soil studies (Bull et al. 2000; Jandl et al. 2012), oceanic and lacustrine sediment research (Eglinton and Eglinton 2008) and palaeoclimate investigations (Bingham et al. 2010; Nott et al. 2000) using established gas chromatographic (GC) methods.

The re-establishment of a near-natural forest is the ultimate objective of reclamation strategies for the AOSR. Because of the fundamental role of SOM in ecosystem functioning (Schmidt et al. 2011), we hypothesised that the progression of long chain *n*-alkane signatures in reconstructed soils towards those of soils

from natural sites of the same ecotype would indicate the advancing state of SOM development along chronosequences since reclamation began. Specifically, we expected to observe a transition from the dominance of peat biomarkers (C_{23} and C_{25}) to woody biomarkers (C_{27}) from early (1 yr) to mid (< 30 yr) succession, that would correlate with the O-alkyl/alkyl ratio values described by Turcotte et al. (2009) and provide evidence for the source species contributing to the change in NMR spectra. Therefore, to test our hypothesis, we extracted and quantified *n*-alkanes from a chronosequence of soils from the AOSR to investigate the relationship between specific environmental variables (including ecosite classification, soil type, dominant vegetation and stand age) and *n*-alkane abundance and distribution.

2.2. Material and methods

2.2.1. Field sampling and preparation

Detailed site descriptions and sampling procedures were described in full by Turcotte et al. (2009). Briefly, soil samples from natural and novel ecosystems were collected from the LTMPs to span the range of ecological conditions in the region within a 100 km radius of Fort McMurray (Alberta, Canada; Table 2-1). Ecosites representing natural ecosystems were classified based on the Central Mixedwood Subregion of the Boreal Forest Region and included a range of tree covers with *P. tremuloides*, *P. glauca* and *P. banksiana* in pure or mixed stands (Beckingham and Archibald 1996). Ecosites were chosen to span a range of nutrient regimes from less productive ecosites (e.g., a1, b1), on drier, nutrient poor, coarse textured Dystric Brunisol (FAO: Dystric Cambisol) soils, to those with a greater moisture content and richer nutrient regime (e.g., d1, d2), on fine textured Gray Luvisols (FAO: Albic Luvisols) (Soil Classification Working Group 1998). Reclaimed stands representing novel ecosystems on reconstructed soils were classified according to the six representative treatments of reclamation practices in the AOSR, and included a similar range of tree covers. All treatments were capped with 20 cm of a mixture of salvaged peat (25-50 % by volume) and mineral material. Treatments varied in their composition of material below 20 cm

with combinations of: pure tailings sand (H), salvaged mineral soil and tailings sand (A), salvaged mineral soil and geological substrate (E), pure geological substrate (I), and peat and mineral soil combination directly placed from an undisturbed site to the reconstructed landscape (B). Three plots of each ecosite type (a1, b1, b3, d1, d2, d3) were randomly selected (n = 3) from the LTMPs, to represent natural ecosystems and at least four reclaimed sites of each treatment were selected (A, B, E, H, I) to represent novel ecosystems (Table 2-1). At each plot, surface soil samples (0-10 cm from below the fresh litter layer) were randomly collected from 10 locations around the edge of the permanent LTMP using a trowel. The soils were then composited to one representative sample per plot. Soil samples were sieved to 4 mm, oven dried at 65 °C and finely ground by ball mill before analysis of total organic carbon (TOC) concentration by dry combustion using a Costech ECS 4010 Elemental Analyzer (Costech Analytical Technologies Inc. Valencia, USA).

Peat material, representative of the material used in novel ecosystem construction, was collected as a composite sample from the top 10 cm of an Organic or Histosol from a representative *Sphagnum* peatland within the LTMP area in 2003, air dried and stored. Additional vegetation in the peatland included *Picea mariana* (Mill.) Britton, *Larix laricina* (Du Roi) K.Koch, *Vaccinium vitis-idaea* L., *Betula glandulosa* Michx. and *Salix spp*. Leaves were collected directly from a random selection of a dozen *P. tremuloides* trees on a novel ecosystem site within the radius of LTMPs in September 2010, air dried and ground using a ball mill to a fine powder. Needles of *P. banksiana* and *P. glauca* were collected from trees of Canadian origin from Bedgebury Pinetum (UK) in November 2012, oven dried at 85 °C and finely ground with a ball mill.

2.2.2. Extraction and analysis

The leaves and soils were extracted according to Bull et al. (2000). Samples (0.5 to 3.0 g) were Soxhlet extracted with dichloromethane (DCM) and acetone (9:1) for 24 h. A known amount of C_{34} *n*-alkane was added as an internal standard

before extraction. The extract was evaporated to dryness then hydrolysed with 0.5 M NaOH (100 °C, 1 h) before neutralisation (1.0 M HCl) and extraction with Et_2O. The extract was separated into three fractions using silica gel flash-column chromatography. Elution of the first fraction with hexane gave the aliphatic hydrocarbons. The fraction was redissolved in hexane and analysed using an Agilent 7890A GC (Agilent, Berkshire, UK) fitted with an FID equipped with an Agilent HP-5 column (30 m x 320 µm i.d. x 0.25 µm film thickness). The oven temperature was programmed from 40 °C (held 1 min) to 130 °C at 20 °C min^{-1} to 300°C (held 10 min) at 4°C min^{-1}. Peak identification was confirmed via GC-mass spectrometer (GC-MS) using an Agilent 6890 GC instrument coupled to an Agilent 5973 MS equipped with an Agilent DB-5ms column (30 m x 250 µm i.d. x 0.25 µm film thickness). The dominant fragment ions (base peaks) were represented by *m/z* 57 and the diagnostic ions (*m/z*) 282 (C_{20}), 296 (C_{21}), 324 (C_{23}), 338 (C_{24}), 352 (C_{25}), 366 (C_{26}), 380 (C_{27}), 394 (C_{28}), 408 (C_{29}), 422 (C_{30}), 436 (C_{31}), 450 (C_{32}), 464 (C_{33}), 478 (C_{34}, internal standard). The concentrations of individual *n*-alkanes were determined relative to the C_{34} internal standard. The total concentration of the biomarker *n*-alkanes in a sample was calculated as Σ (C_{20}, C_{21}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}).

2.2.3. Data analysis

Sample mean, standard deviation and t-tests were performed using R (version 2.15.1, the R Foundation for Statistical Computing). Total *n*-alkane concentrations were normally distributed and were tested for differences between ecosystems via a t-test. Proportions of *n*-alkanes between C_{20} and C_{33} were examined for patterns of similarity in the data in response to environmental variables via Non-metric Multidimensional Scaling (NMS). This technique organizes large data sets to two or three dimensions by searching for underlying patterns by examining the distance between data points, using numerous iterations, and grouping similarities and distancing dissimilarities (McCune and Grace 2002). An individual sample, with its multiple responses, is presented as a point on a two or three dimensional plot with directionless axes; rather the axes indicate the fit of pattern. Therefore,

points closer together on the ordination are more similar than points further apart. Proportional data were relativized by a Hellinger transformation (Legendre and Gallagher 2001) prior to ordination with a Sorenson (Bray-Curtis) distance measurement. Variation in the ordination expressed along the axis was explained by correlation with the secondary matrix of environmental variables (including ecosite classification, soil type, dominant vegetation, site age and soil TOC concentration in addition to *n*-alkane concentration). Patterns in the data were tested by a non-parametric test, multi-response permutation procedures (MRPP), which evaluates differences between discrete groups; environmental categories included in this study were: ecosystem, ecosite, soil and vegetation (Table 2-1). MRPP testing of grouping variables gives three values: an overall significance (p), a term indicating the degree of separation between groups (T) and the separation within groups (A). NMS and MRPP were performed with PC-ORD software version 5 (MjM Software Design, Gleneden Beach, USA). Multi-variate Regression Tree (MRT) analysis of transformed *n*-alkane proportional data was performed with a Bray-Curtis distance measure to evaluate the influence of environmental variables used in the NMS using the R software package with the mvpart library. The amount of variation described by the tree is given by the inverse of the Error, while CV Error is an indicator of the predictive ability of the tree with values close to zero are perfect and those close to one are poor predictors (De'ath 2002).

2.3. Results and discussion

2.3.1. Soil total organic carbon concentrations

There was no significant difference between the TOC concentrations of the natural or reconstructed soils. Mean TOC concentrations were 77.9 ±49.3 mg g^{-1} dry weight soil (range 17.0 to 188.9 mg g^{-1} dry weight soil) and 85.3 ±59.4 mg g^{-1} dry weight soil (range 12.3 and 243.6 mg g^{-1} dry weight soil) for the natural and novel stands, respectively (Table 2-2). This is similar to the values previously reported by Turcotte et al. (2009) where the authors also determined that there was no significant difference between the mean total SOC concentration of the

novel site forest soils (0 – 10 cm) vs. the natural forest soil. Thus, measurements of SOM stocks as an indicator of ecosystem recovery may not be appropriate for these ecosystems. Instead this suggests that other indicators of soil regeneration, such as the biomarker approach described herein, are essential in understanding the progression of reconstructed systems to a target natural ecosystem.

2.3.2. *n*-Alkanes in vegetation input

The peat material, which was obtained from a regional source representative of the organic matter (OM) used to reconstruct soils in the AOSR, contained a high proportion of *Sphagnum* spp. This enabled the potential use of the peat biomarker (C_{23}) to differentiate inputs, because the biomarker is comparatively uncommon in terrestrial environments (Eglinton and Hamilton 1967). The total concentration of *n*-alkanes (223 μg g^{-1} dry wt. or 521 μg g^{-1} C) was within the range reported for a series of *Sphagnum* spp. (Baas et al. 2000). However, the peat-derived OM sample revealed a mixture of *Sphagnum* biomarkers (C23 and C25; Baas et al. 2000; Bingham et al. 2010; Nott et al. 2000) and a predominance of 'higher plant' biomarkers (C31 and C33; Eglinton and Hamilton 1967). This may suggest: (i) a mixed provenance for the OM (peat plus OM from another source) or (ii) the emergence of *Sphagnum* spp. adapted to dry conditions. For example, *S. magellanicum*, widely distributed throughout circumpolar North America (Johnson et al. 1995), has a higher abundance of C_{31} *vs.* the C_{25} in peat from drier regions (Bingham et al. 2010). Samples of *S. magellanicum* from the Canadian Beaufort Sea region analysed by Yunker *at al*. (1993) also exhibited dominant C_{31} distributions. Similarly, C_{33} is widely reported to be either absent from or present in low abundance in *Sphagnum* spp. and is used as a biomarker of non-*Sphagnum* peat vegetation; however, Pancost et al. (2002) observed that *S. rubellum* (which may also display prominent C_{25} or C_{31}) also contained substantial C_{33} concentrations. Evidence for peat versus vascular plant input to soils may be achieved by further investigation of other compounds derived from plants, e.g. monosaccharides (Jia et al., 2008) and sterols (Ronkainen et al., 2013) which have

been successfully applied to the investigation of palaeoclimatic influence on the evolution of peat profiles.

Although the peat material was characterised by 'higher plant' biomarkers (C_{31} and C_{33}), Fig. 2-1 shows that the distributions for leaves and needles of trees used in the reclaimed stands differed substantially from the composite peat sample because they all contained a non-detectable concentrations (*P. glauca and P. banksiana*) or much lower abundances (*P. tremuloides*) of C_{31} and C_{33}. The deciduous species, *P. tremuloides,* had the highest concentrations, e.g. > 900 µg g^{-1} dry wt. or 1900 µg g^{-1} C for C_{25} (most abundant), while both conifers had much smaller concentrations, e.g. ~10 µg g^{-1} dry wt. or ~20 µg g^{-1} C for C_{29}. Similar differences in concentration for deciduous vs. conifer leaves were also reported by Marseille et al. (1999) and Diefendorf et al. (2011) and the distributions of Pinaceae and Picea have also been characterised by strong C_{29}, C_{27} and C_{25} abundances (Corrigan et al. 1978; Herbin and Robins 1968; Oros et al. 1999). Needles from *Picea abies* were reported to be dominated by C_{27} with strong abundances of C_{25} and C_{29} (Marseille et al. 1999). In contrast, Maffei et al. (2004) reported C_{31} to be the most abundant in a survey of leaf wax *n*-alkanes in conifers; including *P. abies* and *P. glauca*.

2.3.3. *n*-Alkanes in natural soils

The influence of tree vegetation type on the *n*-alkanes in the soils of the natural plots differed between ecosites (Table 2-2). Soils in ecosites with *P. tremuloides* as the dominant, or co-dominant, canopy component (b1, b3, d1, d2) had corresponding high concentrations of C_{27} and C_{25} (Fig. 2-2). Soils from plots d1 and d2 on Gray Luvisols had greater abundances of C_{27} and C_{25} which may be related to an increase in biomass input: a medium to rich nutrient regime is required to support and promote the growth of *P. tremuloides* (Beckingham and Archibald 1996). Similarly, ecosite a1, which was dominated by *P. banksiana,* had a reduced abundance of longer *n*-alkane chain lengths, which was similar to the *n*-alkane distribution in the conifer needles. A predominance of C_{29} for

coniferous soil was also found for the French Massif Central region (Lavrieux et al. 2012) and the litter layer under Corsican pines in The Netherlands (Nierop et al. 2006). Soils from b1 ecosites were interesting as they had overall higher concentrations of *n*-alkanes, with inputs from *P. tremuloides* and *P. banksiana*, than soils from other ecosites however this could have been due in part to the soils having the lowest range of TOC (Table 2-2).

There was a greater diversity of *n*-alkane homologues in the natural soils compared with the sampled tree leaves and needle inputs, which is probably derived from the additional input of understorey species, i.e. Ericaeae, particularly *Vaccinium* sp. and forest floor grasses and forbs (Rowland et al. 2009). Vegetation in the understorey is reported to comprise < 2 % of the OM budget in mature natural *P. tremuloides* ecosystems (Gower et al. 2000). On deciduous stands in the western boreal forest there is greater shrub cover (48 %) compared to mixed wood (20 %) or conifer (16 %) stands (Macdonald and Fenniak 2007). Soils from the natural ecosystems had greater concentrations of C_{31} which were presumably derived from understory vegetation, because the comparative concentrations were less in the tree leaves and needles. An *n*-alkane distribution dominated by C_{31} (and C_{33}) is characteristic of inputs from grasses and forbs (Bull et al. 2000; Maffei 1994; 1996a; b).

2.3.4. *n*-Alkanes in novel soils

The concentration of biomarker *n*-alkanes in the natural soils was significantly greater than in the soils from the novel plots (577.1 ±578 vs. 193.9 ±116 μg g^{-1} C; $p = 0.01$). Studies have indicated that the novel soils differ from their natural counterparts in many ecosystem process indicators, e.g. nitrogen availability (Hemstock et al. 2010; Rowland et al. 2009), soil microbial biomass (McMillan et al. 2007), microbial communities (Dimitriu et al. 2010) and OM quality (Turcotte et al. 2009). Although the concentration of *n*-alkanes in the soils of the novel ecosystems was less than in the natural soils, their signature (i.e. *n*-alkane distribution) more closely resembled that of the tree inputs than in the natural

ecosystems (Fig. 2-3). Overall, the distributions of *n*-alkanes varied between soils derived from peat reconstruction material or soil from natural ecosystems, with three times the mean concentration of C_{27} (the biomarker for woody biomass input) in natural systems (i.e., mean concentration of 130.5 versus 36.4 μg g^{-1} C, respectively). Variation in *n*-alkane signatures among soils under different tree species was also determined. For example, soils from *P. banksiana* dominated ecosites (H) contained relatively even amounts of C_{27} and C_{29} characteristic of the *P. banksiana* needles, and those planted with a combination of *P. glauca* and *P. tremuloides* contained a predominance of C_{27} with contributions from C_{25} and C_{29}. Of the reclaimed stands H alone featured a strong C_{31} signal, the signature of the *Sphagnum* material used in the soil construction of the novel ecosites or, as reported by Maffei et al. (2004), the most abundant *n*-alkane for *P. glauca*. While present, C_{31} was in low abundance in the novel ecosystem soils under *P. glauca*, in contrast to the higher abundances in natural ecosites b3 and d3 also under *P. glauca*. On similar reclaimed sites to those used here, Hahn and Quideau (2012) determined that the ground cover in sites that were 6 to 12 years old was either bare with few to no shrubs or dominated by forbs. A vegetation survey of LTMPs on regenerating AOSR soils also reported bare ground, grasses and forbs on novel ecosystem sites contrasting with the accumulation of moss, shrubs, and woody debris on natural ecosystem sites (Rowland et al., 2009). Therefore, it is postulated that the reduced abundance of C_{31} in the novel ecosystems in this study was due to a lack of inputs from understorey vegetation.

2.3.5. *n*-Alkanes and ecosystem development

A major objective of this study was to determine if a transition in *n*-alkane signatures was observable with ecosystem development, adding to the body of work that has identified distinct differences in SOM from novel and natural ecosystems in the western boreal forest (Quideau et al. 2013; Turcotte et al. 2009). The use of *n*-alkane biomarkers in soils to elucidate vegetation transitions, particularly in grassland to forest transitions, has both determined separation of samples based on dominant OM inputs to be possible (Bird et al. 1995; Jansen et

al. 2006; Trendel et al. 2010; van Bergen et al. 1997) and to be inconclusive (Lavrieux et al. 2012; Marseille et al. 1999). One method of determining vegetation transitions from biomarkers in soil is by the use of ordination as ordination of ecological data has provided a means of investigating and interpreting complex multivariate datasets by relating shifts in community composition to environmental responses (Clarke 1993). Previous applications of ordination techniques have used Principal Component Analysis (PCA) to differentiate plant species using *n*-alkane biomarkers (Maffei 1994; 1996a; b) or carboxylic acids and monosaccharides (Dungait et al. 2011), and non-vascular *versus* vascular plant inputs to peats using monosaccharides (Jia et al. 2008). Redundancy Analysis (RDA) has been used to differentiate between lipid biomarkers (*n*-alkanes and sterols) of fen mosses and vascular plants (Ronkainen et al. 2013). However an alternative is NMS, an ordination technique which requires neither normal distribution nor linear relationships among the variables (McCune and Grace 2002). NMS has been effective in investigating, for example: spider assemblages (Oxbrough et al. 2005); soil microbial diversity (Hannam et al. 2006); vegetation composition (Small and McCarthy 2002); lipid geochemistry of lake sediments (Pearson et al. 2007); and, biogeochemical processes in novel ecosystems (Quideau et al. 2013; Turcotte et al. 2009).

Differences between *n*-alkane biomarker signatures of natural and novel ecosystems in our study were clearly identified using NMS (Fig. 2-4). Using *n*-alkane proportional data, a three-dimensional ordination solution (stress 10.8 after 67 iterations) was determined. The first and second axes represented 49 % and 27 % of the variation respectively while the third axis accounted for 16 %; hence, for clarity, the data were presented with respect to the first and second axes of the ordination in Figure 2-4. Testing data grouping by MRPP based on the environmental variables illustrated the clear differences between the natural and novel ecosystems (ecosystem, $p < 0.001$, $T = -8.11$, $A = 0.09$; soil type, $p < 0.001$, $T = -5.75$, $A = 0.18$; ecosite classification, $p < 0.001$, $T = -5.18$, $A = 0.22$; and vegetation, $p < 0.05$, $T = -1.91$, $A = 0.05$). When correcting for multiple pairwise

comparisons, the differences were not strong enough among ecosites to be significant. For soil type, while there were no differences within ecosystem soils there were differences between natural (Dystric Brunisol and Gray Luvisol) and novel ecosystem soils (PM/S/TS and PM/OB). For vegetation, soils with *P. banksiana* as the input were significantly different from sites of mixed *P. glauca* and *P. tremuloides*. In an effort to explain the spread of the data, results were correlated to stand age, TOC concentration and *n*-alkane concentrations; none of these variables correlated with an r^2=0.5. Our results did, somewhat, separate out by vegetation type; however the grouping of results was best explained by the categorical classification of ecosystem, which was based on a combination of dominant vegetation input and soil type. These results agree with previous studies which indicated a change in soil biomarkers with changing vegetation input (Jansen et al. 2006; van Bergen et al. 1997) and an increase in C_{27} *n*-alkane for forested sites (Trendel et al. 2010). Comparisons with previous work however must be done cautiously due to the anthropogenic origin of the novel ecosystems and the survey approach used here to investigate these novel and natural ecosystems.

To further explore the data and determine possible grouping parameters for future investigations of these ecosystems the distributions of *n*-alkane were then investigated by MRT. MRT is a method of cluster analysis which models species and environment relationship by splitting by dissimilarity where divisions are based on minimizing dissimilarities within groups and has become widely used in ecological analysis (De'ath 2002; Sorenson et al. 2011). Processing data via MRT determined that vegetation type was the strongest grouping variable (Error:0.84, CV Error:1.3, SE:0.23; Fig. 2-5). The split of dissimilarity driven by vegetation separated pure stands of *P. banksiana* from *P. tremuloides*, *P. glauca*, and mixtures with *P. banksiana*. Novel and natural ecosystems were not distinct in their *n*-alkane distributions; rather those soils below pure stands of *P. banksiana* were more similar to each other and these included novel ecosystems with tailings sands closer to the surface (H and B) and soils from natural ecosystems of a lower

nutrient regime (e.g., a1; Beckingham and Archibald 1996). Other research on novel and natural ecosystems reported that ecosite type was a strong grouping feature in terms of soil nutrients, organic matter and microbial communities Quideau et al. (2013). Despite both the low data variation described and the low predictive ability of the MRT, our analysis indicated that vegetation best described *n*-alkane proportions rather than ecosite type, stand age or TOC concentration.

2.4. Conclusion

Specific *n*-alkane biomarkers for different vegetation types were used to characterize and investigate the combined effect of vegetation and land -use change on soil OM development in western Canadian boreal soils. Novel ecosystem soils were found to have similar TOC concentrations to natural systems (77.9 mg g^{-1} and 85.3 mg g^{-1} dry weight soil, respectively), but lower concentrations of *n*-alkane biomarkers (summed concentrations were 577.1 and 193.9 μg g^{-1} C respectively). The distributions of *n*-alkanes varied between soils derived from peat reconstruction material or soil from natural ecosystems, with twice the mean concentration of C_{27} (the biomarker for woody biomass input) in natural systems (i.e. mean concentration of 130.5 versus 36.4 μg g^{-1} C, respectively). However, although the *n*-alkane biomarker signatures were different between ecosystems, a specific robust biomarker of SOM development from litter input was not determined. The biomarker signatures related to different vegetation types were better predictors than stand age or SOC concentration. Further research, particularly more comprehensive assessments of the *n*-alkane inputs from stand vegetation and litter types, is required to refine the approach.

Table 2.1

Characteristics of ecosites in the LTMP in the AOSR. Ecosites were ecological units defined by similar moisture and nutrient regimes. Reclamation types represent six treatments of reclamation practices in the region.

Ecosystem	Ecosite or reclamation type[a]	Soil[b]	Vegetation	Age (yr.)
Natural	a1	Dystric Brunisol	*P. banksiana*	50
	a1	Dystric Brunisol	*P. banksiana*	80
	a1	Dystric Brunisol	*P. banksiana*	87
	b1	Dystric Brunisol	*P. tremuloides* and *P. banksiana*	49
	b1	Dystric Brunisol	*P. tremuloides* and *P. banksiana*	64
	b1	Dystric Brunisol	*P. tremuloides* and *P. banksiana*	64
	b3	Dystric Brunisol	*P. glauca* and *P. tremuloides*	103
	b3	Dystric Brunisol	*P. glauca* and *P. tremuloides*	50
	b3	Dystric Brunisol	*P. tremuloides*	56
	d1	Gray Luvisol	*P. tremuloides*	77
	d1	Gray Luvisol	*P. tremuloides*	59
	d1	Gray Luvisol	*P. tremuloides*	56
	d2	Gray Luvisol	*P. glauca* and *P. tremuloides*	71
	d2	Gray Luvisol	*P. glauca* and *P. tremuloides*	83
	d2	Gray Luvisol	*P. glauca* and *P. tremuloides*	72
	d3	Gray Luvisol	*P. glauca*	165
	d3	Gray Luvisol	*P. glauca*	107
	d3	Gray Luvisol	*P. glauca*	105
Novel	A	PM/S/TS	*P. glauca*	6
	A	PM/S/TS	*P. glauca*	20
	A	PM/S/TS	*P. glauca* and *P. tremuloides*	11
	A	PM/S/TS	*P. glauca* and *P. tremuloides*	3
	A	PM/S/TS	*P. glauca* and *P. tremuloides*	12
	A	PM/S/TS	*P. glauca* and *P. tremuloides*	1
	B	DP/TS	*P. banksiana*	8
	B	DP/TS	*P. tremuloides* and *P. banksiana*	14
	B	DP/TS	*P. glauca* and *P. tremuloides*	4
	B	DP/TS	*P. tremuloides* and *P. banksiana*	5
	B	DP/TS	*P. glauca* and *P. tremuloides*	6
	E	PM/S/OB	*P. glauca*	7
	E	PM/S/OB	*P. tremuloides*	6
	E	PM/S/OB	*P. glauca* and *P. tremuloides*	18
	E	PM/S/OB	*P. tremuloides*	0
	H	PM/TS	*P. banksiana*	9
	H	PM/TS	*P. banksiana*	9
	H	PM/TS	*P. glauca*	30
	H	PM/TS	*P. banksiana*	29
	I	PM/OB	*P. glauca* and *P. banksiana*	17
	I	PM/OB	*P. glauca*	30
	I	PM/OB	*P. glauca* and *P. tremuloides*	18
	I	PM/OB	*P. glauca*	21
	I	PM/OB	*P. glauca* and *P. tremuloides*	1

[a]Ecosites were ecological units defined by similar moisture and nutrient regimes; reclamation types represent six treatments of reclamation practices in the region; [b] Canadian System of Soil Classification; disturbed soil abbreviations: PM, 25-50 % by volume peat mixed with mineral material; S, salvaged secondary mineral material; TS, tailings sand which is residual sand following extraction; OB, overburden of geologic substrate above oil sands (Turcotte et al. 2009)

Table 2.2

Biomarker *n* -alkane concentration (μg g^{-1} C) for each composite sample of replicate soil samples (0 – 10 cm; n = 10) from each ecosite plot described in Table 1.

Ecosystem		Age	Organic Carbon	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	Total
	Ecosite	(yr.)	(mg g^{-1})	(μg g^{-1} C)														
	a1	50	66.4	3.6	5.3	1.7	3.1	1.9	5.0	2.8	12.2	4.8	70.3	12.3	119.9	7.2	46.1	296.2
	a1	80	17.0	29.3	16.5	0.0	10.6	0.0	14.8	0.0	30.5	10.6	53.0	27.5	84.7	0.0	38.7	316.2
	a1	87	52.1	5.9	4.3	3.4	4.1	2.8	6.5	2.9	15.0	6.7	21.7	10.1	19.9	0.0	7.5	110.9
	b1	49	32.1	10.4	15.1	16.3	25.2	21.9	60.5	22.9	123.4	23.2	98.4	31.0	182.3	21.4	42.8	694.6
	b1	64	20.3	39.4	28.6	10.3	31.9	21.5	107.2	31.7	241.8	18.9	430.6	52.3	607.3	25.1	204.7	1851.3
	b1	64	31.1	56.0	40.5	19.0	52.8	36.6	298.5	55.1	674.8	65.9	250.7	69.9	393.0	33.4	157.4	2203.5
	b3	103	105.2	4.9	8.5	3.4	13.2	9.2	39.7	7.4	89.8	7.0	58.9	12.9	128.1	31.0	94.3	508.4
	b3	50	60.5	8.5	9.7	3.4	16.2	8.9	51.6	11.3	96.1	8.7	65.9	20.4	114.0	8.9	42.2	466.0
Natural	b3	56	64.6	2.7	6.5	2.3	4.0	3.6	9.4	3.5	15.6	3.5	25.9	9.7	48.4	5.5	17.1	157.6
	d1	77	188.9	1.7	5.7	3.2	30.7	13.5	49.5	7.9	90.8	4.1	19.6	6.6	49.7	5.9	30.7	319.7
	d1	59	62.7	3.1	6.6	2.5	14.6	6.1	57.8	8.6	103.1	4.0	28.2	3.1	25.4	2.6	18.4	284.2
	d1	56	91.6	3.5	4.0	4.0	21.8	11.0	80.2	14.2	110.9	10.7	47.5	18.0	58.0	8.9	40.7	433.4
	d2	71	186.8	2.2	1.6	1.5	16.1	5.6	55.2	9.1	105.2	6.0	42.6	5.0	71.6	5.1	0.0	327.1
	d2	83	73.5	14.3	15.8	7.3	37.7	22.2	167.6	29.5	301.5	19.1	62.2	16.3	75.6	9.4	198.7	977.1
	d2	72	51.2	4.4	6.3	4.6	12.2	6.5	40.5	8.0	68.2	6.1	32.2	3.6	29.0	0.0	10.3	231.8
	d3	165	70.0	8.9	11.6	4.8	23.1	13.1	74.6	17.5	139.4	14.9	68.2	18.1	237.2	0.0	51.1	682.5
	d3	107	107.8	6.0	6.2	0.0	19.0	7.6	63.0	13.5	126.6	11.1	57.0	19.0	118.4	10.1	45.7	503.3
	d3	105	119.6	0.8	1.3	0.6	0.9	0.6	2.1	0.8	4.2	0.0	3.9	0.8	5.1	0.0	3.7	24.8
	Reclaimed	(yr.)		(μg g^{-1} C)														
	A	6	53.4	2.6	10.4	3.9	13.7	5.3	25.0	5.0	46.3	9.0	66.7	9.5	44.3	3.7	22.0	267.3
	A	20	43.8	11.5	32.6	12.0	49.0	15.0	74.7	10.6	83.6	10.6	76.5	79.8	48.7	4.0	19.4	528.1
	A	11	113.0	4.0	8.1	3.3	13.4	5.0	21.9	3.3	32.7	3.0	25.2	3.2	14.9	1.3	11.4	150.5
	A	3	178.2	4.2	13.9	6.8	20.5	5.2	23.3	4.5	24.3	4.7	22.5	26.7	15.4	10.9	5.7	188.7
	A	12	108.7	5.0	5.1	4.6	8.6	4.3	18.6	5.7	36.5	5.2	29.0	3.5	25.2	0.0	11.2	162.4
	A	1	147.7	3.5	5.7	2.9	7.3	2.2	9.5	1.5	11.0	1.2	8.6	20.3	10.7	6.6	6.5	97.6
	B	8	33.4	4.4	4.8	2.8	5.2	3.3	9.5	3.7	24.8	0.0	28.1	5.9	43.0	5.7	37.5	178.8
	B	14	47.3	2.8	0.0	0.0	3.6	2.4	5.4	0.0	8.8	0.0	16.2	0.0	10.9	0.0	6.7	56.7
	B	4	16.7	7.8	12.6	9.8	20.5	13.5	45.5	17.4	105.0	12.8	67.2	11.0	23.9	10.1	19.8	376.7
	B	5	38.4	5.8	6.3	5.7	9.2	4.4	12.8	4.0	17.3	0.0	17.8	2.6	17.2	0.0	10.2	113.2
	B	6	25.1	9.4	6.8	7.6	10.1	6.1	15.1	5.7	25.6	4.1	42.8	4.2	25.2	2.1	27.7	192.5
	E	7	109.0	4.3	7.2	3.8	16.0	4.3	19.1	2.6	21.3	3.1	23.0	4.9	20.9	0.5	11.2	141.9
Novel	E	6	243.6	1.7	4.4	2.4	10.3	3.8	17.4	5.5	28.0	3.7	20.3	1.8	13.2	0.5	6.7	119.9
	E	18	112.8	5.5	10.9	7.8	25.3	8.7	33.9	9.8	43.1	8.6	40.1	17.0	35.8	3.1	15.0	264.6
	E	0	32.1	2.1	2.7	0.0	3.5	2.7	6.2	0.0	11.6	0.0	13.5	15.1	12.9	2.9	14.9	88.1
	H	9	77.0	9.3	11.4	0.0	20.9	0.0	27.9	0.0	34.9	0.0	31.5	0.0	20.4	8.6	4.4	169.4
	H	9	51.1	1.6	2.6	1.8	2.8	2.2	7.5	1.9	9.0	2.2	13.0	2.4	15.2	0.0	7.9	70.0
	H	30	42.4	12.1	7.5	4.0	5.7	9.0	18.5	3.0	36.0	4.1	55.7	2.3	49.8	0.0	14.2	222.1
	H	29	12.3	0.0	0.0	0.0	2.9	0.0	4.5	3.0	8.1	3.3	10.1	3.9	11.6	0.0	19.3	66.9
	I	17	147.8	6.2	10.2	8.2	25.9	12.1	43.6	9.5	55.7	8.0	40.5	1.6	27.4	0.0	9.2	258.1
	I	30	52.9	9.0	11.0	0.0	14.9	8.1	23.0	0.0	39.8	0.0	29.5	7.8	22.1	2.5	10.1	177.8
	I	18	128.6	5.4	13.9	8.1	28.4	12.3	52.0	9.5	106.4	11.7	122.0	4.8	32.7	0.9	13.7	421.6
	I	21	75.8	0.0	8.9	0.0	11.1	0.0	14.1	0.0	17.7	0.0	21.4	0.0	11.3	9.2	10.7	104.4
	I	1	157.6	7.5	8.4	9.2	16.3	6.3	29.7	8.4	45.3	7.4	39.4	4.4	33.4	1.8	18.1	235.6

Figures

Figure 2-1. Distribution of odd *n*-alkanes extracted from dominant vegetation inputs to SOM in the western boreal forest.

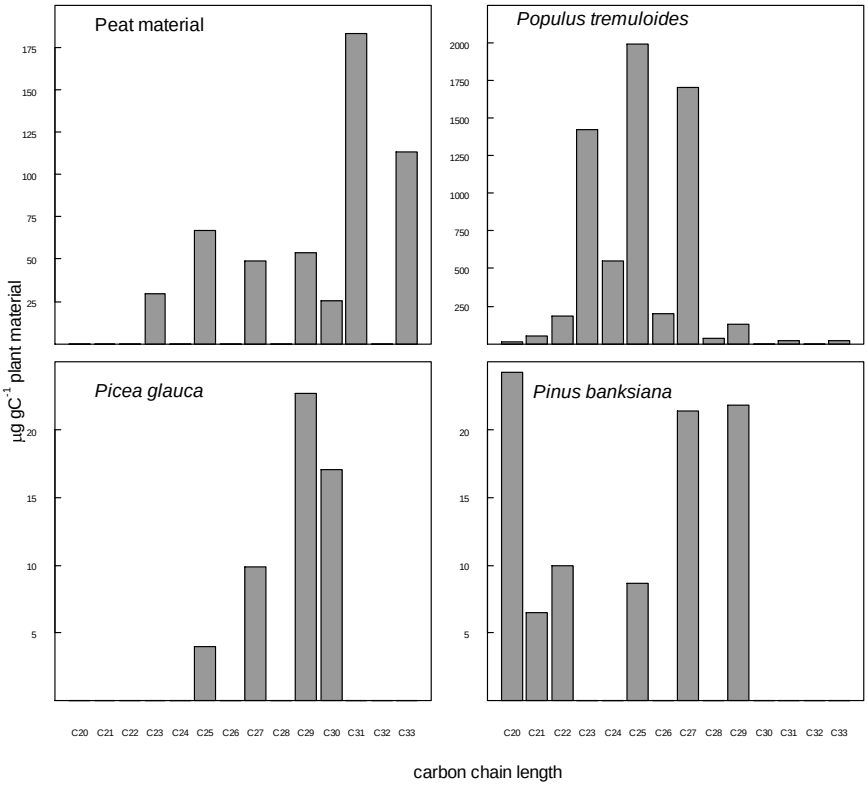


Figure 2-2. Distribution of *n*-alkanes in natural ecosystems within the LTMPs in the AOSR, Canada. Error bars reflect one standard deviation from the mean (n= 3).

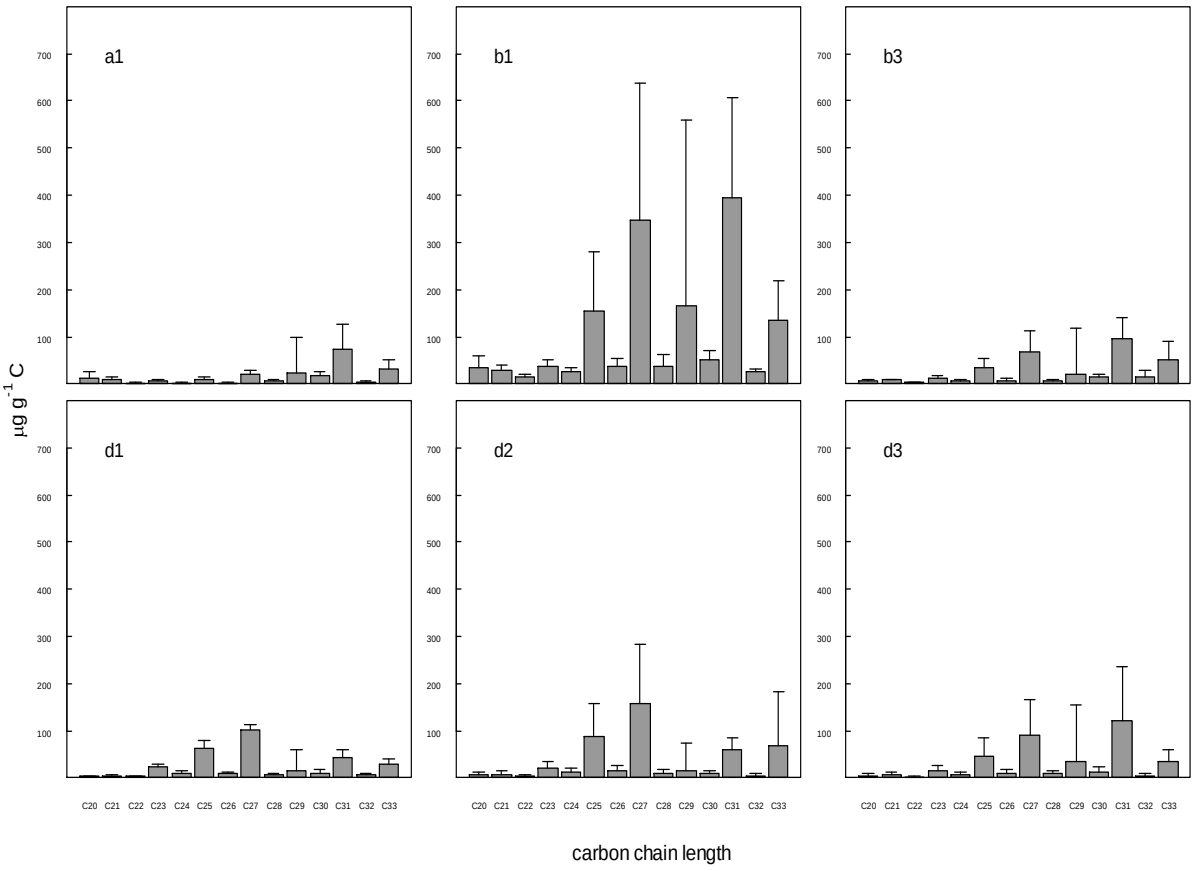


Figure 2-3. Distribution of *n*-alkanes in novel ecosystems in LTMPs in the AOSR, Canada. Error bars reflect one standard deviation from the mean (n= 4-6, number of replicates per reclamation treatment are indicated in Table 2-1).

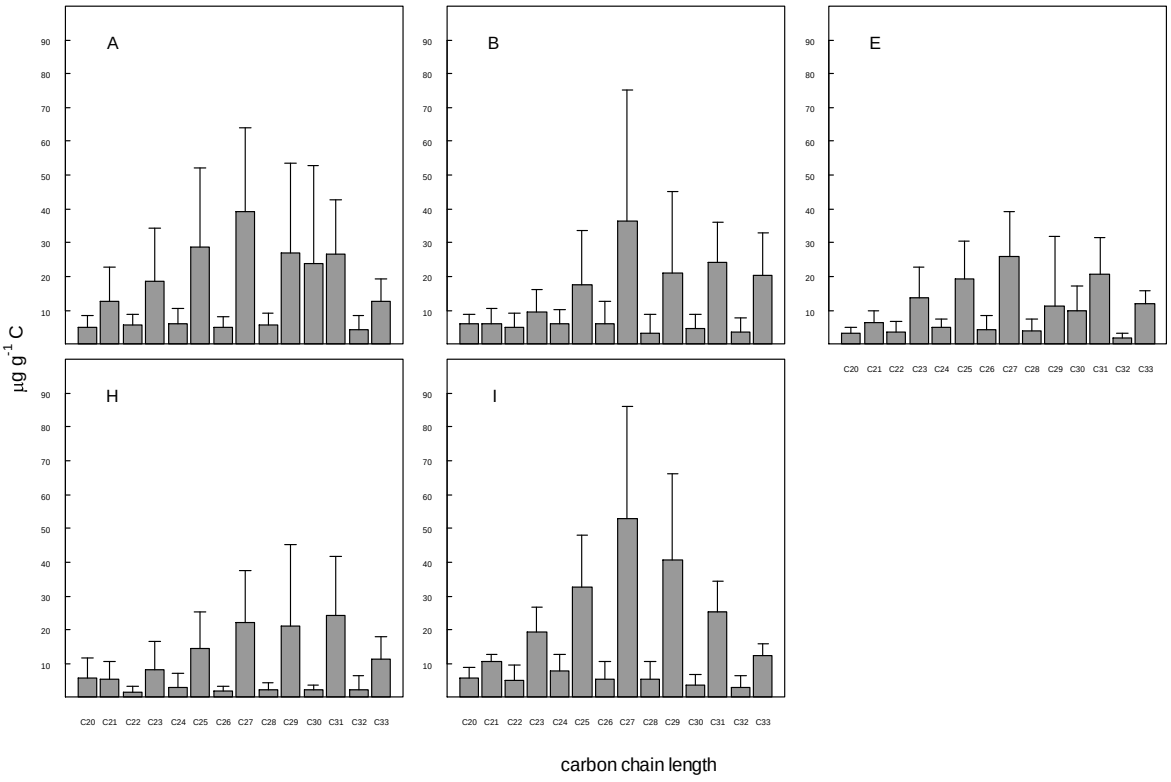


Figure 2-4. NMS ordination of the *n*-alkane distribution data (%) from SOM of novel and natural ecosystems within the LTMPs in the AOSR, Canada. Each point corresponds to one site and points clustered together are more similar than points which are further apart. Points are categorized by natural or novel ecosystem type as these ecosystems were determined to be different ($p < 0.001$). Axes are presented to indicate the fit of the pattern but have no direction or meaning themselves.

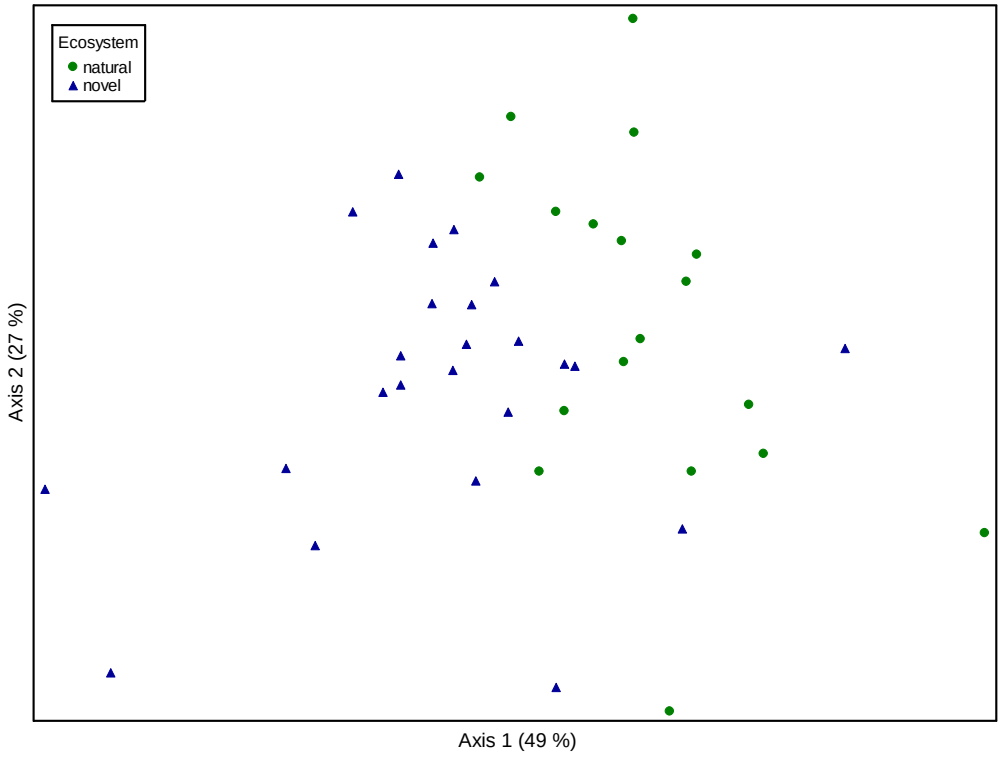
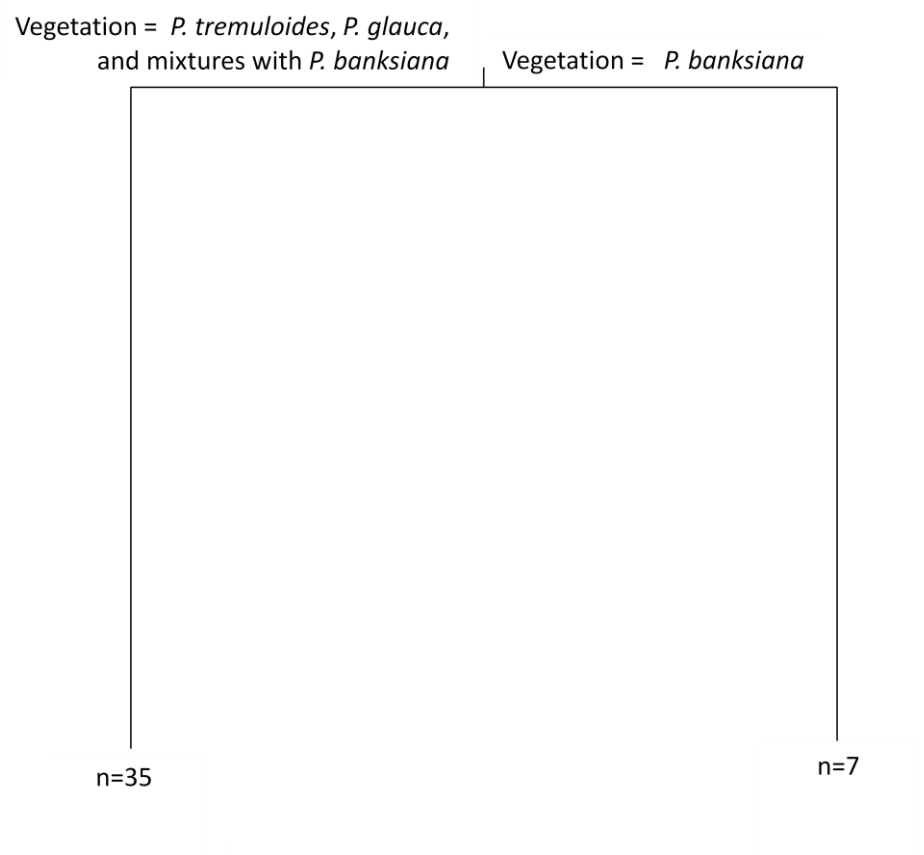


Figure 2-5. MRT analysis of the *n*-alkane distribution data (%) from SOM of novel and natural ecosystems in the LTMPs in the AOSR, Canada. Groups are based on ecosite, soil type, vegetation, stand age and soil carbon concentration listed in Table 2-1. Variation in the data (16 %) is explained by cluster division based on vegetation type with n indicating the number of sites per cluster.



References

Akala, V.A., Lal, R. 2000. Potential of mine land reclamation for soil organic carbon sequestration in Ohio. Land Degradation and Development 11, 289-297.

Baas, M., Pancost, R., van Geel, B., Sinninghe Damsté, J.S. 2000. A comparative study of lipids in *Sphagnum* species. Org. Geochem. 31, 535-541.

Banning, N.C., Grant, C.D., Jones, M.D., Murphy, D.V. 2008. Recovery of soil organic matter, organic matter trunover and nitrogen cycling in a post-mining forest-rehabilitation chronosequence. Soil Biol. Biochem. 40, 2021-2031.

Beckingham, J.D., Archibald, J.H. 1996. 'Field Guide to the Ecosites of Northern Alberta.' (Natural Resources Canada, Canadian Forest Service: Edmonton).

Bingham, E.M., McClymont, E.L., Väliranta, M., Mauquoy, D., Roberts, Z., Chambers, F.M., Pancost, R.D., Evershed, R.P. 2010. Conservative composition of *n*-alkane biomarkers in *Sphagnum* species: Implications for palaeoclimate reconstruction in ombrotrophic peat bogs. Org. Geochem. 41, 214-220.

Bird, M.I., Summons, R.E., Gagan, M.K., Roksandic, Z., Dowling, L., Head, J., Fifield, K.L., Cresswell, R.G., Johnson, D.P. 1995. Terrestrial vegetation change inferred from *n*-alkane $\delta^{13}C$ analysis in the marine environment. Org. Geochem. 59, 2853-2857.

Bull, I.D., van Bergen, P.F., Nott, C.J., Poulton, P.R., Evershed, R.P. 2000. Organic geochemical studies of soils from the Rothamsted classical experiments-V. The fate of lipids in different long-term experiments. Org. Geochem. 31, 389-408.

Clarke, K.R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18, 117-143.

Corrigan, D., Timoney, R.F., Donnelly, D.M.X. 1978. *n*-Alkanes and ω-hydroxyalkanoic acids from the needles of twenty-eight *Picea* species. Phytochemistry 17, 907-910.

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

Derenne, S., Largeau, C. 2001. A review of some important families of refractory macromolecules: Composition, origin and fate in soils and sediments. Soil Sci. 166, 833-847.

Diefendorf, A.F., Freeman, K.H., Wing, S.L., Graham, H.V. 2011. Production of *n*-alkyl lipids in living plants and implications for the geologic past. Geochim. Cosmochim. Acta 75, 7472-7485.

Dimitriu, P.A., Prescott, C.E., Quideau, S.A., Grayston, S.J. 2010. Impact of reclamation of surface-mined boreal forest soils on microbial community composition and function. Soil Biol. Biochem. 42, 2289-2297.

Dungait, J.A.J., Docherty, G., Straker, V., Evershed, R.P. 2011. Variation in bulk tissue, fatty acid and monosaccharide $\delta^{13}C$ values between autotrophic and heterotrophic plant organs. Phytochemistry 72, 2130-2138.

Eglinton, G., Hamilton, R.J. 1967. Leaf epicuticular waxes. Science 156, 1322-1335.

Eglinton, T.I., Eglinton, G. 2008. Molecular proxies for paleoclimatology. Earth and Planetary Science Letters 275, 1-16.

Fung, M.Y.P., Macyk, T.M. 2000. Reclamation of oil sands mining areas, in: Barnhisel, R.I., Darmody, R.G., Daniels, W.L. (Eds.) Reclamation of Drastically Disturbed Lands. American Society of Agronomy, Inc., Madison, WI. pp. 755-774.

Government of Alberta 1993. Environmental Protection and Enhancement Act: Conservation and Reclamation Regulation.

Gower, S.T., Hunter, A., Campbell, J., Vogel, J., Veldhuis, H., Harden, J., Trumbore, S., Norman, J.M., Kucharik, C.J. 2000. Nutrient dynamics of the southern and northern BOREAS boreal forests. Écoscience 7, 481-490.

Hahn, A.S., Quideau, S.A. 2012. Long-term effects of organic amendments on the recovery of plant and soil microbial communities following disturbance in the Canadian boreal forest. Plant Soil, 1-14.

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

Hemstock, S.S., Quideau, S.A., Chanasyk, D.S. 2010. Nitrogen availability from peat amendments used in boreal oil sands reclamation. Can. J. Soil Sci. 90, 165-175.

Herbin, G.A., Robins, P.A. 1968. Studies on plant cuticular waxes - III. The leaf wax alkanes and ω-hydroxy acids of some members of the Cupressaceae and Pinaceae. Phytochemistry 7, 1325-1337.

Jandl, G., Baum, C., Blumschein, A., Leinweber, P. 2012. The impact of short rotation coppice on the concentration of aliphatic soil lipids. Plant Soil 350, 163-177.

Jansen, B., Nierop, K.G.J., Hageman, J.A., Cleef, A.M., Verstraten, J.M. 2006. The straight-chain lipid biomarker composition of plant species responsible for the dominant biomass production along two altitudinal transects in the Ecuadorian Andes. Org. Geochem. 37, 1514-1536.

Jia, G., Dungait, J.A.J., Bingham, E.M., Valiranta, M., Korhola, A., Evershed, R.P. 2008. Neutral monosaccharides as biomarker proxies for bog-forming plants for applications to palaeovegetation reconstruction in ombrotrophic peat deposits. Org. Geochem. 39, 1790-1799.

Johnson, D., Kershaw, L., MacKinnon, A., Pojar, J. 1995. 'Plants of the Western Boreal Forest and Aspen Parkland.' (Lone Pine Publishing: Edmonton).

Larney, F.J., Angers, D.A. 2012. The role of organic amendments in soil reclamation: A review. Can. J. Soil Sci. 92, 19-38.

Lavrieux, M., Bréheret, J.-R., Jacob, J., Le Milbeau, C., Zocatelli, R. 2012. Preservation of an ancient grassland biomarker signature in a forest soil from the French Massif Central. Org. Geochem. 51, 1-10.

Legendre, P., Gallagher, E.D. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271-280.

Lockheart, M.J., van Bergen, P.F., Evershed, R.P. 1995. Variations in the stable carbon isotope compositions of individual lipids from the leaves of modern angiosperms: Implications for the study of higher plant-derived sedimentary organic matter. Org. Geochem. 26, 137-153.

Macdonald, E., Quideau, S., Landhäusser, S. 2012. Rebuilding boreal forest ecosystems after industrial disturbance, in: Vitt, D., Bhatti, J. (Eds.) Restoration and Reclamation of Boreal Ecosystems. Cambridge University Press. pp. 123-160.

Macdonald, S.E., Fenniak, T.E. 2007. Understory plant communities of boreal mixedwood forests in western Canada: Natural patterns and response to variable-retention harvesting. For. Ecol. Manage. 242, 34-48.

Maffei, M. 1994. Discriminant analysis of leaf wax alkanes in the Lamiaceae and four other plant families. Biochemical Systematics and Ecology 22, 711-728.

Maffei, M. 1996a. Chemotaxonomic significance of leaf wax alkanes in the Gramineae. Biochemical Systematics and Ecology 24, 53-64.

Maffei, M. 1996b. Chemotaxonomic significance of leaf wax *n*-alkanes in the Umbelliferae, Cruciferae and Leguminosae (subf. Papilionoideae). Biochemical Systematics and Ecology 24, 531-545.

Maffei, M., Bandino, S., Bossi, S. 2004. Chemotaxonomic significance of leaf wax *n*-alkanes in the Pinales (Coniferales). Journal of Biological Research 1, 3-19.

Marseille, F., Disnar, J.R., Guillet, B., Noack, Y. 1999. n-Alkanes and free fatty acids in humus and A1 horizons of soils under beech, spruce and grass in the Massif-Central (Mont-Lozère), France. Eur. J. Soil Sci. 50, 433-441.

McCune, B., Grace, J.G. 2002. 'Analysis of ecological communities.' (MJM Software Design: Gleneden Beach, OR).

McMillan, R., Quideau, S.A., MacKenzie, M.D., Biryukova, O. 2007. Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. J. Environ. Qual. 36, 1470-1478.

Nierop, K.G.J., Jansen, B., Hageman, J.A., Verstraten, J.M. 2006. The complementarity of extractable and ester-bound lipids in a soil profile under pine. Plant Soil 286, 269-286.

Nott, C.J., Xie, S., Avsejs, L.A., Maddy, D., Chambers, F.M., Evershed, R.P. 2000. *n*-Alkane distributions in ombrotrophic mires as indicators of vegetation change related to climatic variation. Org. Geochem. 31, 231-235.

Oros, D.R., Standley, L.J., Chen, X., Simoneit, B.R.T. 1999. Epicuticular wax compositions of predominant conifers of western North America. Zeitschrift für Naturforschung 54c, 17-24.

Otto, A., Simpson, M.J. 2006. Sources and composition of hydrolysable aliphatic lipids and phenols in soils from western Canada. Org. Geochem. 37, 385-407.

Oxbrough, A.G., Gittings, T., O'Halloran, J., Giller, P.S., Smith, G.F. 2005. Structural indicators of spider communities across the forest plantation cycle. For. Ecol. Manage. 212, 171-183.

Pancost, R.D., Baas, M., van Geel, B., Damsté, J.S.S. 2002. Biomarkers as proxies for plant inputs to peats: an example from a sub-boreal ombrotrophic bog. Org. Geochem. 33, 675-690.

Pearson, E.J., Farrimond, P., Juggins, S. 2007. Lipid geochemistry of lake sediments from semi-arid Spain: Relationships with source inputs and environmental factors. Org. Geochem. 38, 1169-1195.

Quideau, S.A., Swallow, M.J.B., Prescott, C.E., Grayston, S.J., Oh, S.-W. 2013. Comparing soil biogeochemical processes in novel and natural boreal forest ecosystems. Biogeosciences Discussions 10, 7521-7548.

Ronkainen, T., McClymont, E.L., Väliranta, M., Tuittila, E.-S. 2013. The *n*-alkane and sterol composition of living fen plants as a potential tool for palaeoecological studies. Org. Geochem. 59, 1-9.

Rowland, S.M., Prescott, C.E., Grayston, S.J., Quideau, S.A., Bradfield, G.E. 2009. Recreating a functioning forest soil in reclaimed oil sands in Northern Alberta: An approach for measuring success in ecological restoration. J. Environ. Qual. 38, 1580-1590.

Schmidt, M.W.I., Torn, M.S., et al. 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49-56.

Schnitzer, M., McArthur, D.F.E., Schulten, H.-R., Kozak, L.M., Huang, P.M. 2006. Long-term cultivation effects on the quantity and quality of organic matter in selected Canadian prairie soils. Geoderma 130, 141-156.

Shrestha, R.K., Lal, R. 2007. Soil carbon and nitrogen in 28 year old land uses in reclaimed coal mines of Ohio. J. Environ. Qual. 36, 1775-1783.

Six, J., Conant, R.T., Paul, E.A., Paustian, K. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil 241, 155-176.

Small, C.J., McCarthy, B.C. 2002. Spatial and temporal variability of herbaceous vegetation in an eastern deciduous forest. Plant Ecology 164, 37-48.

Soil Classification Working Group 1998. The Canadian System of Soil Classification, in: Agriculture and Agri-Food Canada- Research Branch (Eds.). NRC Research Press. pp. 53 - 59.

Sorenson, P.T., Quideau, S.A., MacKenzie, M.D., Landhäusser, S.M., Oh, S.-W. 2011. Forest floor development and biochemical properties in reconstructed boreal forest soils. Appl. Soil Ecol. 49, 139-147.

Trendel, J.M., Schaeffer, P., Adam, P., Ertlen, D., Schwartz, D. 2010. Molecular characterisation of soil surface horizons with different vegetation in the Vosges Massif (France). Org. Geochem. 41, 1036-1039.

Turcotte, I., Quideau, S.A., Oh, S.-W. 2009. Organic matter quality in reclaimed boreal forest soils following oil sands mining. Org. Geochem. 40, 510-519.

van Bergen, P.F., Bull, I.D., Poulton, P.R., Evershed, R.P. 1997. Organic geochemical studies of soils from the Rothamsted Classical Experiments - I. Total lipid extracts, solvent insoluble residues and humic acids from Broadbalk Wilderness. Org. Geochem. 26, 117-135.

Vancampenhout, K., Wouters, K., De Vos, B., Buurman, P., Swennen, R., Deckers, J. 2009. Differences in chemical composition of soil organic matter in natural ecosystems from different climatic regions - A pyrolysis-GC/MS study. Soil Biol. Biochem. 41, 568-579.

Vetterlein, D., Hüttl, R. 1999. Can applied organic matter fulfil similar functions as soil organic matter? Risk-benefit analysis for organic matter application as a potential strategy for rehabilitation of disturbed ecosystems. Plant Soil 213, 1-10.

Yunker, M.B., Macdonald, R.W., Cretney, W.J., Dallimore, S.R., McLaughlin, F.A. 1993. Geochemistry and fluxes of hydrocarbons to the Beaufort Sea shelf: A multivariate comparison of fluvial inputs and coastal erosion of peat using principle component analysis. Geochim. Cosmochim. Acta 55, 255-273.

Chapter 3. Processing of ^{13}C glucose in mineral soil from aspen, spruce and novel ecosystems in the Athabasca Oil Sands Region

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3.1. Introduction

Forest stand dynamics in the western Canadian boreal forest are driven by large scale natural disturbances often caused by fire and insects. However, a new, anthropogenic disturbance is currently affecting this region – oil sands mining. To date, an area of 715 km^2 has been mined in the Athabasca Oil Sands Region (AOSR), and 104 ha have been officially reclaimed (Government of Alberta 2011). Reclamation certification is given once the area is found to be functioning at an equivalent land capability to what was present prior to disturbance (Government of Alberta 1993). Determining land capability can be challenging. One approach is to evaluate whether soils are self-sustaining in terms of central ecosystem processes such as nutrient cycling. In turn, nutrient cycling may be evaluated by examining soil microbial community dynamics.

Although it is difficult to determine if soil microorganisms reflect or initiate changes in an ecosystem, they are recognized as a critical component that drives its nutrient cycling (Harris 2009). The analysis of soil derived phospholipid fatty acids (PLFAs) may be used to broadly characterize and quantify soil microbial communities (Frostegård et al. 2011). This analysis is an indirect and culture independent method of determining the soil microbial composition (White and Ringelberg 1998). The PLFAs extracted from soils are an integral part of microbial cell membranes which readily degrade upon cell death and, therefore, may be used to quantify the number of living microorganisms present in soils. Furthermore, a pattern of microbial community structure may be acquired when examining the proportion all of PLFAs together (Frostegård et al. 2011). Using the PLFA method, a body of knowledge has emerged which demonstrates that soil

microbial community structure is influenced by the type of above ground vegetation in mature undisturbed natural stands, e.g. in the Malaysian Borneo (Ushio et al. 2008), across forest regions in western Canada (Brockett et al. 2012; Grayston and Prescott 2005), and in the Finnish (Priha et al. 2001) and central Canadian (Hannam et al. 2006) boreal forests. In disturbed stands, the PLFA method has identified soil microbial communities that differed from their undisturbed counterparts following harvest (Mummey et al. 2010), fire (Williams et al. 2012), and in surface mine reclamation areas (Dimitriu et al. 2010; Mummey et al. 2002b). The method has also established that mine reclamation chronosequences display a change in their microbial community structure with time (Banning et al. 2011; Claassens et al. 2008; Mummey et al. 2002a), which in some cases were found to be correlated with changing vegetative inputs (Hahn and Quideau 2012). While the microbial community structure has been shown to vary among differing systems, there is a lack of knowledge on the functioning of the community, specifically in terms of nutrient cycling.

The reconstruction of novel upland boreal forest soils in mine reclamation of the AOSR uses two main organic matter amendments, peat and forest floor. The use of forest floor material was beneficial through its inclusion of plant propagules from the native seed bank (Mackenzie and Naeth 2010), which helped in the establishment of native vegetation and promoted a microbial community more similar to that of natural soils (Hahn and Quideau 2012). However, peat is a much more plentiful organic matter supply in the AOSR, therefore, it is preferentially used as an organic amendment during reclamation. Additionally, peat may be preferred for decreasing bulk density, increasing moisture retention and boosting the organic nutrient bank of the newly constructed soils (Fung and Macyk 2000).

Trembling aspen (*Populus tremuloides* Michx.) is one of the native tree species in the AOSR which quickly pioneers disturbed sites while slower growing species, such as white spruce (*Picea glauca* (Moench) Voss), emerge as the stand ages (Perala 1990). Therefore, aspen and spruce dominated sites are two of the desired objectives of the forest reclamation treatments on novel sites from the

region. Within the boreal forest of western Canada, aspen and spruce-dominated stands harbor forest floors with different microbial biomass and structure (Hannam et al. 2006; Swallow et al. 2009). This variation in soil microbial community and its relation to the dominant organic matter input has also been demonstrated for the AOSR. Previous work determined that the novel sites differed from forest floor in natural aspen stands and exhibited lower microbial biomass carbon (McMillan et al. 2007), lower organic carbon alkyl/O-alkyl (Turcotte et al. 2009), lower rate of organic matter decomposition (Rowland et al. 2009) and decreased enzyme activity (Dimitriu et al. 2010). When comparing among novel sites, the sampling time (MacKenzie and Quideau 2010), percent canopy cover (Sorenson et al. 2011) and type of organic matter amendments used in soil reconstruction (Hahn and Quideau 2012), were all found to affect the soil microbial community.

Novel upland soils constructed as part of the landscape reclamation efforts following mining in the AOSR host microbial communities that are different from those in aspen or spruce dominated forest stands in the region (Hahn and Quideau 2012). However, little is known on how the newly reclaimed soils compare in function to undisturbed aspen or spruce stands. Consequently, we used a stable isotope tracer (^{13}C) to assess microbial community processing of a simple carbon substrate by three different mineral soil types, including a novel soil, and two natural soils from an aspen-dominated and a spruce-dominated stand. We determined ^{13}C enrichment and overall values for respiration rates, the change in microbial biomass and structure, and organic carbon stabilization within the soil matrix following the addition of ^{13}C labelled glucose during a laboratory incubation experiment. We expected that the movement of ^{13}C among the different organic matter pools would be different between the two natural soils under different stand covers, as well as between the natural and novel soils.

3.2. Methods

3.2.1. Soil Collection

The three mineral soils were collected from northeastern Alberta in the Athabasca Oil Sands Region (AOSR) of the western boreal forest. The aspen and spruce soils were part of the same continuous forest landscape, and within 2 km of each other. The novel soil, from a reclaimed site, was within 20 km of the natural soils and all three were located on the Syncrude Mine Site north of Fort McMurray, AB. Natural stands were greater than 70 years old and the soils were classified as Gray Luvisols (Soil Classification Working Group 1998), or as Albic Luvisols according to the FAO classification (Food and Agriculture Organization of the United Nations 2006). The novel soil was an anthropogenic soil (Technosol) created in 1998, and was composed of a mixture of peat organic matter and mineral soil for the top 0-15 cm underlain by a fine-textured material salvaged from the top 1 meter of mineral soils prior to mining (Hahn and Quideau 2012; McMillan et al. 2007).

The three soil types were collected in preparation of the laboratory incubation in August 2009. Live vegetation, and the forest floor when present, was removed prior to collection of about 1 kg of soil. The top 0-10 cm of the novel soil was randomly collected from three locations within 5 m of one another, and all three samples were composited to yield a representative and homogeneous sample from this reclaimed site. For the aspen and spruce soils, the top 0-5 cm of mineral soil was collected from directly under the respective tree canopies at three locations and then composited. Soil samples were kept cool and transported to the laboratory within five days at which time they were air dried, sieved to 2 mm, and stored.

3.2.2. Laboratory Analyses

General Methods

Homogenized subsamples from each soil type (n=5) were analyzed for pH with an Ag/AgCl pH electrode, using a soil to 0.01 M calcium chloride solution ratio of 1:2 and a settling time of 30 minutes (Kalra and Maynard 1991). Soil texture was determined by the hydrometer method for particle size distribution (Sheldrick and Wang 1993). Subsamples of the soils were finely ground using a Retsch MM200 ball mill grinder (Retsch Inc. Newtown, USA) for measurement

of total organic carbon and nitrogen values by dry combustion on a Costech ECS 4010 Elemental Analyzer equipped with a thermoconductivity detector (Costech Analytical Technologies Inc. Valencia, USA), and for ^{13}C and ^{15}N isotopic composition by coupling the Costech ECS 4010 to a Finnigan Deltaplus Advantage Isotopic Ratio Mass Spectrometer (IRMS; ThermoFinnigan, Bremen, Germany). Results were expressed in both the δ-notation, part per thousand variations from the standard Pee Dee Belemnite, and as atom %.

Incubation Experiment

Eighteen experimental units were constructed for each homogenized soil type; with soil moistened to 30 kPa and allowed to equilibrate for seven days prior to commencing the experiment. Each experimental unit consisted of a 1 L glass jar containing three glass vials where each vial contained 30.0 g of soil. This design was chosen to allow for immediate destructive sampling of the soil, eliminating time delays due to subsampling while maximizing the opportunity to capture soil respiration responses. The incubation began with the addition to each vial of 5 mg of D-glucose-1-^{13}C and 0.5 mg of L-alanine-^{15}N in 1 ml of deionized water. Experimental units were destructively sampled, in triplicate, for soil fractionation and phospholipid fatty acid analysis (PLFA) prior to the addition of glucose for initial conditions (0 hour) and at 5 sampling points during the incubation (22, 46, 70, 142, and 310 hours; n=3). One vial from each unit immediately underwent soil fractionation, one was frozen at -20 °C to be later freeze dried for the PLFA procedure, and the third was used for microbial biomass quantification by the chloroform-fumigation-extraction method (data not shown).

Soil Respiration

Experimental units were non-destructively measured for soil respiration under initial conditions and in an incremental fashion across six sampling points (2, 18.5, 27, 44.5, 68.5, and 140 hours). For each measurement, the amount of CO_2 generated between sampling points was determined by randomly selecting,

sealing and incubating five units of each soil type from one sampling time to the next e.g., from 44.5 to 68.5 hours (except 18.5 and 27 hours which were sealed for 1 and 5 hours respectively). Two headspace subsamples were taken from each unit. The first 5 ml was transferred to an evacuated 12 ml borosilicate Labco Exetainer (Labco Limited, High Wycombe, UK) for quantitative CO_2 measurement on an HP 5890 Series II gas chromatograph (GC) with a 1 m Poropak Q column and an HP 3396 Series II integrator (Hewlett Packard, Santa Clara, USA), using helium as the carrier gas. The second subsample was transferred to a helium flushed positive pressure 12 ml borosilicate Labco Exetainer for ^{13}C isotopic composition measurement via a CTC Combi PAL (CTC Analytics AG, Zwingen, CH) leading to a Porapak Q column in a Finnigan GasBench II (Thermo Electron Corporation, Waltham, USA) attached to the IRMS with a helium gas carrier. Total respiration for the entire incubation (mg CO_2 and mg CO_2-^{13}C) was calculated as the sum of respiration from each sampling time.

Phospholipid Fatty Acid Analysis

Freeze dried soil samples (2.00 g) were extracted using the Bligh and Dyer (1959) methanol:chloroform:buffer extraction ratio of 1:1:0.9 with a 0.15 M citrate buffer (Frostegård et al. 1991). Extracts were separated on SPE columns (Agilent Technologies, Santa Clara, USA) of 500 mg of silica in 6 ml tubes (Zelles and Bai 1993). Purified PLFAs were re-dissolved in 1:1 chloroform:methanol prior to a mild alkaline methanolysis and subsequent extraction with hexane to synthesize fatty acid methyl esters, a modified procedure from White and Ringelberg (1998). Two standards were used for each sample: a surrogate standard of 19:0 (1,2-dinonadecanoyl-sn-glycero-3-phosphocholine, Avanti Polar Lipids Inc, Alabaster, USA) was added to the soil sample prior to the first extraction, and an instrument standard of 10:0Me (methyl decanoate, Aldrich, St. Louis, USA) was added prior to identification and quantification analysis (Bird et al. 2011). PLFA markers were identified and quantified on a HP 5890A Series II GC equipped with a HP 7673 Injector, an

Agilent Ultra 2 column (Crosslinked 5 % PhMeSilicone) of 25 m length and 0.33µm film thickness, and an FID detector (Hewlett Packard, Santa Clara, USA). Peaks were identified by matching retention times against a known standard solution using the Sherlock Microbial Identification System Version 4.5 software (MIDI, Inc., Newark, USA). PLFA samples were then examined for compound specific stable isotope ratio via a CTC Combi PAL joined to a 6890N Agilent GC (Agilent Technologies, Santa Clara, USA). The GC was equipped with an HP-Ultra 2 column (50 m length, 0.2 mm i.d. and 0.33 µm film by J & W Scientific Columns from Agilent Technologies), and was linked to a Thermo Finnigan GC Combustion III (Thermo Finnigan, Bremen, Germany) and the IRMS.

PLFA biomarkers between 14 and 20 carbon units in length and greater than 0.5 % in abundance were included in the analysis. Molar concentrations of individual PLFAs were adjusted for both the instrument and surrogate standards. Abundance of individual PLFAs in each sample was expressed as nmol PLFA g^{-1} dry soil and as nmol % of total microbial biomass. Total microbial biomass in a sample was calculated for each sampling point by summing the molar concentrations (nmol PLFA g^{-1} dry soil) of all reported PLFAs. The PLFA nomenclature used follows standard formatting (Maxfield and Evershed 2011).

Soil Fractionation

The soil separation was based on Norris *et al.* (2011), but was amended for the following four categories: sand, silt, clay-sized particles, and the dissolved organic carbon (DOC) fraction. Briefly, soil samples were transferred to 250 ml plastic bottles with 100 ml of deionized water, shaken for 1 hour and passed through a 53 µm sieve to isolate the sand fractions. The filtrates were left to settle and the silt-sized fractions were removed after siphoning off the clay suspensions. Suspensions were then flocculated with KCl, the supernatants were collected, made to a known volume, and identified as the DOC fractions. Potassium chloride was removed from the clay fractions by dialysis and the fractions were then, as with the sand and silt fractions, dried down with a forced air oven (40 °C). Once dried and weighed, the mineral fractions were homogenized with a ball mill

grinder prior to total carbon, nitrogen and isotopic analysis as described for the whole soils. Total organic carbon of the DOC fractions was determined on a Shimadzu TOC-V with TN option (Mandel Scientific Company Inc., Guelph, CA) after which the samples were freeze dried, homogenized with mortar and pestle and analyzed as above for isotopic composition.

3.2.3 Data analysis

Total soil respiration and total initial and final soil microbial biomass were statistically compared for differences among soil types. These data were normally distributed and were analyzed using a one-way analysis of variance (ANOVA) with pair-wise comparisons using a Tukey's adjustment for multiple inferences and an α of 0.05. All analyses (descriptive statistics, sample means and standard deviations) and graphing were performed in R (version 2.11.0, the R Foundation for Statistical Computing). The Non-metric MultiDimensional Scaling (NMDS) method was employed to examine Hellinger transformed PLFA data using a Sorenson (Bray-Curtis) distance measurement followed by Multi-Response Permutation Procedures (MRPP) with the PC-ORD software version 5 (MjM Software Design, Gleneden Beach, USA) with the addition of a constant (100) to the isotope data (Legendre and Gallagher 2001). Correlations with the secondary matrix of environmental variables (including pH, respiration, DOC and PLFA concentrations) were used to explain variations in the ordination expressed along the axes. Ordering of the sample units by interpoint differences in the NMDS ordination indicated which categories or groups needed to be tested further. Grouping variables were then tested for overall and pair-wise comparisons using MRPP, a non-parametric test, which yielded three values: an overall significance (p), a term indicating the degree of separation between groups (T), and the separation within groups (A). Groups were also examined for the presence of indicator PLFA species using PC-ORD.

3.3. Results

General Soil Characteristics

Aspen and spruce mineral soils were collected from mature forest stands (> 70 years) in the western boreal forest. In comparison, the novel soil was obtained from a site created 11 years ago and was without tree cover although there was some coverage by forbs and graminoids (see Site 1 PM in Hahn and Quideau 2012). All three mineral soils were comparable in texture; both the aspen and spruce soils were silt loams while the novel soil had a loamy texture, although it contained a clay concentration almost double that of the other two soils (Table 3-1). A gradient of increasing pH (4 to 6) was evident from aspen to spruce to the novel soil. The total organic carbon and nitrogen concentrations in the novel soil were almost 5 times higher than in the aspen and spruce soils. Because the incubation was set up on a dry soil mass basis, experimental units for the novel soil contained more total carbon than the aspen and spruce units. Furthermore, with greater concentrations of carbon and ^{13}C atom %, the novel soil initially contained more ^{13}C in its incubation vials compared to the aspen and spruce soils (0.019 g vs 0.004 g). Thus, the addition of ^{13}C labelled glucose only represented an isotope addition of 2 % for the novel soil, while this corresponded to 9 and 10 % for aspen and spruce, respectively.

Soil Respiration

Total respiration by overall soil mass was greater for the novel soil than for either spruce or aspen for both total mg CO_2-C and CO_2-^{13}C (Table 3-2), as could be expected from the greater quantity of total carbon and ^{13}C in this soil. However, total respiration was not fivefold greater, as might have been anticipated from their respective total carbon concentrations. Rather, the aspen samples showed the largest total soil respiration per gram of carbon, followed by spruce, and finally the novel soil (Table 3-2). This was true for both total carbon and ^{13}C. Respiration rates for the three different soil types followed a similar pattern over the course of the incubation, with all soils rapidly increasing in rate two hours following the addition of labelled substrate, and then steadily declining for both total CO_2-C and CO_2-^{13}C (Figure 3-1). Interestingly, aspen and spruce diverged in the timing of their peak respiration rates, with the greatest activity

occurring 2 hours after substrate addition for aspen and 18 hours for spruce. The novel soil samples were notable for their lower respiration during the entire incubation experiment for both ^{13}C and overall CO_2.

Phospholipid Fatty Acid Analysis

Total soil microbial biomass as determined by PLFA analysis differed among the three soil types (Table 3-2). This remained true throughout the incubation experiment. Total microbial biomass was greater for the aspen than the novel soil, and was lowest in the spruce soil. Not only did microbial biomass differ among soil types, the microbial community structure was also distinct among the three soils and remained so for the duration of the experiment (Figure 3-2). The NMDS on the data of 36 PLFAs resulted in a two dimensional ordination solution (stress of 7.83 after 92 iterations), where subsequent MRPP testing showed that the three soil types were separate ($p<0.001$, $T = -29.29$, $A=0.64$). Pair-wise comparisons further revealed that each type was unique ($p<0.001$). Four PLFA biomarkers (with an r^2 of 0.55) were found to explain the separation along the Axis 1 (81 %). Three biomarkers increased towards the novel soil (16:1ω5c, 16:1ω9c, and 17:1ω8c), while one increased towards the spruce (16:1 isoH). Indicator species analysis of aspen, spruce and novel PLFAs determined five key biomarkers (indicator value > 55 %, $p \leq 0.001$). Biomarkers 15:0 3OH and 16:1 2OH were key for aspen soil, while 16:1 isoH and 16:1ω11c were indicative of spruce soils, and 16:1 2OH was key in novel soils.

Isotopic compound specific analysis was determined for the extracted microbial PLFAs. Due to methodological limitations, results are limited to 14 of 36 PLFAs. An NMDS on the nmol % of the 14 PLFAs yielded the same grouping pattern as when using all 36 PLFAs (data not shown). The same separation was evident in the ordination of $\delta^{13}C$ PLFA values (Figure 3-3) as in the ordination of nmol % (Figure 3-2). An ordination solution of two dimensions was obtained after 56 iterations with a stress of 5.63. Axis 1 explained 89 % of the separation yet none of the secondary matrix values, including PLFA concentration, were found to correlate and explain the separation. MRPP analysis indicated that the soil

groups were separate (T=-11.12, A=0.31, $p<0.001$) and unique ($p<0.001$) but differences were weakened as there was less distinction between groups and a lower agreement value than in the overall ordination. These results indicate that the microbial communities that utilized the added substrate were not as structurally distinct as the overall communities. Indicator species analysis of the isotopic data only identified two key biomarkers, both associated with the novel soil, namely 16:1ω5c and 18:2ω6,9c/*a*18 sum feature 5 (indicator value > 55 %, $p \leq 0.001$). Incorporation of ^{13}C, as evidenced by the enrichment of a representative PLFA 18:1ω7c (Figure 3-4), indicated differences among soil types in the processing of ^{13}C labelled glucose. Microbial incorporation was similar in the aspen and novel soils but the spruce soil microorganisms assimilated twice as much of the stable isotope with most enrichment occurring by the first sampling time.

Not presented in our results were fungal:bacterial ratios as our PLFA identification procedure, Sherlock Microbial Identification System, did not resolve between the two peaks of 18:2ω6,9c and *a*18:0. The PLFA 18:2ω6,9c is a recognized fungal biomarker while *a*18:0 is regarded as a biomarker for Gram positive bacteria (Ruess and Chamberlain 2010). The unresolved 18:2ω6,9c /*a*18:0 peak was found in all of our samples and we believe that both lipids were present in varying proportions. The PLFA 18:1ω9c, often considered a fungal marker, was extracted from all three soils; however, it is also found in Gram positive bacteria (Ruess and Chamberlain 2010). A third fungal marker, 18:3ω3,6,9c, was extracted from both aspen and spruce soils but not the novel soil. Therefore, due to ambiguous assignment of 18:2ω6,9/*a*18:0 peak, we hesitate to calculate fungal:bacterial ratios. We suspect that when 18:2ω6,9/*a*18:0 was determined as an indicator species of the novel soil on the second ordination, this may have derived from the bacterial rather than the fungal component biomarker.

Soil Fractionation

The distribution of organic carbon across the fractions was similar for the three soil types with 68 – 80 % in the sand sized fraction, 17 – 26 % in silt, 1 – 6 % in clay, and about 1 % for DOC (Table 3). The same pattern of association was observed for ^{13}C, with a decreasing proportion of the total isotope with decreasing particle size (Table 3-3 and Figure 3-5). All soil fractions were enriched immediately after the addition of the labelled glucose substrate and, once enriched above background levels, the ^{13}C isotopic signature remained elevated (Figure 3-6). The greatest variation in ^{13}C isotopic enrichment was observed in the clay and DOC fractions. In the clay fraction, the novel soil was enriched beyond the background level, but both the aspen and spruce soils had a greater enrichment after one day, which was followed by a steady decline in subsequent sampling times. In the DOC fractions, ^{13}C atom % was greater in spruce than in the other two soil types throughout the incubation. Across the incubated soils, the sand fractions increased in proportion of ^{13}C while the silt and clay sized fractions appeared to be either unchanged or decrease during the course of the incubation, particularly for the novel soil (Figure 3-5).

3.4. Discussion

Processing of a simple labelled compound (^{13}C glucose) by the microbial community in mineral soils from aspen, spruce and novel ecosystems in the Athabasca Oil Sands Region (AOSR) was determined to be unique to each soil type. These soil types had previously been reported to be distinct in terms of their microbial biomass carbon (McMillan et al. 2007), organic matter macromolecular composition (Turcotte et al. 2009), and microbial community structure (Hahn and Quideau 2012). In the previous studies, sample collection was by depth; however, as there was minimal forest floor development on the reclaimed sites compared to natural stands, this study focused on comparing reconstructed soils to the mineral soils of mature natural stands. Mineral soils of the upland forests in the experimental area are dominated by fine textured material supporting trembling aspen, white spruce or a mixture of the two stands (Turcotte et al. 2009). Hence, fine textured (< 36% sand) soils from both dominant natural stand types as well

from a novel ecosystem were represented in this experiment. While the aspen and spruce soils, both from climax forest stands, were similar in organic carbon (1.1 and 1.2 %) and organic nitrogen (0.07 %) concentrations, the reconstructed soil, from a site which had been reclaimed eleven years earlier and had no canopy cover, was higher in both organic carbon and nitrogen (5.8 and 0.33 %; Table 3-1). The pH was also higher in the novel soil (6), compared to aspen (5) and spruce (4). Differences in pH and organic nutrient concentrations can largely be attributed to the substrates used to build the novel soil. The fine textured mineral material used to construct the soil was glaciolacustrine-derived and rich in carbonates (resulting in a higher pH) and, while peat material was added to improve the physical properties of the mineral material and increase the overall carbon content, it also raised the organic nutrient concentrations above those of soils in natural stands (Fung and Macyk 2000). Our pH and carbon concentration results were similar to those from previous studies, which illustrated characteristic differences between spruce and aspen forest floors (Hannam et al. 2004), as well as between aspen forest floor and reconstructed soils (Hahn and Quideau 2012).

Incubation of the three mineral soils with a labelled substrate enriched all of the soil fractions in ^{13}C, with the ^{13}C in all fractions remaining above background levels for the duration of the incubation (Figure 3-6). Of the four fractions, the largest proportional increase in ^{13}C was observed in sand-sized fractions (Figure 3-5). Of particular note was that both the clay and DOC fractions from the spruce soil had the highest levels of absolute enrichment (Figure 3-6). Soil separation based on density and particle size categories imposes some order on its highly heterogeneous nature and may reflect organic matter stabilization processes. For instance, clay and silt-sized separates that are dominated by microbially altered, recalcitrant organic carbon (Guggenberger et al. 1995), have reported mean residence times based on ^{14}C analysis of 800-1660 years for silt and 75-4400 years for clay (von Lützow et al. 2007). In contrast, with no means of stabilization, the sand-sized fraction encompasses a labile pool with rapid turnover, which shows little chemical alteration from fresh plant residues (Christensen 1992). In addition to individual sand particles, this fraction

may include sand-sized aggregates, as our fractionation scheme did not discriminate between the two. Indeed, the increased proportion of ^{13}C within the sand-sized fraction could have been due to the formation of aggregates. Microbial exudates and any unutilized glucose may be stabilized in the soil physical environment by complexation with mineral particles or other macromolecular fragments forming aggregates which then alter the soil physical environment (Christensen 2001). As our soils were fine textured, aggregate formation would have been favored, and was the likely reason for increased proportion of ^{13}C in the sand fraction.

Further differences among mineral soils were observed in the microbial community composition (Figure 3-2) and total biomass (Table 3-2). Our results agree with previous reports from forest floor materials which established clear structural differences in the microbial communities between aspen and spruce dominated stands (Hannam et al. 2006; Swallow et al. 2009) and between novel soils and soils from aspen dominated stands (Hahn and Quideau 2012). Vegetation is known to be an important soil forming factor which may drive microbial community composition (Grayston and Prescott 2005; Priha et al. 2001), and such separation was evident between the deciduous-dominated (aspen) and conifer-dominated (spruce) stands (Figure 3-2 and Table 3-2). Similarly to our results, forest floor from aspen dominated stands has been shown to have greater microbial biomass compared to both newly reclaimed soils (Dimitriu et al. 2010; Hahn and Quideau 2012) and spruce forest floors (Hannam et al. 2006). Of note, the novel soil, which had the greatest carbon concentration, did not have the largest microbial biomass. Rather, other factors such as soil organic matter composition may be controlling total microbial biomass in these soils.

While the three soils harbored microbial communities with distinct overall structures, they also functioned differently as evidenced by the soil respiration results (Figure 3-1 and Table 3-2). The largest total amounts of carbon and ^{13}C respired were observed in the novel soil. However, when expressed in terms of the initial carbon content, the aspen soil respired the highest amount of carbon and both natural soils respired proportionately more ^{13}C than the novel soil. While the

microbial communities that incorporated the isotope were different (Figure 3-3), there was greater commonality in the microorganisms that assimilated the glucose than in the overall communities (Figure 3-2). Taken together, these results indicate that each soil type was unique. Spruce soil in particular was distinct from the other two soil types, with a more diverse community structure (demonstrated by a more spread-out pattern on the NMS ordinations- Figures 3-2 and 3-3), lower total microbial biomass (Table 3-2), and higher ^{13}C enrichment in the DOC and clay fractions (Figure 3-6). These results suggest that the lower density of the spruce soil microorganisms was likely reflected in decreased proximity to the labeled glucose and was therefore the reason for the observed response delay in terms of the respiration rates (Figure 3-1). However, spruce soil microorganisms were highly efficient at incorporating ^{13}C (Figure 3-4), not necessarily for increased biomass (Table 3-2), but rather perhaps for cell maintenance with greater production of extracellular enzymes or metabolites as suggested by the greater enrichment levels in the DOC and clay-sized fractions (Figure 3-6). This greater enrichment in the spruce soil could have been due to the fact that there was less competition for the substrate in spruce as indicated by the lower microbial biomass in this soil.

What we may have observed with our results was the priming effect and differences in r- and K- strategist populations. The priming effect refers to the increase in decomposition of soil organic matter after the addition of easily-decomposable organic substances due to a certain proportion of the microbial population being in a resting but metabolically alert state (De Nobili et al. 2001). Although spruce microorganisms were slower to respond, once they responded, they were very efficient at utilizing the substrate. Therefore, the microbial community in the spruce soil may have had a higher proportion in this metabolically alert state (r-strategists). Additionally, Bird et al. (2011) concluded that Gram positive bacteria (K-strategists) are more wide spread in bulk soil, degrading complex substrates such as humified soil organic matter, while Gram negative bacteria (r-strategists typically found in higher concentrations in the rhizosphere) rely on simpler carbon sources such as exudates. Consequently, the

higher enrichment of PLFA 18:1ω7c , commonly assigned to Gram negative bacteria (Dungait et al. 2011), that we observed in our study (Figure 3-4) might be the result of positive priming (Gude et al. 2012). If we take the view that a proportion of the microbial biomass maintains a state of metabolic alertness (Paterson et al. 2011) as a strategy to exploit transient nutrient availability (De Nobili et al. 2001), then the novel soil, with a low microbial population and low diversity, may also be low in r-strategists and thus unresponsive to the addition of glucose.

Response to the addition of a simple labelled compound illustrated how each of the three soil types was unique, not only structurally, but also functionally. While each soil type was different, there was no stand level replication of soils in this study; therefore, we must be cautious in our result interpretation and potential extrapolation to the landscape level. Previous studies in boreal forests examining microbial functional diversity have failed to establish a definite relationship between substrate utilization (White et al. 2005), enzyme rates (Dimitriu et al. 2010) and above ground stand types. By using ^{13}C-glucose as a tracer we were able to determine that microorganisms incorporating the labelled substrate were distinct on these three soil types; and we conclude that this was due to the differing vegetation covers and soil types. As forest stand type is known to affect soil microbial composition in the boreal forest (Hannam et al. 2006), it is possible that the differences observed between natural and reconstructed soils is due to the lack of canopy cover and therefore fresh litter inputs on the reclaimed site. Long term exclusion of plant inputs may put selective pressure on the soil microbial community and result in altered community structure and functionality (Paterson et al. 2011). Therefore, we further presume that differences in the microbial functional diversity between natural and reconstructed soils may continue as long as fresh litter inputs remain limited on reclamation sites.

Table 3-1: Soil identification, general site characteristics, locations and soil properties for three mineral soils from the Athabasca Oil Sands Region.

Identification	Soil Classification		Canopy	Location		Stand Age	Soil Texture (%)			Textural class		TN	TOC	^{13}C
	Canadian	FAO				(years)	Sand	Silt	Clay		pH	(%)	(%)	(atom %)
aspen	Gray Luvisol	Albic Luvisol	aspen	N 56 57.5	W 111 38.9	>70	35	55	10	silt loam	4	0.07	1.20	1.0752
spruce	Gray Luvisol	Albic Luvisol	spruce	N 56 56.6	W 111 44.3	>70	30	56	14	silt loam	5	0.07	1.09	1.0756
novel	---	Technosol	none	N 57 06.1	W 111 40.2	11	33	43	24	loam	6	0.33	5.79	1.0764

Table 3-2: Soil microbial response to addition of ^{13}C-glucose in a 310 hour incubation. Numbers in parentheses represent one standard deviation from the mean (n=3). Statistical differences between the soils are represented by different letters with alpha=0.05.

Soils	Total Respiration								Total microbial biomass (nmol g^{-1} dry soil) at specific sampling times (hours).					
	mg CO_2 -C			mg CO_2 -^{13}C		mg CO_2 -C g^{-1}C	mg CO_2 -^{13}C g^{-1} C		0	22	46	70	142	310
aspen	2.8	(0.3)	b	0.085	(0.002) ab	2.54 (0.30) a	0.078 (0.001) a		499 (47) a	682 (97)	744 (85)	661 (225)	666 (49)	446 (71) a
spruce	1.9	(0.2)	c	0.073	(0.007) b	1.89 (0.18) b	0.074 (0.007) a		263 (13) b	275 (37)	274 (23)	222 (20)	205 (13)	200 (7) b
novel	4.1	(0.3)	a	0.095	(0.006) a	0.78 (0.05) c	0.018 (0.001) b		321 (22) b	459 (70)	328 (33)	457 (47)	356 (50)	338 (30) a

Table 3-3: Initial and final total organic carbon contents (mg) for the soil fractions from the ^{13}C-glucose addition incubation. Numbers in parentheses represent one standard deviation from the mean (n=3).

soil	time	sand†		silt‡		clay§		DOC#		sum	
		total organic carbon (mg)									
aspen	initial	300.3	(29.3)	72.0	(3.9)	12.1	(6.7)	3.0	(0.2)	387.3	(32.0)
	final	304.8	(80.0)	65.6	(5.3)	8.1	(3.4)	2.6	(0.1)	381.1	(78.7)
spruce	initial	205.3	(17.8)	74.8	(16.7)	17.7	(10.0)	2.4	(0.0)	300.3	(18.3)
	final	258.5	(54.2)	83.1	(12.4)	7.8	(3.7)	2.2	(0.1)	351.6	(59.9)
novel	initial	1068.5	(146.3)	385.0	(10.4)	50.7	(8.3)	12.1	(5.0)	1516.3	(133.0)
	final	1211.2	(155.8)	329.6	(12.9)	19.0	(4.4)	4.3	(0.3)	1564.1	(157.3)
		total ^{13}C organic carbon (mg)									
aspen	initial	3.2	(0.3)	0.8	(0.0)	0.13	(0.07)	0.03	(0.00)	4.2	(0.3)
	final	3.3	(0.9)	0.7	(0.1)	0.09	(0.04)	0.03	(0.00)	4.2	(0.9)
spruce	initial	2.2	(0.2)	0.8	(0.2)	0.19	(0.11)	0.03	(0.00)	3.2	(0.2)
	final	2.8	(0.6)	0.9	(0.1)	0.09	(0.04)	0.02	(0.00)	3.8	(0.6)
novel	initial	11.5	(1.6)	4.2	(0.1)	0.55	(0.09)	0.13	(0.05)	16.4	(1.4)
	final	13.1	(1.7)	3.6	(0.1)	0.21	(0.05)	0.05	(0.00)	16.9	(1.7)

†sand 53-2000 µm, ‡silt 2-53 µm, §clay <2 µm, #DOC - dissolved organic carbon

Figure 3-1. Respiration rates for three mineral soils incubated with ^{13}C-glucose. Total carbon respiration rate is indicated on side A and ^{13}C respiration rate is on side B. Error bars reflect one standard deviation from the mean (n=5).

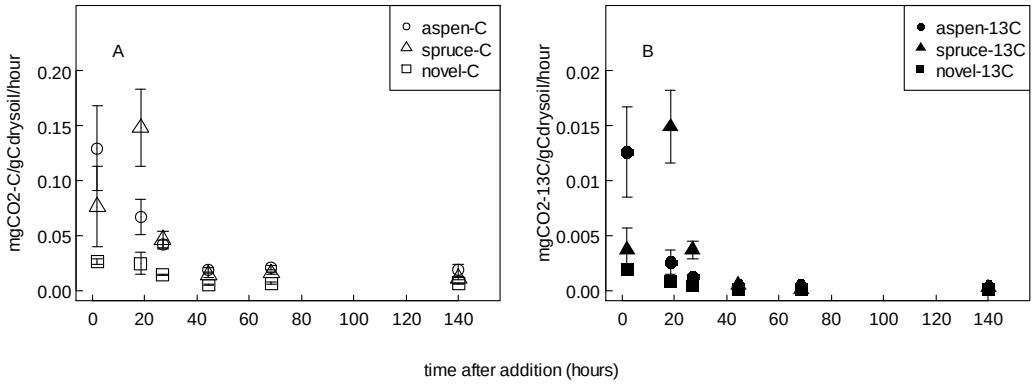


Figure 3-2. NMS ordination of PLFA profiles in three mineral soils from the western boreal forest (aspen, spruce and novel) incubated with ^{13}C-glucose. Grouping based on soil type and MRPP analysis is highlighted with circles.

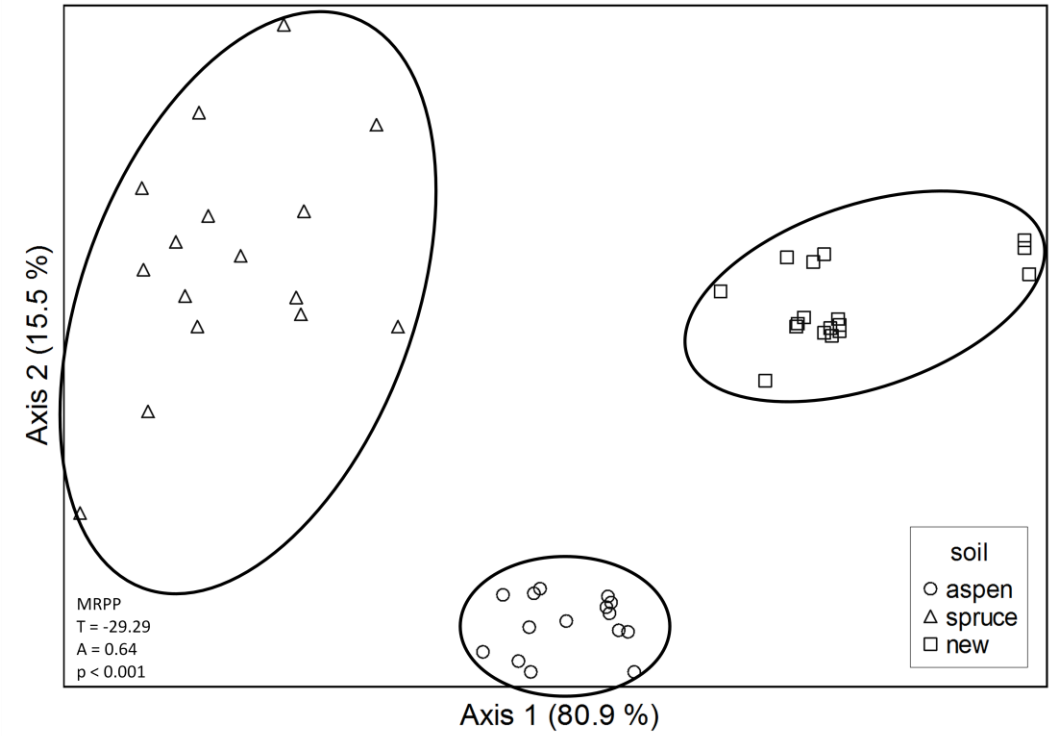


Figure 3-3: NMS ordination of $\delta^{13}C$ PLFA profiles in three mineral soils from the western boreal forest (aspen, spruce and novel) incubated with ^{13}C-glucose. Grouping based on soil type and MRPP analysis is highlighted with circles.

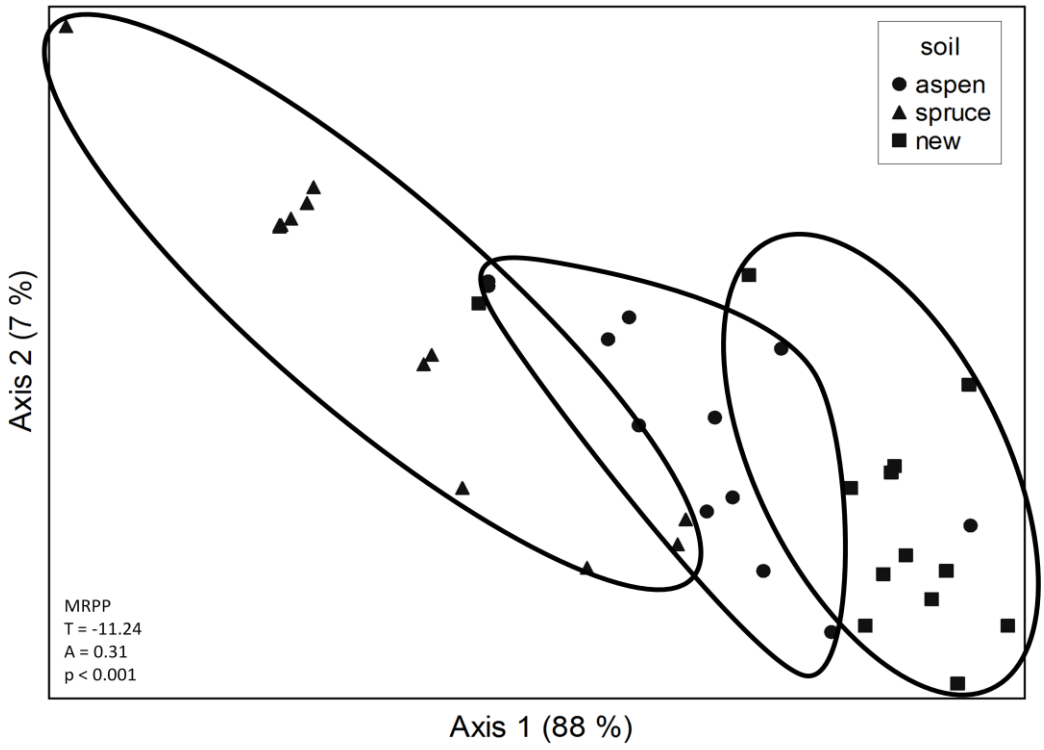


Figure 3-4. $\delta^{13}C$ enrichment of the 18:1ω7c PLFA in three mineral soils from the western boreal forest (aspen, spruce and novel) incubated with ^{13}C-glucose. Error bars reflect one standard deviation from the mean (n=3).

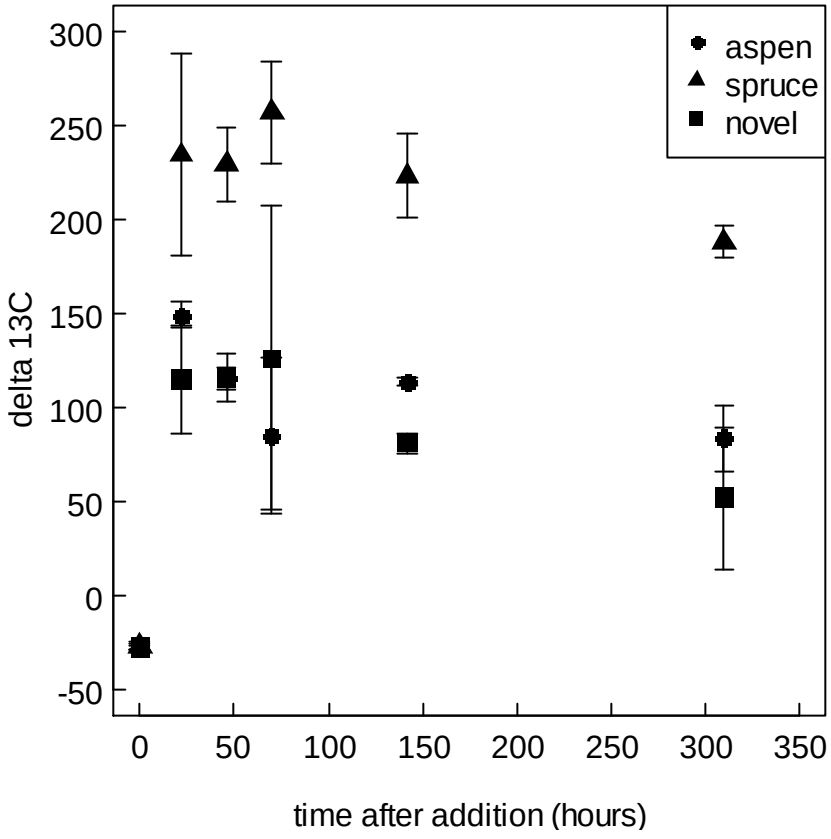
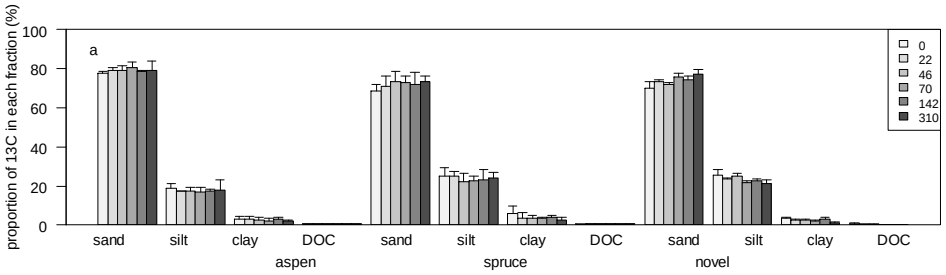


Figure 3-5. a) Proportion of ^{13}C in four soil fractions from three mineral soils from the western boreal forest (aspen, spruce and novel) incubated with ^{13}C-glucose. Time (hours) since beginning of the experiment is shown in the legend. Error bars reflect one standard deviation from the mean (n=3). b) DOC fraction enlarged for comparison.



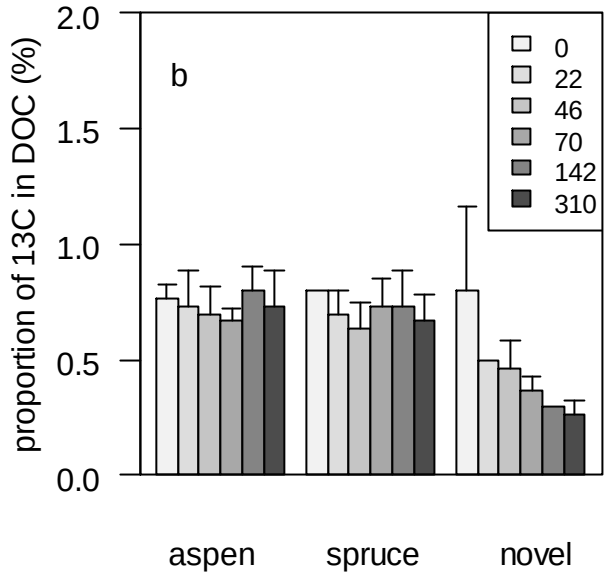
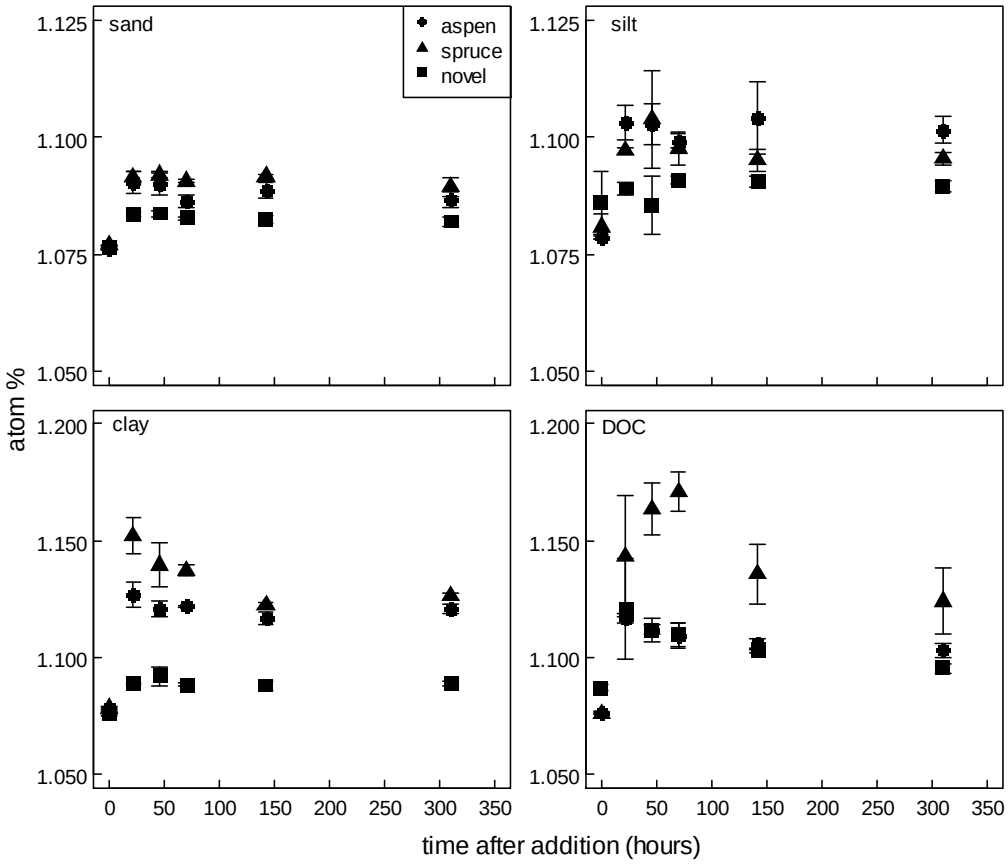


Figure 3-6. Enrichment of ^{13}C (atom %) in four soil fractions from three mineral soils from the western boreal forest (aspen, spruce and novel) incubated with ^{13}C-glucose. Error bars reflect one standard deviation from the mean (n=3).



References

Banning, N.C., Gleeson, D.B., Grigg, A.H., Grant, C.D., Andersen, G.L., Brodie, E.L., Murphy, D.V. 2011. Soil microbial community successional patterns during forest ecosystem restoration. Appl. Environ. Microbiol. 77, 6158-6164.

Bird, J.A., Herman, D.J., Firestone, M.K. 2011. Rhizosphere priming of soil organic matter by bacterial groups in a grassland soil. Soil Biol. Biochem. 43, 718-725.

Bligh, E.G., Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

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

Christensen, B.T. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates, in: Stewart, B.A. (Eds.) Advances in Soil Science. Springer-Verlag, New York. pp. 1-90.

Christensen, B.T. 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. Eur. J. Soil Sci. 52, 345-353.

Claassens, S., Jansen van Rensburg, P.J., Maboeta, M.S., van Rensburg, L. 2008. Soil microbial community function and structure in a post-mining chronosequence. Water Air Soil Pollut. 194, 315-329.

De Nobili, M., Contin, M., Mondini, C., Brookes, P.C. 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate. Soil Biol. Biochem. 33, 1163-1170.

Dimitriu, P.A., Prescott, C.E., Quideau, S.A., Grayston, S.J. 2010. Impact of reclamation of surface-mined boreal forest soils on microbial community composition and function. Soil Biol. Biochem. 42, 2289-2297.

Dungait, J.A.J., Kemmitt, S.J., Michallon, L., Guo, S., Wen, Q., Brookes, P.C., Evershed, R.P. 2011. Variable responses of the soil microbial biomass to trace concentrations of ^{13}C-labelled glucose, using ^{13}C-PLFA analysis. Eur. J. Soil Sci. 62, 117-126.

Food and Agriculture Organization of the United Nations 2006. Guidelines for soil description. Rome. pp. 1-109.

Frostegård, Å., Tunlid, A., Bååth, E. 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. J. Microbiol. Methods 14, 151-163.

Frostegård, Å., Tunlid, A., Bååth, E. 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43, 1621-1625.

Fung, M.Y.P., Macyk, T.M. 2000. Reclamation of oil sands mining areas, in: Barnhisel, R.I., Darmody, R.G., Daniels, W.L. (Eds.) Reclamation of Drastically Disturbed Lands. American Society of Agronomy, Inc., Madison, WI. pp. 755-774.

Government of Alberta 1993. Environmental Protection and Enhancement Act: Conservation and Reclamation Regulation.

Government of Alberta 2011. Alberta's oil sands: Protecting the environment. http://www.oilsands.alberta.ca/FactSheets/Protecting_the_environment_July_20_2011%281%29.pdf

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

Gude, A., Kandeler, E., Gleixner, G. 2012. Input related microbial carbon dynamic of soil organic matter in particle size fractions. Soil Biol. Biochem. 47, 209-219.

Guggenberger, G., Zech, W., Haumaier, L., Christensen, B.T. 1995. Land-use effects on the composition of organic matter in particle-size separates of soils: II. CPMAS and solution ^{13}C NMR analysis. Eur. J. Soil Sci. 46, 147-158.

Hahn, A.S., Quideau, S.A. 2012. Long-term effects of organic amendments on the recovery of plant and soil microbial communities following disturbance in the Canadian boreal forest. Plant Soil, 1-14.

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

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

Harris, J. 2009. Soil microbial communities and restoration ecology: facilitators or followers. Science 325, 573-574.

Kalra, Y.P., Maynard, D.G. 1991. 'Methods Manual for Forest Soil and Plant Analysis.' (Forestry Canada).

Legendre, P., Gallagher, E.D. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271-280.

Mackenzie, D.D., Naeth, M.A. 2010. The role of the forest soil propagule bank in assisted natural recovery after oil sands mining. Restor. Ecol. 18, 418-427.

MacKenzie, M.D., Quideau, S.A. 2010. Microbial community structure and nutrient availability in oil sands reclaimed boreal soils. Appl. Soil Ecol. 44, 32-41.

Maxfield, P.J., Evershed, R.P. 2011. Phospholipid fatty acid stable isotope probing techniques in microbial ecology, in: Murrell, J.C., Whiteley, A.S. (Eds.) Stable isotope probing and related technologies. ASM Press, Washington, DC.

McMillan, R., Quideau, S.A., MacKenzie, M.D., Biryukova, O. 2007. Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. J. Environ. Qual. 36, 1470-1478.

Mummey, D.L., Clarke, J.T., Cole, C.A., O'Connor, B.G., Gannon, J.E., Ramsey, P.W. 2010. Spatial analysis reveals differences in soil microbial community interactions between adjacent coniferous forest and clearcut ecosystems. Soil Biol. Biochem. 42, 1138-1147.

Mummey, D.L., Stahl, P.D., Buyer, J.S. 2002a. Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation. Appl. Soil Ecol. 21, 251-259.

Mummey, D.L., Stahl, P.D., Buyer, J.S. 2002b. Soil microbiological properties 20 years after surface mine reclamation: spatial analysis of reclaimed and undisturbed sites. Soil Biol. Biochem. 34, 1717-1725.

Norris, C.E., Quideau, S.A., Bhatti, J.S., Wasylishen, R.E. 2011. Soil carbon stabilization in jack pine stands along the Boreal Forest Transect Case Study. Glob. Change Biol. 17, 480-494.

Paterson, E., Sim, A., Osborne, S.M., Murray, P.J. 2011. Long-term exclusion of plant-inputs to soil reduces the functional capacity of microbial communities to mineralise recalcitrant root-derived carbon sources. Soil Biol. Biochem. 43, 1873-1880.

Perala, D.A. 1990. *Populus tremuloides* Michx.(Eds.) Silvics of North America.

Priha, O., Grayston, S.J., Hiukka, R., Pennanen, T., Smolander, A. 2001. Microbial community structure and characteristics of the organic matter in soils

under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. Biol. Fert. Soils 33, 17-24.

Rowland, S.M., Prescott, C.E., Grayston, S.J., Quideau, S.A., Bradfield, G.E. 2009. Recreating a functioning forest soil in reclaimed oil sands in Northern Alberta: An approach for measuring success in ecological restoration. J. Environ. Qual. 38, 1580-1590.

Ruess, L., Chamberlain, P.M. 2010. The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biol. Biochem. 42, 1898-1910.

Sheldrick, B.H., Wang, C. 1993. Particle size distribution, in: Carter, M.R. (Eds.) Soil sampling and methods of analysis. Lewis Publishers, Boca Raton, FL. pp. 499-511.

Soil Classification Working Group 1998. The Canadian System of Soil Classification, in: Agriculture and Agri-Food Canada- Research Branch (Eds.). NRC Research Press. pp. 53 - 59.

Sorenson, P.T., Quideau, S.A., MacKenzie, M.D., Landhäusser, S.M., Oh, S.-W. 2011. Forest floor development and biochemical properties in reconstructed boreal forest soils. Appl. Soil Ecol. 49, 139-147.

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

Turcotte, I., Quideau, S.A., Oh, S.-W. 2009. Organic matter quality in reclaimed boreal forest soils following oil sands mining. Org. Geochem. 40, 510-519.

Ushio, M., Wagai, R., Balser, T.C., Kitayama, K. 2008. Variations in the soil microbial community composition of a tropical montane forest ecosystem: Does tree species matter? Soil Biol. Biochem. 40, 2699-2702.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B. 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. Soil Biol. Biochem. 39, 2183 - 2207.

White, C., Tardif, J.C., Adkins, A., Staniforth, R. 2005. Functional diversity of microbial communities in the mixed boreal plain forest of central Canada. Soil Biol. Biochem. 37, 1359-1372.

White, D.C., Ringelberg, D.B. 1998. Signature lipid biomarker analysis, in: Burlage, R.S., Atlas, R., Shahl, D., Geesey, G., Sayler, G. (Eds.) Techniques in microbial ecology. Oxford University Press, New York, NY.

Williams, R.J., Hallgren, S.W., Wilson, G.W.T. 2012. Frequency of prescribed burning in an upland oak forest determines soil and litter properties and alters soil microbial community. For. Ecol. Manage. 265, 241-247.

Zelles, L., Bai, Q.Y. 1993. Fractionation of fatty acids derived from soil lipids by solid phase extraction and their quantitative analysis by GC-MS. Soil Biol. Biochem. 25, 495-507.

Chapter 4. Tracking Stable Isotope Enrichment in Tree Seedlings with Solid-State NMR Spectroscopy

A version of this chapter has been published:

Norris, C.E., Quideau, S.A., Landhäusser, S.M., Bernard, G.M. and Wasylishen, R.E. (2012) Tracking stable isotope enrichment in tree seedlings with solid-state NMR spectroscopy. Scientific Reports. 2:719. DOI:10.1038/srep00719.

4.1. Introduction

Terrestrial ecosystems are changing due to anthropogenic pressures, leading to far-reaching effects on global biogeochemical cycles. Assessing the net CO_2 flux between terrestrial ecosystems and the atmosphere remains one of the largest uncertainties in understanding of the global carbon cycle. Mechanisms controlling the persistence of soil organic matter are complex and reflect the interacting influences of abiotic conditions, nutrient supply, microbial community structure, and organic matter composition (Schmidt et al. 2011). Soils globally contain twice as much carbon as the atmosphere and world's vegetation combined, yet how this major carbon pool will respond to global environmental change is not well understood. Carbon storage in boreal forest soils, which alone represents close to 25% of the global soil carbon stocks, is particularly susceptible as northern regions are anticipated to experience warming of up to 5 ˚C by 2100 due to climate change (Christensen et al. 2007).

Accurate and quantitative rates of soil carbon fluxes are increasingly required to better constrain predictions of soil carbon turnover. Carbon transfer from plant residues to soils occurs both as surface litterfall or below-ground *via* root exudates and turnover. Carbon-13 (natural abundance 1.07 %) and ^{15}N (natural abundance 0.364 %) have been used to track both of these elements as they are mineralized from the decomposing enriched plant materials, taken up by the soil microbial biomass, stabilized into organic matter, or alternatively respired back to the atmosphere (Moore-Kucera and Dick 2008a; Swanston and Myrold 1997). Vascular plants, particularly woody perennial species, contain a range of complex tissues made up of varying amounts of different plant biopolymers

(Hancock et al. 2007; Preston et al. 2000) depending on environmental conditions (Donaldson et al. 2006). In addition these biopolymers display different decay rates, at least initially, with compounds such as lignins and waxes taking longer to decompose in the soil environment compared to proteins, soluble sugars, or cellulose (Kögel-Knabner 2002). Consequently, molecular characterization of labeled litter inputs should be combined with isotopic flux measurements if the fluxes are to be accurately interpreted in the broader context of ecosystem biogeochemical cycling.

Historically, isotope labeling of plant material originated with the use of radioactive carbon (^{14}C) (Svejcar et al. 1990; Warembourg and Kummerow 1991). With increased availability of stable isotopes (notably ^{13}C and ^{15}N) and improved quantitative analytical instrumentation, stable isotopes have become an important tool for tracer studies in terrestrial ecosystems (Dawson et al. 2002). Experimentally, enrichment is either achieved by continuous addition of the stable isotope or by applying pulses. Since the introduction of pulse chase labeling (Svejcar et al. 1990), the method has been adopted as the technique of choice compared to the more cumbersome continuous labeling apparatus. Numerous studies have used stable isotope enriched organic matter to examine carbon (Arevalo et al. 2010; Bird et al. 2003; Bottner et al. 2000) and nitrogen biogeochemical cycles (Bird et al. 2003). However, for methodological reasons, most studies have been restricted to non-woody fast growing graminoids that show little complexity in tissues and their associated biopolymers. Here, we selected trembling aspen (*Populus tremuloides* Michx.), a dominant upland tree species in North-American boreal forests (Little Jr. 1971), which is closely related to the equally common *Populus tremula* in the northern hemisphere of Eurasia. Aspen leaf tissues display a diverse and complex composition including cellulosic, aromatic and alkyl carbon species (Preston et al. 2000). The objective of the present study was to determine how pulse-labeling with $^{13}CO_2(g)$ and $K^{15}NO_3(l)$ affected isotopic enrichment in aspen seedlings at both the elemental and biopolymer level. To provide the greatest contrast in tissue composition, we present results on both foliage and woody root tissues for isotopic (^{13}C, ^{15}N)

abundance in response to three pulses and two chase periods (two hours and seven days). In addition, we quantified and characterized isotope assimilation in plant biopolymers using solid-state ^{13}C and ^{15}N nuclear magnetic resonance (NMR) analysis.

4.1. Methods

4.2.1. Seedling Growth and Enrichment

Thirty three one-year-old aspen seedlings, grown from an open pollinated aspen seed source at a commercial nursery (Smoky Lake Nursery, Smoky Lake, Alberta), were used in this study. Dormant seedlings were planted into pots (15 cm wide and 17 cm high) filled with a mixture of two thirds peat and one third sand. Seedlings were grown in a greenhouse with an average air temperature of 21 °C, a photoperiod of 16 hours, and daily watering. Seedlings flushed after 7 days and seedlings were fertilized once with a liquid application of 2.0 g L^{-1} of 20-20-20 N-P-K with chelated micronutrients (Plant Products Co., Brampton, Ontario). After 5 weeks of growth, three seedlings were harvested to establish initial baseline measurements and three additional seedlings were transferred to a separate greenhouse with identical growing conditions to provide the source for the final, unenriched (control) measurements.

Seedlings were labeled once per week for three consecutive weeks. Following each pulse (label), harvesting occurred after two distinct chase periods: two hours and seven days after the pulse (except for the third pulse). Labeled nitrogen (^{15}N) was added to the seedlings via an application of 50 ml $K^{15}NO_3$ (0.207 M of N, 60 atom % ^{15}N) solution (Sigma-Aldrich Chemicals, St. Louis, MO, USA). Enrichment of carbon (^{13}C) was achieved by adding 100 ml of 99.9 % ^{13}C CO_2 (Cambridge Isotope Laboratories, Inc. Andover, MA, USA) to each seedling enveloped in a Mylar tubular bag (36 cm diameter with the length varying depending on the seedling height (VacPac®, Baltimore MD). A 50 ml gastight syringe (Hamilton, Reno NV) was used to inject $^{13}CO_2$ gas.

Seedlings were allowed to take up the $^{13}CO_2$ gas for 30 minutes before the bags were removed. At sampling time (two hours and seven days after the pulse), three seedlings were randomly selected for harvesting and separated into three components: leaves, stem, and roots. Plant components were oven dried at 60 °C, weighed, coarsely ground and stored until further analysis. The complete aspen seedling enrichment experiment was run twice.

4.2.2. Laboratory Analyses

Samples were finely ground using a Retsch MM200 ball mill grinder (Retsch Inc. Newtown, USA) and analysed for total carbon and nitrogen contents on a Costech ECS 4010 Elemental Analyzer (Costech Analytical Technologies Inc. Valencia, USA). The isotopic composition of the samples was measured on a Costech ECS 4010 Elemental Analyzer equipped with a thermoconductivity detector joined to a Finnigan Deltaplus Advantage Isotopic Ratio Mass Spectrometer (ThermoFinnigan, Bremen, Germany).

4.2.3. Nuclear Magnetic Resonance

Samples for ^{13}C were analyzed on a Chemagnetics CMX Infinity 200 [magnetic field ($\mathbf{B}_0$) = 4.70 T, Larmor frequency (ν_L) (^{13}C) = 50.3 MHz] spectrometer with a 7.5 mm double-resonance MAS probe capable of high-power ^1H decoupling (Varian, Ft. Collins, CO, USA). Samples were loaded into a 7.5 mm OD zirconium oxide rotor with Kel-F drive tips and end caps, and spacers made of Teflon (DuPont, Circleville, OH, USA). The magic angle was set to 54.74° by maximizing the ^{79}Br NMR signal for spinning sidebands of KBr. Carbon-13 spectra were referenced to tetramethylsilane (0 ppm) by setting the high-frequency adamantane peak to 38.56 ppm (Earl and VanderHart 1982).

Direct polarization ^{13}C with a spin echo pulse (90 - τ - 180 - τ -ACQ- τ = 400 s) was used to acquire spectra (Bryce et al. 2001; Smernik and Oades 2001). A total of 500 transients (2000 for the control initial leaves) were acquired for each free induction decay (FID) with: 5.0 kHz spinning rate, 4.5 µs 90° pulse width, and 100 s recycle time. Processing of the resulting FID was performed with

WIN-NMR version 6.0 (Bruker, Germany). Processing the spectra included zero filling to 1K, line broadening of 100 Hz with phase and baseline correction followed by integration of the spectra. Previously published descriptions on peak identification and classification of integral regions were used to separate the following six regions (Kögel-Knabner 2002; Preston et al. 2000): alkyl (0-47 ppm), *O*-alkyl (47-112 ppm), di-*O*-alkyl (93-112 ppm), aromatic (112-140 ppm), phenolic (140-165 ppm), and carbonyl (165 – 185 ppm).

Nitrogen-15 NMR spectra of magic angle spinning samples were acquired on an Avance 500 NMR spectrometer, operating at 50.69 MHz for ^{15}N. Spectra were acquired with ramped CP, a 4.0 μs ^{1}H $\pi/2$ pulse, a 1 ms contact time and a recycle delay of 3.0 s. Samples were spun at 6 kHz. Spectra were obtained by adding 8 000 to 20 000 transients and were referenced such that $\delta_{iso} = 0$ for the isotropic ^{15}N resonance of methyl nitrate by setting the ^{15}N isotropic peak of glycine-^{15}N (98 % ^{15}N) to -347.54 ppm (Hayashi and Hayamizu 1991). The latter sample was used to optimize acquisition parameters. Spectra of ^{15}N enriched samples contained 4 distinct regions attributed to amide (−220 to −288 ppm), arginine sidechain (−288 to −324 ppm) and amine (−324 to −365 ppm) nitrogen sites (Smernik and Baldock 2005); the much more intense signal from the amide site contained spinning sidebands whose integrated intensities were added to that of the isotropic peak. In addition, to confirm the ^{13}C spectra acquired on the CMX 200, select samples were analyzed with either ramped CP or Bloch decay pulses sequences on the Avance 500 spectrometer operating at a frequency of 125.81 MHz. for ^{13}C.

4.2.4. Statistics

Descriptive statistics, sample means and standard deviations were determined using R version 2.11.0 (The R Foundation for Statistical Computing).

4.3. Results

The ^{13}C and ^{15}N concentrations increased in the seedling tissues with each successive pulse label; however, timings and patterns of isotope enrichment were

different (Figure 4-1). Two hours after the first $^{13}CO_2$ pulse, leaf tissues had doubled in ^{13}C concentrations compared to background levels. Although not all ^{13}C was retained within the leaves, a steady enrichment to 2.7 atom % from a background concentration of 1.1 atom % was observed with successive pulses. Root tissues did not show any detectable ^{13}C enrichment two hours after the first pulse, but a significant increase in ^{13}C concentration was evident seven days later (1.4 atom %). In contrast to ^{13}C enrichment patterns, ^{15}N enrichment was first noted in the roots two hours after the first pulse, with subsequent labels further increasing the concentration from 0.4 to 10.7 atom % ^{15}N. Enrichment of ^{15}N in the leaves could be detected seven days after the first pulse label. Final concentrations of ^{13}C and ^{15}N for unlabeled leaves and roots were similar to the initial ones; i.e., without pulse-labeling the proportion of ^{13}C to ^{12}C and ^{15}N to ^{14}N did not change during the experiment.

Variation in the biopolymer allocation of ^{13}C in aspen seedlings with repeated pulses and multiple chase times depended on the tissue type (Figure 4-2). As can be seen on the ^{13}C spectra of leaves, the *O*-alkyl signal (corresponding to oxygen-bonded or carbohydrate type carbons) was strongest two hours following labeling, and corresponded to 41 – 48 % of the total integrated spectral area (Figure 4-3). In comparison, the alkyl carbon signal (from aliphatic carbons in polymethylene such as cutin) was originally much smaller, but increased with time, so that the *O*-alkyl and alkyl peaks were closer in intensity with a longer (seven days) chase period (Figure 4-2). The resolution of peaks attributed to aromatic and phenolic carbons (arising from lignin and tannins) improved with ^{13}C enrichment, and the intensity of both increased in proportion with the longer chase time.

Allocation of ^{15}N to plant biopolymer groups, as with ^{13}C, varied depending on the chase time (Figure 4-4). Nitrogen characterization was limited to a subset of labeled samples as the signal from nuclei other than amides was indistinguishable from the noise on the control samples with natural abundance ^{15}N levels. Results are qualitative as amine nitrogen is known to be under-represented, and nonprotonated nitrogen is difficult to detect (Smernik and

Baldock 2005). After two pulses and a chase time of 7 days, the ^{15}N NMR spectra for leaves and roots both had NMR peaks characteristic of amide and amine nitrogen well above the background noise (Figure 4-4).

4.4. Discussion

Solid-state NMR spectroscopy may be used as an analytical tool to characterize the environment surrounding nuclei of interest (e.g., ^{13}C or ^{15}N) from which the chemical composition of plant or soil organic matter may be identified (Norris et al. 2011; Preston et al. 2000). The magic angle spinning technique (MAS) entails spinning samples rapidly about the magic angle (54.7°) relative to the applied magnetic field. The method greatly reduces the effects of chemical shift anisotropy, resulting in significant improvements in resolution compared to what is obtained for solid-state NMR spectra of stationary samples. The method also reduces the effects of homonuclear (e.g., ^{13}C-^{13}C) dipolar coupling. This technique is often combined with cross polarization (CPMAS) whereby the magnetization is transferred from abundant spins (usually ^{1}H) to less abundant spins; i.e., ^{13}C or ^{15}N. The latter technique provides a signal enhancement as great as the ratio of the magnetogyric ratios of the nuclei, ≈4 for ^{13}C and ≈10 for ^{15}N. However, since in most cases the less abundant spins also have a much longer spin-lattice relaxation time (T_1) then do the ^{1}H nuclei, the greatest benefit of the CP technique is usually that the repetition time of the experiment is controlled by the T_1 value of the latter. Hence, one may acquire many more transients in a given period of time, greatly improving the signal/noise ratios of the spectra (Bryce et al. 2001). However, it is important to realize that spectra obtained with CPMAS are not quantitative, since the signal arising from the nuclei are not all enhanced to the same extent. An alternative to the CPMAS technique is to obtain spectra with MAS using direct polarization (DP), which provides qualitative results provided the recycle time is significantly greater than the greatest T_1 value for the nuclei of interest (Simpson and Preston 2007). For the samples in the present study, ^{13}C spectra were obtained by DP; to eliminate the interference from natural abundance ^{13}C in either the probe or the rotor drive tips and end caps, a modified DP spin-

echo pulse sequence was used (Smernik and Oades 2001). The ^{15}N enrichment was qualitatively characterized by the CPMAS technique since obtaining suitable ^{15}N DP spectra was not practical.

Chemistry of seedling roots was different from leaves in that the samples did not exhibit much variation with the different levels of enrichment (Figures 4-2 and 3). Hence, the roots showed fairly uniform ^{13}C enrichment across the different biopolymers. For the leaves, the shift in intensity of the *O*-alkyl carbon peak between the two chase times likely reflects the initial fixation of carbon as glucose followed by a partial redistribution to more complex compounds such as cutin or lignin. On the other hand, uniform ^{13}C enrichment within the root biopolymers reflects non-discriminatory incorporation into new structural root tissues, including cellulose and lignin, rather than preferential accumulation into *O*-alkyl C in the form of soluble sugars or starch reserves. While the ^{15}N NMR spectra reflected the acquisition and allocation of nitrate by the seedling; two hours after the $K^{15}NO_3$ pulse, roots had taken up and reduced the labelled nitrate into amine groups. Roots contain nitrogen reductase and other enzymes for amino acid and amide formation, which allows the roots to synthesize organic solutes for nitrogen distribution throughout the plant (Daniel-Vedele et al. 2010; Pate 1973). Our results support this pathway as evidenced by the relatively higher amide/amine peak ratio on the root spectra after a 7 day chase period (Figure 4-4).

For biogeochemical studies aimed at elucidating organic matter processes and ecosystem fluxes, a distinction between plant-labeled material and native soil organic matter needs to be made. Hence knowledge of the isotope enrichment levels across different tissues and biopolymers is essential. Here we demonstrated that both the allocation of ^{13}C and ^{15}N in leaves and roots was dependent on the chase time (Figure 4-1). Similar results have been reported during pulse labeling of birch (*Betula papyrifera*) and fir (*Pseudotsuga menziesii*) trees, where the initial increase in foliage ^{13}C concentration decreased while that of roots increased with a longer chase time(Simard et al. 1997). The level of enrichment also needs to be sufficient for planned biogeochemical tracer studies. Enrichment of ^{13}C at the end of our labeling experiment was 2.7 % in leaves and 1.4 atom % in roots.

This is comparable to levels previously reported for needles (1.6 % ^{13}C) and roots (1.4 atom % ^{13}C) of Douglas-fir seedlings, which were demonstrated to be sufficient to follow tracers during a soil incubation study (Moore-Kucera and Dick 2008b). The ^{15}N enrichment levels for leaves (8.7 % ^{15}N) and roots (10.7 atom % ^{15}N) also were comparable to enriched ponderosa pine tissues used in a field decomposition study (Bird and Torn 2006). The NMR spin-active isotopes of carbon (^{13}C) and nitrogen (^{15}N) make up 1.1 and 0.4 %, respectively of the total number of atoms at natural abundance levels in our control samples. With tissue enrichment, there was a subsequent increase in both spin-1/2 atoms, which directly translated into an enhanced signal-to-noise ratio and improved characterization. Using this technique, we were able to identify that uniform labeling required a one-week chase period for both the leaves and the roots. Combining NMR characterization with stable isotopic labeling has been successfully applied in other fields, notably plant metabolomics (Sekiyama et al. 2010), but, to our knowledge, this represents the first use of this rapid, non-destructive method to quantify molecular isotope allocation using DP ^{13}C NMR and ^{15}N CPMAS spectroscopy for stable and non-reactive tracers in biogeochemical studies. Further work using multi-dimensional NMR methods or a combined instrument approach (e.g., solid-state NMR, solution-state NMR, compound specific isotope analysis with isotope ratio mass spectrometry), as in the area of plant metabolomics (Sekiyama et al. 2010), would continue to refine our knowledge on the allocation of the stable isotopes within the tissues. With knowledge of these methods not only could enriched plant organic matter for decomposition in biogeochemical studies be better characterized but also investigations on the abiotic influences (e.g., water stress) or biotic interactions (e.g., insect grazing) on root exudates and plant physiology could be elucidated.

Figures

Figure 4-1. Atom percent concentration of ^{13}C and ^{15}N in seedling leaves and roots at different pulse and chase times. Chase time of control, 2 hour or 7 day is indicated by shading in the box. The number of pulses (0 – 3) is indicated by a number at the base of the barplot. Error bars represent one standard deviation (n=6).

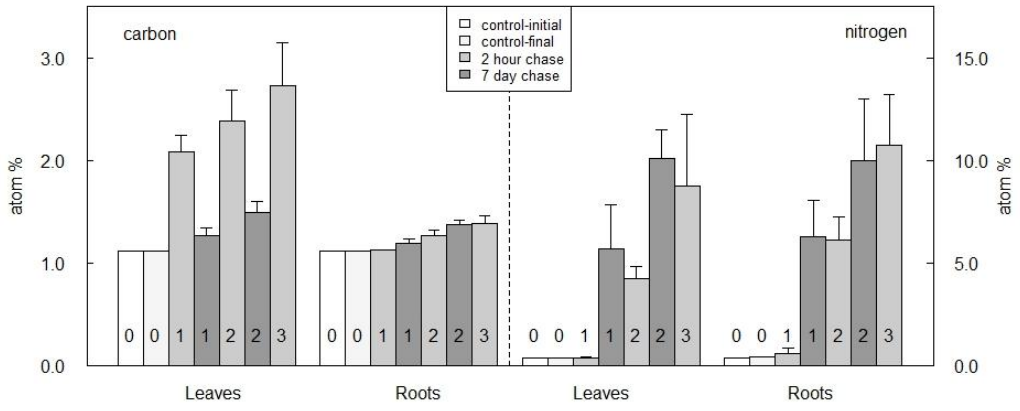


Figure 4-2. Direct pulse ^{13}C NMR spectra with control-initial, one pulse and 2 hour chase and one pulse 7 day chase ^{13}C enriched seedling leaves and roots. Common integral regions representing the different magnetic environments of carbon are shown on the spectra.

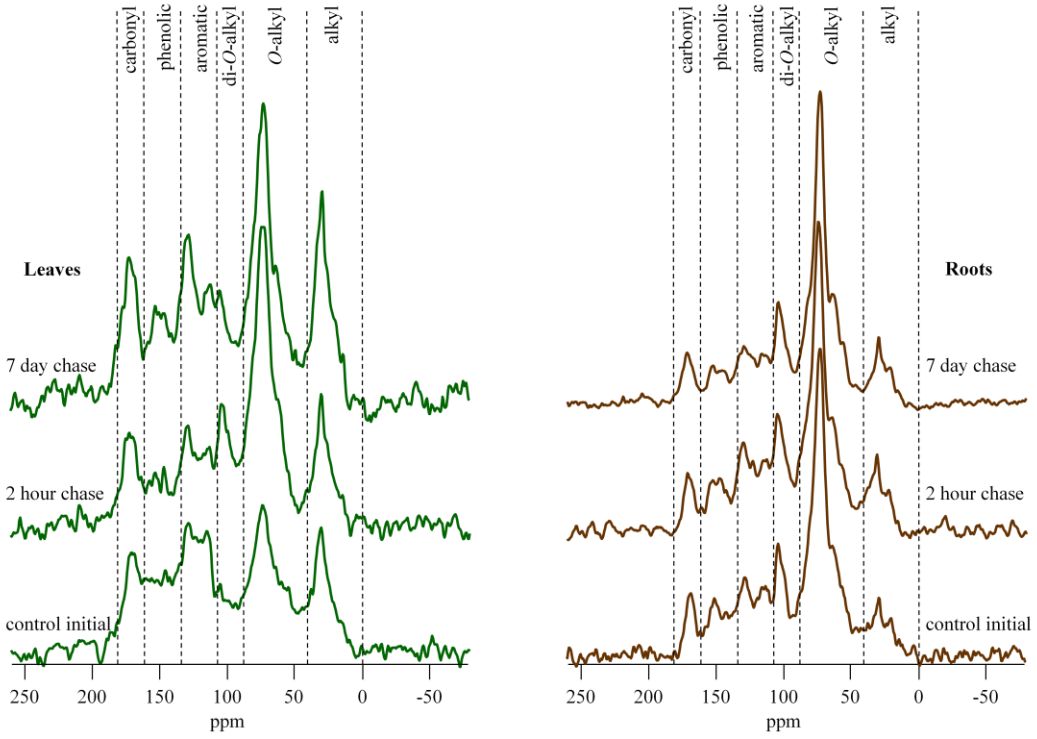


Figure 4-3: Relating the proportion of *O*-alkyl carbon to ^{13}C enrichment for the multi-pulse and multi-chase stable isotope labeling of seedling leaves and roots. One standard deviation for *O*-alkyl carbon (n=2) and carbon-13 atom % (n=6) are indicated by error bars on the graph.

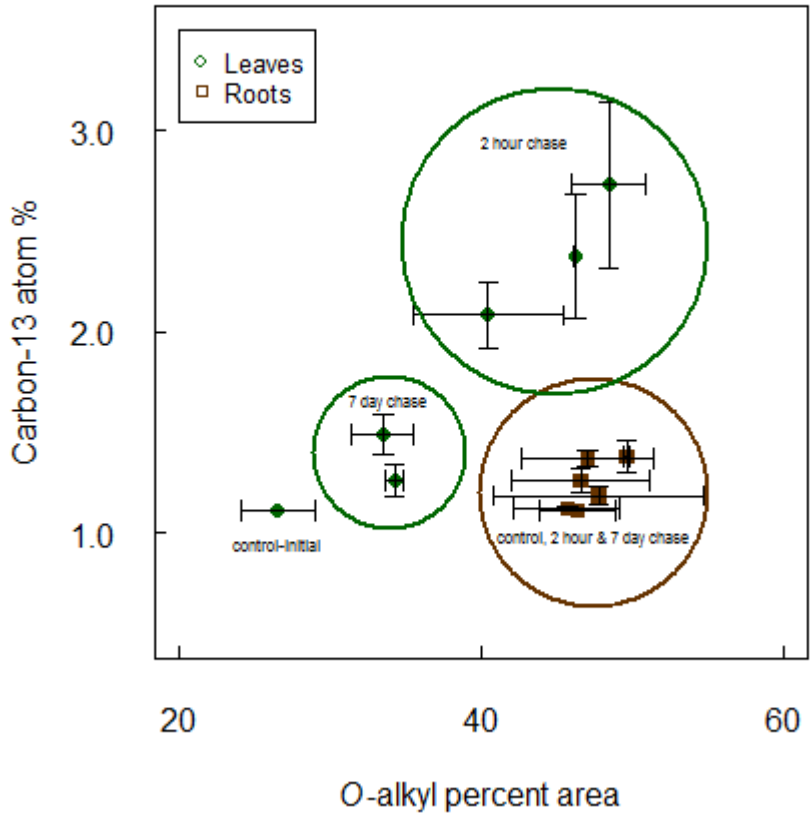
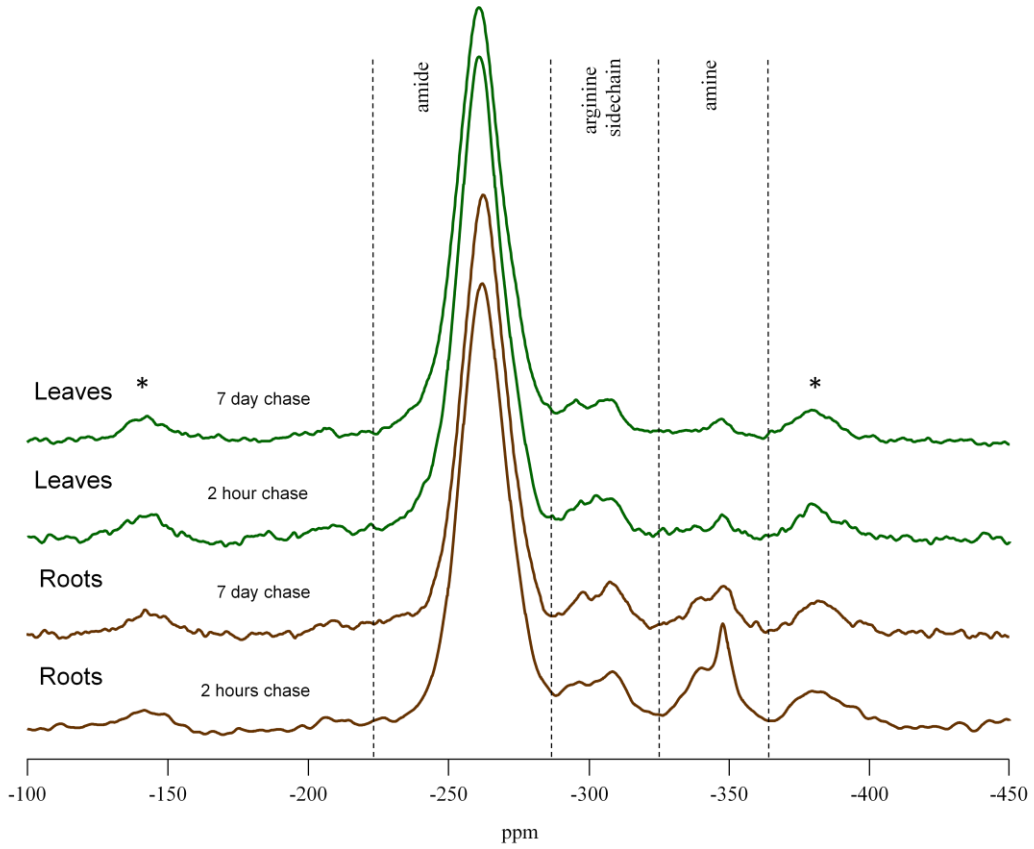


Figure 4-4. Cross polarization ^{15}N NMR spectra of seedling leaves and roots indicating qualitative differences between the two chase times (2 hour and 7 day). The 7 day chase samples had experienced 2 pulses while the 2 hour samples had 3 pulse of $^{13}CO_2$. Common integral regions representing the different magnetic environments of nitrogen are shown on the spectra.



REFERENCES

Arevalo, C.B.M., Bhatti, J.S., Chang, S.X., Sidders, D. 2010. Distribution of recent photosynthates in saplings of two hybrid poplar clones. Comm. Soil Sci. Plant Anal. 41, 1004-1015.

Bird, J.A., Torn, M.S. 2006. Fine roots vs. needles: a comparison of ^{13}C and ^{15}N dynamics in a ponderosa pine forest soil. Biogeochemistry 79, 361-382.

Bird, J.A., van Kessel, C., Horwath, W.R. 2003. Stabilization of ^{13}C-carbon and immobilization of ^{15}N-nitrogen from rice straw in humic fractions. Soil Sci. Soc. Am. J. 67, 806-816.

Bottner, P., Coûteaux, M.-M., Anderson, J.M., Björn, B., Billès, G., Bolger, T., Casabianca, H., Romanyá, J., Rovira, P. 2000. Decomposition of ^{13}C-labelled plant material in a European 65-40° latitudinal transect of coniferous forest soils: simulation of climate change by translocation of soils. Soil Biol. Biochem. 32, 527-543.

Bryce, D.L., Bernard, G.M., Gee, M., Lumsden, M.D., Eichele, K., Wasylishen, R.E. 2001. Practical aspects of modern routine solid-state multinuclear magnetic resonance spectroscopy: one-dimensional experiments. Can. J. Anal. Sci. Spectros. 46, 46-82.

Christensen, J.H., Hewitson, B., et al. 2007. Regional climate projections, in: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge. pp. 847-892.

Daniel-Vedele, F., Krapp, A., Kaiser, W.M. 2010. Cellular biology of nitrogen metabolism and signaling, in: Hell, R., Mendel, R.-R. (Eds.) Plant Cell Monographs. pp. 145-172.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., Tu, K.P. 2002. Stable isotopes in plant ecology. Annu. Rev. Ecol. Syst. 33, 507-559.

Donaldson, J.R., Kruger, E.L., Lindroth, R.L. 2006. Competition- and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (*Populus tremuloides*). New Phytol. 169, 561-570.

Earl, W.L., VanderHart, D.L. 1982. Measurement of ^{13}C chemical shifts in solids. J. Magn. Reson. 48, 35-54.

Hancock, J.E., Loya, W.M., Giardina, C.P., Li, L., Chiang, V.L., Pregitzer, K.S. 2007. Plant growth, biomass partitioning and soil carbon formation in response to altered lignin biosynthesis in *Populus tremuloides*. New Phytol. 173, 732-742.

Hayashi, S., Hayamizu, K. 1991. Chemical shift standards in high-resolution solid-state NMR (2) ^{15}N Nuclei. Bull. Chem. Soc. Jpn. 64, 688-690.

Kögel-Knabner, I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol. Biochem. 34, 139-162.

Little Jr., E.L. 1971. 'Atlas of Unites States trees.' (U.S. Dept. of Ag. For. Serv.: Washington, DC).

Moore-Kucera, J., Dick, R.P. 2008a. Application of ^{13}C-labeled litter and root materials for *in situ* decomposition studies using phospholipid fatty acids. Soil Biol. Biochem. 40, 2485-2493.

Moore-Kucera, J., Dick, R.P. 2008b. A pulse-chase method to ^{13}carbon-label Douglas-fir seedlings for decomposition studies. Soil Sci. 173, 46-53.

Norris, C.E., Quideau, S.A., Bhatti, J.S., Wasylishen, R.E. 2011. Soil carbon stabilization in jack pine stands along the Boreal Forest Transect Case Study. Glob. Change Biol. 17, 480-494.

Pate, J.S. 1973. Uptake, assimilation and transport of nitrogen compounds by plants. Soil Biol. Biochem. 5, 109-119.

Preston, C.M., Trofymow, J.A., Canadian Intersite Decomposition Experiment Working Group 2000. Variability in litter quality and its relationship to litter decay in Canadian forests. Can. J. Bot. 78, 1269-1287.

Schmidt, M.W.I., Torn, M.S., et al. 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49-56.

Sekiyama, Y., Chikayama, E., Kikuchi, J. 2010. Profiling polar and semipolar plant metabolites throughout extraction processes using a combined solution-state and high-resolution magic angle spinning NMR approach. Anal. Chem. 82, 1643-1652.

Simard, S.W., Durall, D.M., Jones, M.D. 1997. Carbon allocation and carbon transfer between *Betula papyrifera* and *Pseudotsuga menziesii* seedlings using a ^{13}C pulse-labeling method. Plant Soil 191, 41-55.

Simpson, M.J., Preston, C.M. 2007. Soil organic matter analysis by solid-state ^{13}C nuclear magnetic resonance spectroscopy, in: Carter, M.R., Gregorich, E.G.

(Eds.) Soil Sampling and Methods of Analysis. CRC Press, Boca Raton USA. pp. 681 - 692.

Smernik, R.J., Baldock, J.A. 2005. Solid-state ^{15}N NMR analysis of highly ^{15}N-enriched plant materials. Plant Soil 275, 271-283.

Smernik, R.J., Oades, J.M. 2001. Background signal in solid state ^{13}C NMR spectra of soil organic matter (SOM)- quantification and minimization. Solid State Nucl. Magn. Reson. 20, 74-84.

Svejcar, T.J., Boutton, T.W., Trent, J.D. 1990. Asessment of carbon allocation with stable carbon isotope labeling. Agron. J. 82, 18-21.

Swanston, C.W., Myrold, D.D. 1997. Incorporation of nitrogen from decomposing red alder leaves into plants and soil of a recent clearcut in Oregon. Can. J. For. Res. 27, 1496-1502.

Warembourg, F.R., Kummerow, J. 1991. Photosynthesis/translocation studies in terrestrial ecosystems, in: Coleman, D.C., Fry, B. (Eds.) Carbon Isotope Techniques. Academic Press, Inc., San Diego. pp. 11-37.

Chapter 5. Improving forest soils during reclamation

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5.1. Introduction

Soil is critical in delivering multiple ecosystem services. Restoring soil organic matter, for example, improves the regulation of water flow and soil fertility for plant growth (Banwart 2011; Lal 2004). Organic matter is therefore recognized as a key component of soil (Carter 2002), and when removed, causes a direct decrease in porosity (Powers et al. 2005) and productivity (Paterson et al. 2011). However, decreases in soil quality can be reversed with increased organic matter inputs, as has been shown in both agricultural (Lal et al. 2007) and reclamation settings (Larney and Angers 2012).

The Athabasca Oil Sands Region (AOSR) lies within the western boreal forest north of Fort McMurray, Alberta, Canada. The region is currently being mined for the Athabasca oil sands deposit and reclamation by the mining companies is a requirement, where the disturbed land is returned to an equivalent pre-mining land capability with self-sustaining ecosystems (Government of Alberta 1993). One indicator of successful reclamation is the re-establishment of central ecosystem processes such as soil nutrient cycling.

Upland forests dominated by a native aspen tree community are one of the desired objectives of forest reclamation treatments in the AOSR. Upland soils are constructed from a combination of salvaged mineral material with inputs of either peat, for its ability to decrease bulk density and increase both the moisture holding capacity and organic nutrient bank of the constructed soils, or forest floor material, for its inclusion of plant propagules and promotion of a more natural microbial community (Hahn and Quideau 2012; Macdonald et al. 2012). As the majority of nitrogen in mature natural aspen ecosystems is contained belowground, namely within the mineral soil and its overlying forest floor (Ste-

Marie et al. 2007), nutrient status in constructed soils will be critical in their ability to eventually support a mature aspen forest.

Total nitrogen stocks on reclamation stands are above the natural range (Hemstock et al. 2010; McMillan et al. 2007) due to organic matter additions as part of soil contruction practices. Yet, the amount of nitrogen available for vegetation growth is of concern as soil microbial biomass (McMillan et al. 2007) and net mineralization rates (Hemstock et al. 2010) are less than in natural upland aspen forests. Further work in the AOSR reported differences in forest floor organic matter chemistry (Turcotte et al. 2009), a lower rate of organic matter decomposition (Rowland et al. 2009) and decreased enzyme activity (Dimitriu et al. 2010) at the reclaimed sites compared to the natural undisturbed stands. Results clearly demonstrate that nutrient cycling differs between reclaimed and undisturbed stands. Among reclaimed stands, the percent canopy cover (Sorenson et al. 2011) and type of organic matter amendment used in soil reconstruction (Hahn and Quideau 2012) were found to affect the soil microbial community, which drives nutrient availability and therefore soil quality. Building on this work, and on recent work using stable isotopes in natural systems to follow nutrient fluxes (Bird and Torn 2006; Swanston and Myrold 1997; Zeller et al. 2000), we conducted a field experiment using a stable isotope tracer (^{15}N) to determine if nitrogen from enriched aspen leaf litter applied to soils is readily available to new living biomass on both reclaimed, harvested and undisturbed sites. The stable isotope provides a marker to follow fluxes between litter, microbial community, soil and vegetation and therefore constitutes an unparalleled tool to assess nutrient cycling.

5.2. Material and methods

5.2.1. Generating the nutrient tracer

Seven-year-old aspen saplings (4-5 m tall) growing in the open at the University of Alberta's Ellerslie Research Farm, Edmonton, Alberta were used in this study. Stable isotope enrichment of the sapling leaves occurred by a spray application of 0.207 M of N using 60.0 atom % $K^{15}NO_3$ (Aldrich Chemicals) on June 11, 2009

and then once a week from July 10 to July 31, for a total of five applications. The saplings were harvested on August 31, 2009 and the leaves were immediately removed, air dried and stored at room temperature. Analysis indicated that the aspen leaf litter had been enriched to an average $\delta^{15}N$ 320 ‰ (standard deviation 34 ‰) .

5.2.2. Study area

Four study sites were used in this case study (Figure 5-1, Table 5-1): Young Reclaimed, Older Reclaimed, Harvested, and Undisturbed. All sites were located within a 20 km radius on the Syncrude Canada Mine Lease located 50 km north of Fort McMurray within the AOSR. The Young Reclaimed site was constructed with overburden capped with 20 cm peat mineral mixture over 80 cm of secondary material in 1998 and planted to alternating rows of aspen and spruce seedlings. The Older Reclaimed site was constructed in 1988 with a 20 cm peat mineral mixture over secondary material and tailings sand and planted to pure aspen. The Harvested site was clearcut in 1992 and since then has recovered with natural re-growth of aspen and spruce trees. The Undisturbed site was a mature healthy aspen dominated stand approximately 80 years old. Soils of both reclaimed sites are Technosols while the remaining two, Harvested and Undisturbed, are Albic Luvisols (Food and Agriculture Organization of the United Nations 2006). On each of the four sites three transects of paired mesocosm cylinders were established (with the exception of Harvested which had two transects due to the small site size). Pairing of mesocosms was chosen as a means to minimize both site heterogeneity and sample numbers. Mesocosm pairs were 1 m apart from their centres, and 5 m from the next pair with a total of four pairs along a transect and at least 10 m between transect rows (Figure 5-1). Each mesocosm, was made of SDR 35 PVC, of 20 cm in diameter and 11 cm deep. Each mesocosm was covered with 50 cm by 50 cm of 1.5 cm by 2.0 cm plastic mesh netting fixed by stakes. To one of each pair of mesocosms, 20 g of enriched aspen leaf litter was added in May and September 2010. Field sampling occurred four times: immediately (May 2010), 4 months (September 2010), 12 months

(May 2011), and 16 months (September 2011) after installation of the field incubation experiment.

5.2.3 Abiotic measurements

Air and soil temperatures from Older Reclaimed and Undisturbed sites were monitored at 10 cm above-ground and 15 cm below-ground with a HOBO Micro Station (Onset Computer Corporation, Bourne, MA). Young Reclaimed and Harvested sites were instrumented for temperature measurements at 2 m above-ground and 4 cm below-ground with Campbell 107-L temperature probes. Canopy cover was determined by convex densiometer (Older Reclaimed) or WinSCANOPY (Regent Instruments, QC, Canada). Annual litter accumulation was collected in October from 0.5 m^2 screen traps in the vicinity of the mesocosms, air dried and weighed. The hydrometer method was used to determine soil texture.

5.2.4 Biotic measurements

A pair of mesocosms was destructively collected from each transect at each sampling time for quantification of the following organic matter pools: live vegetation, dead vegetation (litter), and soil (to 5 cm below the litter layer). Samples were stored at 4 °C until transported to the lab where the vegetation was immediately air dried and soil was sieved to 4 mm, subsampled for microbial biomass extraction and roots, and then air dried. Samples were analyzed for total organic carbon and nitrogen by dry combustion on a Costech ECS 4010 Elemental Analyzer equipped with a thermoconductivity detector (Costech Analytical Technologies Inc. Valencia, USA), and ^{15}N isotopic composition by coupling the Costech ECS 4010 to a Finnigan Deltaplus Advantage Isotopic Ratio Mass Spectrometer (IRMS; ThermoFinnigan, Bremen, Germany). Results were expressed in the δ-notation, part per thousand variation from the standard atmospheric N_2.

Microbial biomass of collected soil was determined by the chloroform fumigation extraction method (Voroney et al. 2008). One microbial biomass

subsample (40 g) was immediatly extracted with 80 ml of 0.5 M K_2SO_4 and vacuum filtered (Fisherbrand G4) while the second (40 g) was extracted after a 24 hour incubation with chloroform. All extracts were stored frozen at -20 °C until analysis. For soil moisture, 10 g of field moist sample was oven dried at 120 °C for 24 hours and cooled in a dessicator to constant mass. Microbial biomass, total organic carbon and nitrogen were measured on a Shimadzu TOC-V with TN option (Mandel Scientific Company Inc., Guelph, CA) after which the samples were oven dried at 60 °C, finely ground with mortar and pestle and analyzed (as above) for isotopic composition (Dijkstra et al. 2006).

5.2.5 Data Analysis

Due to the lack of site replication, statistics were not performed on this data set. However, descriptive statistics of sample means, standard deviations and graphs were performed in R (version 2.11.0, the R Foundation for Statistical Computing). Daily air and soil temperature values were averaged from measurements recorded every 5 minutes through the summer and every 10 minutes for the winter on Older Reclaimed and Undisturbed while Young Reclaimed and Harvested data points were recorded every 5 seconds. Biotic response results are presented as ratios of paired mesocosms e.g., R = (gravimetric soil moisture amended)/(gravimetric soil moisture of unamended mesocosm). Therefore ratios greater than 1 represent a greater response of the amended mesocosm compared to that of the paired unamended mesocosm. Proportion of ^{15}N in live vegetation (F_{live}) was determined from a mixing model using applied leaves ($\delta^{15}N_{leaves}$) and soil organic matter ($\delta^{15}N_{soil}$) as the two source materials following: $F_{live} =$
$((\delta^{15}N_{live} - \delta^{15}N_{soil}) / (\delta^{15}N_{leaves} - \delta^{15}N_{soil})) * 100$.

5.3. Results and Discussion

Abiotic responses

With long cold winters and short dry summers water availability can be a constraint to vegetation growth on constructed soils in the AOSR (Macdonald et

al. 2012). Climate normals for the region report a daily average temperature for the year of 0.7 °C and an average yearly precipitation of 455 mm predominantly from summer rainfall (Environment Canada 2013). Site climate data trends for June 2010 to July 2011 were similar to those of the long-term pattern within the region, although measured average air temperature values were higher (Table 5-1). Differences could have been due to the shelter provided by the forest stands or by the placement of sensors within 10 cm of the ground for the Older Reclaimed and Undisturbed sites. Across the two growing seasons, air temperature was comparable in all four sites; however, soil temperature was overall lower and less variable at the Undisturbed site compared to all disturbed sites (Figure 5-2). This was likely due to the insulation provided by the greater canopy cover, developed forest floor and the increased moisture holding capacity. At all three sampling times, soil from the Undisturbed site contained twice the moisture content of the other soils and leaf litter addition generally increased this moisture content (Figure 5-3). The addition of leaves also increased the soil moisture holding capacity at the Harvested and Young Reclaimed sites. Therefore, increasing litter inputs increased water content in the underlying soil layer.

In this study, we used a leaf litter input of 637 g m^{-2} to generate a strong pulse of both organic matter and ^{15}N isotope tracer inputs to the system. Annual aspen leaf litterfall rates in natural ecosystems are typically lower although they can show great variability across the landscape, varying for instance from 140 gm^{-2} yr^{-1} in Utah (Bartos and DeByle 1981) to 215 gm^{-2} in Alberta (Lousier and Parkinson 1976). Leaf litter inputs were measured on three of the four study sites and were at the low end of this range with a strong year to year variability (Table 5-1). These values reflect the low canopy cover at the still young sites, since the Younger Reclaimed stand was reaching canopy closure in 2011, while the Harvested stand was still very open.

Biotic Responses

The soil microbial community is known to drive nutrient cycling in forest ecosystems. With a greater microbial biomass more nutrients are available and

soil quality is increased. Total soil microbial biomass at reclaimed sites has been reported to rise with increased canopy cover (Sorenson et al. 2011) and time since reclamation (Hahn and Quideau 2012); yet it was still markedly less than at the natural sites. In boreal forests, reserves of nutrients such as nitrogen and phosphorus, which are essential for plant growth, are mostly contained within soil organic matter. Their release is largely controlled by the microbial biomass, a biomass that has been shown to have not only decreased quantity (Hahn and Quideau 2012; McMillan et al. 2007), but also decreased activity on reclaimed sites compared to natural sites in the AOSR (Dimitriu et al. 2010; Hemstock et al. 2010). Similar to results from previous studies in the region, we found that soil microbial biomass was twice as high at the Undisturbed site compared to the other sites; this held true for the entire experiment duration (Figure 5-4). However, adding leaf litter tended to increase soil microbial biomass at all sites (Figure 5-3). Therefore, all sites demonstrated improved soil quality with the addition of leaf litter. These results raise an interesting point; while quality indicators such as soil microbial biomass were greater on undisturbed versus reclaimed sites, the response of these indicators was similar for the harvested and reclaimed sites. This study as well as previous research in the AOSR compared reclaimed to natural undisturbed mature stands. Yet, for a true indication of reclamation success, a better analogue for comparison may be natural stands disturbed by other means, such as fire or harvest.

A key soil nutrient, nitrogen, is of concern on constructed soils of the AOSR for two reasons: i) the western boreal forest region is known to be nitrogen limited (Kimmins 1996) and ii) while total soil nitrogen content was determined to be at or above the natural range on disturbed sites in the oil sands region (Hemstock et al. 2010; McMillan et al. 2007; Rowland et al. 2009), net mineralization rates were at the low end of the range of reported rates from mature upland forests (Hemstock et al. 2010). Therefore, while there are abundant nitrogen stocks in the reclaimed soils, little nitrogen may be available as a nutrient source. In this study, we determined that ^{15}N stable isotope tracer from the applied leaf litter was utilized by the soil microbial

community on all sites after one growing season. Specifically, this was evidenced by the ^{15}N ratios that were calculated to be greater than one (Figure 5-5).

Not only did the soil microbial biomass incorporate the ^{15}N isotope tracer, the tracer was also taken up by both the live (above-ground) vegetation and roots (Figure 5-5). Live vegetation collected from within the treatment mesocosms varied from small herbs to grasses and shrubs. Yet, despite bulking these different plants and treating them as one homogeneous organic matter pool, all vegetation growing on mesocosms amended with the tracer showed a similar increase in ^{15}N across all study sites. Roots were similarly treated, i.e., they were coarsely subsampled from the soil and bulked as one homogenous pool; they too increased in ^{15}N (Figure 5-5). Further, seasonal variation was evident in the roots as isotopes were likely leached out or redistributed within the plants between growing seasons. These results indicate that the stable isotope tracer of nitrogen was cycled from the amended leaves through the soil microbial biomass and made available as a nutrient source for growing vegetation with, at least, 0.1 to 1 % of the ^{15}N in live vegetation derived from the added leaf litter.

5.4. Conclusions

While this study is limited in scope with four unreplicated study sites, the results clearly indicate that further work must be done to consider the appropriateness of comparing reclaimed stands to mature undisturbed stands when determining their reclamation recovery trajectory in terms of central ecosystem processes; analogues of fire or harvested disturbed stands may be more appropriate than mature undisturbed stands. In addition, this study illustrates how live vegetation does not rely solely on soil organic matter for its nutrient needs but that nitrogen readily cycles from fresh leaf litter to growing vegetation on AOSR reclamation sites. Not only did the fresh leaf litter provide a labile nutrient bank, it also enhanced several key soil quality parameters such as soil microbial biomass

and moisture content. With improved quality, the soil system will be better able to provide ecosystem services. This was a proof of concept study and further investigation is required to determine best management practices. Further research to determine if these preliminary results are accurate in terms of: accelerating soil organic matter development, enhancing soil quality and reclamation success, could be pursued by increasing seedling stocking density (therefore leaf litter input) on reclaimed sites.

Table 5-1: General characteristics of the four study sites used in this experiment from the Athabasca Oil Sands Region. Canopy cover data was collected in 2009 except for Older Reclaimed which was in 2008. Dashed lines indicate data was not available.

Identification	Disturbance Year	Location		Canopy		Soil		Temperature (2010-2011)		Leaf Litter Rates (gm^{-2}	
				type	cover (%)	classification (FAO)	texture	air (°C)	soil (°C)	2010	2011
Young Reclaimed	1999	N 56 57.4	W 111 43.4	aspen/spruce	38	Technosol	clay loam	1.3	4.3	53.8	143.3
Older Reclaimed	1988	N 56 94.4	W 111 73.9	aspen	23	Technosol	sandy loam	3.9	5.8	--	--
Harvested	1992	N 56 59.1	W 111 43.0	aspen/spruce	0	Albic Luvisol	loam	--	-0.9	82.6	60.7
Undisturbed	pre-1940	N 56 57.5	W 111 38.9	aspen	76	Albic Luvisol	silt loam	4.2	5.1	96.7	129.3

Figure 5-1: Site images from the installation of the soil nutrient field incubation experiment in May 2010: (A) Younger Reclaimed, (B) Older Reclaimed, (C) Harvested and (D) Undisturbed. Note pairing of mesocosms where one received the application of ^{15}N stable isotope enriched aspen leaf litter and the adjacent stayed unamended.

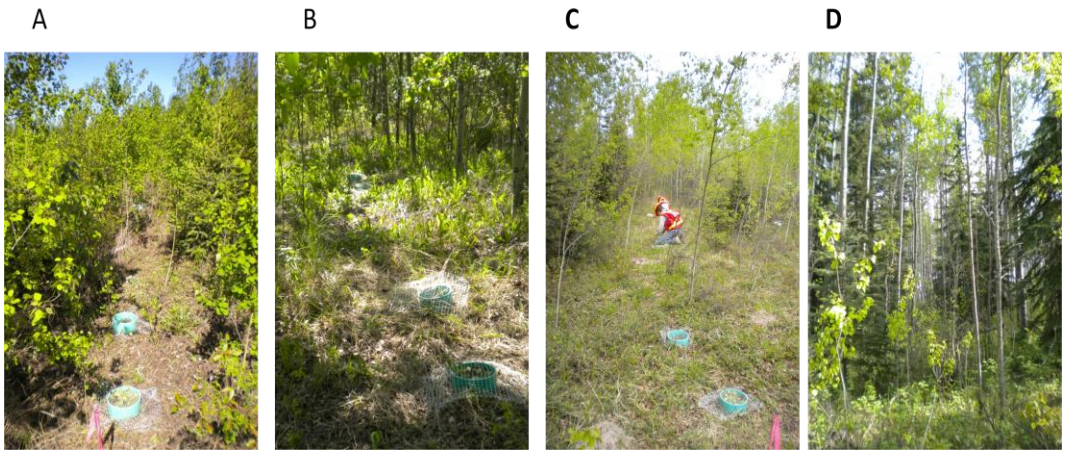


Figure 5-2: Daily average air and soil temperature values across two growing seasons (June 1 – October 1, 2010 and 2011) in the four study sites in the Athabasca Oil Sands Region (AOSR).

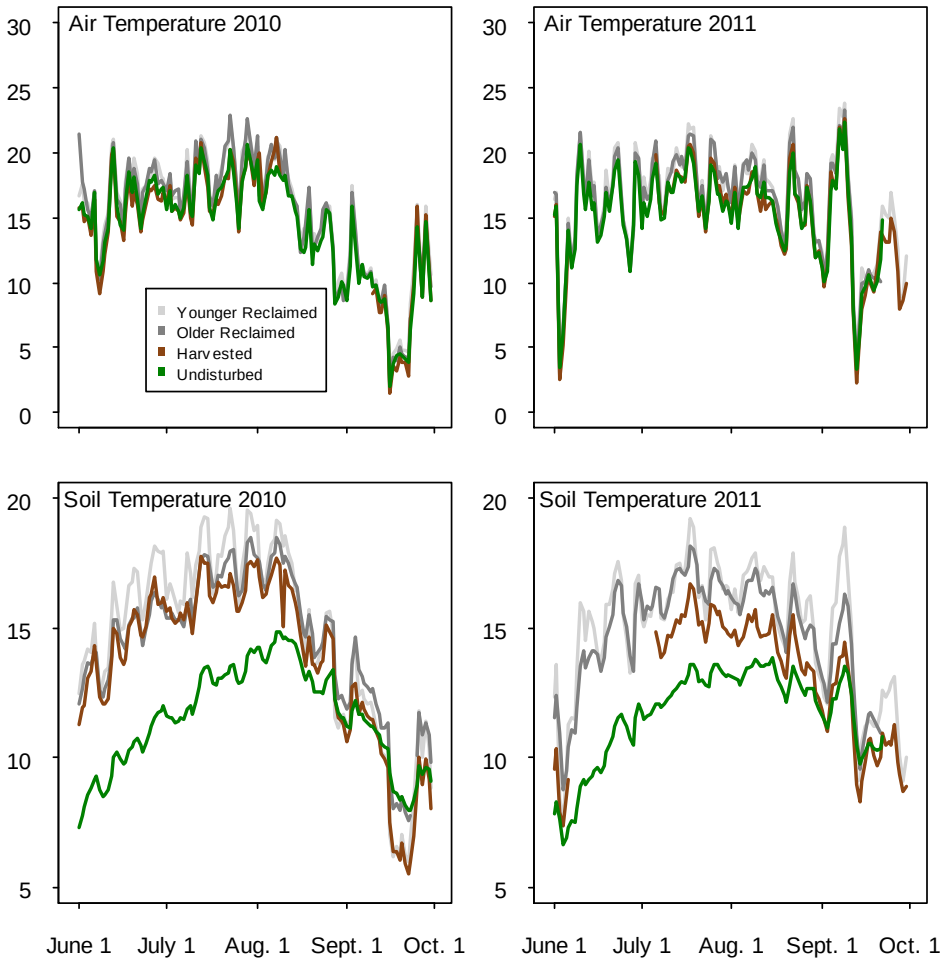


Figure 5-3: Ratio of gravimetric soil moisture (A) and soil microbial biomass carbon (B) for mesocosms amended with leaf litter to unamended mesocosms after four, 12 or 16 months of field incubation across four site types in the AOSR. Values over the dotted line at a ratio of 1 represent enrichment of the amended mesocosms. Standard deviation is indicated by the error bar with n=3 (except Harvested where n=2).

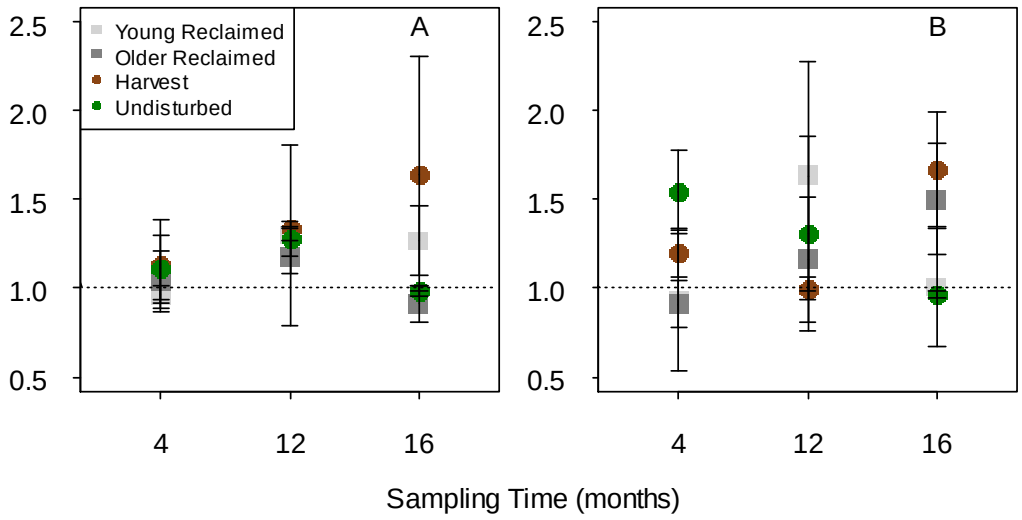


Figure 5-4: Boxplots of soil microbial biomass carbon for all mesocosms and all sampling times across four site types in the AOSR.

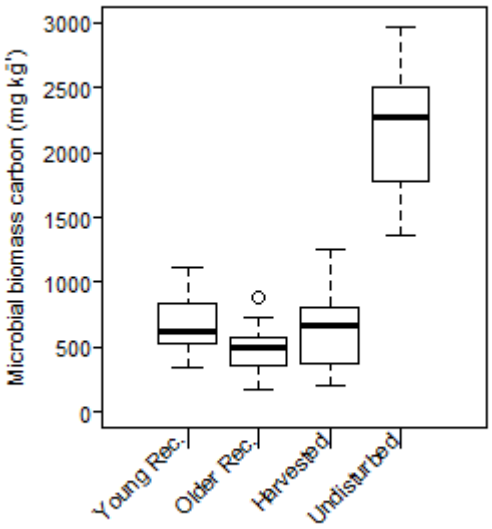
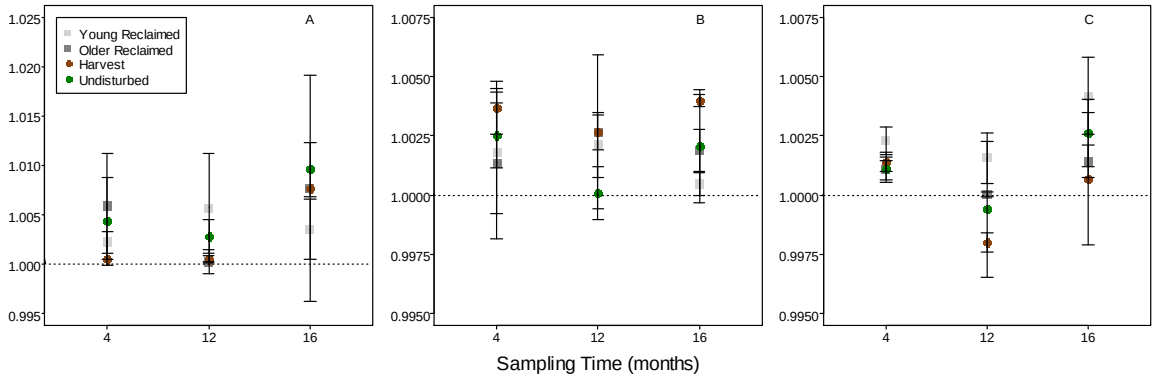


Figure 5-5: Ratio of ^{15}N atom % for live vegetation (A), soil microbial biomass (B) and roots (C) for mesocosms amended with leaf litter to unamended mesocosms after four, 12 or 16 months of field incubation across four site types in the AOSR. Values over the dotted line at a ratio of 1 represent enrichment of the amended mesocosms. Standard deviation is indicated by the error bar with n=3 (except Harvested where n=2).



References

Banwart, S. 2011. Save our soils. Nature 474, 151-152.

Bartos, D.L., DeByle, N.V. 1981. Quantity, decomposition, and nutrient dynamics of aspen litterfall in Utah. Forest Science 27, 381-390.

Bird, J.A., Torn, M.S. 2006. Fine roots vs. needles: a comparison of ^{13}C and ^{15}N dynamics in a ponderosa pine forest soil. Biogeochemistry 79, 361-382.

Carter, M.R. 2002. Soil quality for sustainable land management: Organic matter and aggregation interactions that maintain soil functions. Agron. J. 94, 38-47.

Dijkstra, P., Ishizu, A., Doucett, R., Hart, S.C., Schwartz, E., Menyailo, O.V., Hungate, B.A. 2006. ^{13}C and ^{15}N natural abundance of the soil microbial biomass. Soil Biol. Biochem. 28, 3257-3266.

Dimitriu, P.A., Prescott, C.E., Quideau, S.A., Grayston, S.J. 2010. Impact of reclamation of surface-mined boreal forest soils on microbial community composition and function. Soil Biol. Biochem. 42, 2289-2297.

Environment Canada 2013. Canadian climate normals 1971-2000, Fort McMurray.

Food and Agriculture Organization of the United Nations 2006. Guidelines for soil description. Rome. pp. 1-109.

Government of Alberta 1993. Environmental Protection and Enhancement Act: Conservation and Reclamation Regulation.

Hahn, A.S., Quideau, S.A. 2012. Long-term effects of organic amendments on the recovery of plant and soil microbial communities following disturbance in the Canadian boreal forest. Plant Soil, 1-14.

Hemstock, S.S., Quideau, S.A., Chanasyk, D.S. 2010. Nitrogen availability from peat amendments used in boreal oil sands reclamation. Can. J. Soil Sci. 90, 165-175.

Kimmins, J.P. 1996. Importance of soil and role of ecosystem disturbance for sustained productivity of cool temperate and boreal forests. Soil Sci. Soc. Am. J. 60, 1643-1654.

Lal, R. 2004. Soil carbon sequestrtion impacts on global climate change and food security. Science 304, 1623-1627.

Lal, R., Follett, R.F., Stewart, B.A., Kimble, J.M. 2007. Soil carbon sequestration to mitigate climate change and advance food security. Soil Sci. 172, 943-956.

Larney, F.J., Angers, D.A. 2012. The role of organic amendments in soil reclamation: A review. Can. J. Soil Sci. 92, 19-38.

Lousier, J.D., Parkinson, D. 1976. Litter decomposition in a cool temperate deciduous forest. Can. J. Bot. 54, 419-436.

Macdonald, E., Quideau, S., Landhäusser, S. 2012. Rebuilding boreal forest ecosystems after industrial disturbance, in: Vitt, D., Bhatti, J. (Eds.) Restoration and Reclamation of Boreal Ecosystems. Cambridge University Press. pp. 123-160.

McMillan, R., Quideau, S.A., MacKenzie, M.D., Biryukova, O. 2007. Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. J. Environ. Qual. 36, 1470-1478.

Paterson, E., Sim, A., Osborne, S.M., Murray, P.J. 2011. Long-term exclusion of plant-inputs to soil reduces the functional capacity of microbial communities to mineralise recalcitrant root-derived carbon sources. Soil Biol. Biochem. 43, 1873-1880.

Powers, R.F., Scott, D.A., Sanchez, F.G., Voldseth, R.A., Page-Dumroese, D., Elioff, J.D., Stone, D.M. 2005. The North American long-term soil productivity experiment: Findings from the first decade of research. For. Ecol. Manage. 220, 31-50.

Rowland, S.M., Prescott, C.E., Grayston, S.J., Quideau, S.A., Bradfield, G.E. 2009. Recreating a functioning forest soil in reclaimed oil sands in Northern Alberta: An approach for measuring success in ecological restoration. J. Environ. Qual. 38, 1580-1590.

Sorenson, P.T., Quideau, S.A., MacKenzie, M.D., Landhäusser, S.M., Oh, S.-W. 2011. Forest floor development and biochemical properties in reconstructed boreal forest soils. Appl. Soil Ecol. 49, 139-147.

Ste-Marie, C., Paré, D., Gagnon, D. 2007. The contrasting effects of aspen and jack pine on soil nutritional properties depend on parent material. Ecosystems 10, 1299-1310.

Swanston, C.W., Myrold, D.D. 1997. Incorporation of nitrogen from decomposing red alder leaves into plants and soil of a recent clearcut in Oregon. Can. J. For. Res. 27, 1496-1502.

Turcotte, I., Quideau, S.A., Oh, S.-W. 2009. Organic matter quality in reclaimed boreal forest soils following oil sands mining. Org. Geochem. 40, 510-519.

Voroney, R.P., Brookes, P.C., Beyaert, R.P. 2008. Soil microbial biomass C, N, P, and S, in: Carter, M.R., Gregorich, E.G. (Eds.) Soil sampling and methods analysis. CRC Press, Boca Raton.

Zeller, B., Colin-Belgrand, M., Dambrine, E., Martin, F., Bottner, P. 2000. Decomposition of ^{15}N-labelled beech litter and fate of nitrogen derived from litter in a beech forest. Oecologia 123, 550-559.

Chapter 6. Vegetation influence on soil microbial community structure

6.1. Introduction

Aspen (*Populus tremuloides* Michx.) and white spruce (*Picea glauca* (Moench) Voss) trees lead to very different soil organic matter inputs. The macromolecular chemistry of organic matter inputs to soil is known to differ in types and proportions of biopolymers depending on tissue type (e.g., needle vs. twig; Preston *et al.* 2006), plant type (e.g., lichen vs. jack pine; Norris *et al.* 2011) and environmental conditions (Donaldson *et al.* 2006). Common biopolymers in vegetation include: carbohydrates, lignins, tannins and waxes (Kögel-Knabner 2002). Generally proteins and soluble sugars are readily consumed followed by carbohydrates and more complex compounds (Baldock *et al.* 1997). An increase in macromolecular structure or complexity (e.g., lignin) has been linked to increased stability, or residence time, of the organic carbon contained in the soil (Preston *et al.* 2000). Macromolecular chemistry of spruce litter was noted to have a greater proportion of more complex aromatic carbon than aspen (Preston et al. 2000). These source differences would then be expected to be reflected in both the microbial community and the soil organic matter; indeed this has been demonstrated for both aspen and spruce forest stands (Hannam et al. 2006; Hannam et al. 2004).

In fine textured upland soils from the western Canadian boreal forest, where aspen, spruce or a mixture of the two often co-exist, microbial communities differ between aspen and spruce stands, although mixed stands host a microbial community that more closely resembles that of a pure spruce stand (Hannam et al. 2006). Resolution of these community structures by Hannam et al. (2006) was achieved by extracting and characterizing the soil phospholipid fatty acids (PLFAs), a technique which determines soil microbial community abundance and structure (Frostegård *et al.* 2011). Utilization of this technique has demonstrated that the soil microbial community is influenced in quantity and composition by the above-ground vegetation in natural forests from across the globe (Brockett et al. 2012; Grayston and Prescott 2005; Priha et al. 2001; Ushio et al. 2008).

Indeed, microbial communities vary across the landscape; they may also change with time within a particular location. Primary succession from glacial retreat leads to changes in the microbial community (Hahn 2012) as does secondary succession from fire (Williams *et al.* 2012), harvest (Mummey *et al.* 2010) or reclamation (Hahn and Quideau 2012). To determine the influence of vegetation on soil microbial community development, one approach is to use constructed biosequences, i.e; lysimeter installations, as was done at the San Dimas Experimental Forest. Research from this site demonstrated that of the two tree types, oak and pine, oak had a greater litterfall rate (Quideau *et al.* 1996) and, correspondingly, a greater carbon and nitrogen concentration (Quideau *et al.* 1998). With different quantities and qualities of organic matter inputs, one would expect the soil microbial communities to differ and this may be inferred in part from the different soil organic matter turnover rates (Quideau *et al.* 2001).

Stable isotope compound specific analysis of biomarkers combined with stable isotope tracers provides a means to further probe the influence organic matter inputs have on soil microbial community structure. For example, in early work with PLFAs, Boschker et al. (1998) determined that the sulphate-reducing bacteria, similar to a Gram-positive bacteria and not the more widely studied Gram-negative, consumed a uniformly labelled ^{13}C acetate substrate in a marine sediment environment by comparing the ^{13}C enrichment levels of different representative PLFAs. More recently the technique has been used to investigate soil microbial community response due to the addition of ^{13}C-glucose in terms of: recycling of the label (Ziegler et al. 2005), different soil types (Brant et al. 2006; Dungait et al. 2011), and priming (Garcia-Pausas and Paterson 2011). Reviews on the subject of compound specific stable isotope analysis have been published for several different areas: agricultural research (Amelung et al. 2008), soil food webs (Ruess and Chamberlain 2010), soil microbial ecology (Maxfield and Evershed 2011), general soil science (Glaser 2005), and touched upon in terms of plant ecology (Dawson et al. 2002). However, except for a few cases (e.g., Moore-Kucera and Dick 2008; Rubino et al. 2010) there appears to be a dearth of field scale studies utilizing complex organic matter substrates.

Results from ecosystem succession studies indicate that microbial communities adjust to changing organic matter inputs (Hahn 2012; Hahn and Quideau 2012; Mummey et al. 2010; Williams et al. 2012). With the many existing new and varied pressures on the environment, such as vegetation shifts, what is of most interest is to quantify how quickly organic matter inputs may influence soil microbial communities, and if the soil nutrient status is maintained. To answer this question, we established a field experiment on two stand types (Aspen and Spruce) utilizing double labelled (^{15}N, ^{13}C) aspen leaf litter as the organic matter input. Labelling leaf litter with ^{15}N provided a stable isotope tracer to determine the nutrient functioning of the system, while ^{13}C provided a means of assessing the active microbial community by compound-specific stable isotope analysis of the PLFA biomarkers.

6.2. Methods

6.2.1. Leaf litter input

Enriched aspen (*Populus tremuloides* Michx.) leaves were collected from seven year old saplings growing in the open at the University of Alberta's Ellerslie Research Farm, Edmonton, Alberta. Double labelling of leaves was by a spray application of 0.207 M of N using 60.0 atom % $K^{15}NO_3$ (Aldrich Chemicals) on June 11, 2009 and then once a week with ^{13}C labelling from July 10 to July 31. Carbon-13 labelling was by application of 100 ml of 99.9 % ^{13}C CO_2 gas to a selected tree branch enveloped in a Mylar tubular bag (VacPac®, Baltimore MD) 36 cm in diameter but varied in length depending on branch length. Trees were left to take up the $^{13}CO_2$ for half an hour after which the bags were removed. The saplings were harvested on August 31, 2009 and the leaves were immediately removed, air dried and stored at room temperature. Isotopic analyses indicated that the leaf litter was enriched with $\delta^{13}C$ 23.1 and $\delta^{15}N$ 111.3 ‰ (standard deviation 2.6 and 4.8 ‰, respectively).

6.2.2. Field sites

This field experiment consisted of two mature forest stands (greater than 70 years old) with one site dominated by a canopy of trembling aspen (Aspen) and the second site of white spruce (Spruce; Table 6-1). The sites were located within 3 km of each other in the same continuous western boreal forest landscape in northern Alberta. There is an average of 69 frost-free days, an annual temperature of 0.7 °C and precipitation of 455 mm in northern Alberta (Environment Canada 2013). These conditions inhibit organic matter decomposition and therefore support the development of a thick forest floor (Aspen 7 cm and Spruce 19 cm; Table 6-1). Both stands were growing on Gray Luvisol soils (Soil Classification Working Group 1998) which had developed from glaciolacustrine sedimentary parent material. Both of these fine textured soils supported a wide diversity of understory vegetation, as surveyed by Hahn and Quideau (2012), including: low bush cranberry (*Viburnum edule* Michx.), rose (*Rosa* sp.), green alder (*Aldus crispa* (Ait.) Pursh), buffaloberry (*Sheperdia canadensis* (L.) Nutt.), hairy wild rye (*Elymus innovates* (Beal) Pilg.), bunchberry (*Cornus canadensis* L.), wild sarsaparilla (*Aralia nudicaulis* L., Araliaceae), dewberry (*Rubus pubescens* Raf.) and fireweed (*Epilobium angustifolium* L.) with stair-step moss (*Hylocomium splendens* (Hedw.)), Scheber's moss (*Pleurozium schreberi* (Brid.) Mitt), and knight's plume moss (*Ptilium crista-castrensis* (Hedw.)) on the Spruce site.

Each site was monitored for air (10 cm above-ground) and soil (10 cm below-ground) temperature with a HOBO Micro Station (Onset Computer Corporation, Bourne, MA). On each site, three plots were established roughly 5 m by 5 m and at least 10-15 m apart. However one plot on the Spruce site was destroyed during the first summer due to rodent interference. Each plot consisted of four sets of paired mesocosms where each pair was less than 1 m apart and within 1 m of a dominant tree trunk. Each mesocosm was delineated by a cylinder constructed of SDR 35 PVC plastic to a diameter of 20 cm and a length of 11 cm inserted about 7 cm into the ground. Enriched aspen leaves (30.0 g) were added to one mesocosm of each pair in May 2010. Each mesocosm was then covered with 50 cm by 50 cm of 1.5 cm by 2.0 cm plastic mesh netting fixed by stakes. Field sampling occurred three times: initial (May 2010), 4 months

(September 2010), and 16 months (September 2011) after installation of the field incubation experiment. At each sampling time a pair of mesocosms from each plot was destructively collected for organic matter pools: live vegetation (live), fresh litter (L) and fibric (F) horizon (litter), soil (to 5 cm below the fibric horizon; soil) and soil microbial community. Soil microbial community samples were stored at -20 °C while all other samples were stored at 4 °C until transported, within 5 days, to the lab where the microbial samples were freeze dried, the vegetation was immediately air dried and soil humus was sieved to 4 mm, subsampled for roots (roots), air dried and stored.

6.2.3.General laboratory analyses

Subsamples of the organic matter pools were finely ground using either a Retsch MM200 ball mill grinder (Retsch Inc. Newtown, USA) or a mortar and pestle for analysis. Measurement of total organic carbon and nitrogen was by dry combustion on a Costech ECS 4010 Elemental Analyzer equipped with a thermoconductivity detector (Costech Analytical Technologies Inc. Valencia, USA), and for ^{13}C and ^{15}N isotopic composition by coupling the Costech ECS 4010 to a Finnigan Deltaplus Advantage Isotopic Ratio Mass Spectrometer (IRMS; ThermoFinnigan, Bremen, Germany). Results were expressed in the δ-notation, part per thousand variations from the standard Pee Dee Belemnite.

6.2.4. Phospholipid fatty acid analysis

Soil microbial community samples (0.30 g) were extracted with a 0.15 M citrate buffer (Frostegård et al. 1991) using the Bligh and Dyer (1959) methanol:chloroform:buffer extraction ratio of 1:1:0.9. Separation of the extracts was performed on SPE columns (Agilent Technologies, Santa Clara, USA) of 500 mg of silica in 6 ml tubes (Zelles and Bai 1993). A modified procedure from White and Ringelberg (1998) was used to synthesize fatty acid methyl esters; PLFAs were re-dissolved in 1:1 chloroform:methanol, a mild alkaline methanolysis, and then extraction with hexane. Prior to the first extraction a surrogate standard of 19:0 (1,2-dinonadecanoyl-sn-glycero-3-phosphocholine,

Avanti Polar Lipids Inc, Alabaster, USA) was added to the sample and before analysis an instrument standard of 10:0Me (methyl decanoate, Aldrich, St. Louis, USA) was added to the extract (Bird et al. 2011). Identification and quantification of fatty acid methyl esters was on an Agilent 6890 Series capillary gas chromatograph (GC; Agilent Technologies, Santa Clara, USA) with a 25m Ultra 2 column (Crosslinked 5 % PhMeSilicone) using the Sherlock Microbial Identification System Version 4.5 software (MIDI, Inc., Newark, USA). Samples were then analyzed for compound specific stable isotope ratio via a CTC Combi PAL joined to a 6890N Agilent GC with a 50 m Ultra 2 column linked to a Thermo Finnigan GC Combustion III (Thermo Finnigan, Bremen, Germany) and the IRMS.

PLFA biomarkers greater than 0.5 % in abundance and between 12 and 20 carbon units in length were included in the analysis. Abundance of individual PLFAs in each sample was expressed as nmol PLFA g^{-1} dry soil and as nmol % of total microbial biomass after adjusting the molar concentrations for both the instrument and surrogate standards. Total microbial biomass was determined by summing the concentration of all biomarkers included in the analysis. The PLFA nomenclature used here follows standard formatting (Maxfield and Evershed 2011).

6.2.5. Data analysis

Environmental conditions on Aspen and Spruce sites were recorded with the HOBO Micro Stations every 5 minutes through the summer and every 10 minutes for the winter. Daily air and soil temperature averages were calculated from the data in R (version 2.15.1, the R Foundation for Statistical Computing). R was used to generate the non-ordination figures and to calculate sample means, standard deviations and t-tests. However, due to the lack of site replication, variability within site, temporal factors and missing data points, few other statistics were performed on this data set. Ordination of the soil microbial community structure was determined using Non-metric MultiDimensional Scaling (NMDS) on Hellinger transformed PLFA data (Legendre and Gallagher 2001)

with a Sorenson (Bray-Curtis) distance measurement (McCune and Grace 2002) with the PC-ORD software version 5 (MjM Software Design, Gleneden Beach, USA). Variation in the ordination expressed along the axis was explained by correlation with the secondary matrix of environmental variables (including site, collection time, soil, treatment and PLFA concentrations). Overall, points closer together in the ordination indicated more similar data than points further apart. These grouping patterns were tested for categorical variables by Multi-Response Permutation Procedures (MRPP) for overall and pair-wise comparisons, a non-parametric test, which yielded three values: an overall significance (p), a term indicating the degree of separation between groups (T), and the separation within groups (A). Stronger differences between groups yield a more negative T values while A values closer to 1 indicate greater homogeneity within groups.

6.3. Results

6.3.1. Site conditions

The Aspen and Spruce stands were both located on fine textured Luvisolic soils and both soils had a well developed forest floor; the forest floor of the Spruce site was twice as deep as that of the Aspen site (Table 6-1). Air and soil temperatures for both sites were monitored across the second growing season of the 16 month field incubation experiment (Figure 6-1). Results followed the same trend as those recorded at a nearby weather station (Fort McMurray, AB) within 50 km (see Undisturbed in Chapter 5). Air temperatures in the Spruce stand were slightly cooler than in the Aspen while the soil temperatures in Spruce were on average 2 degrees cooler than in Aspen (Figure 6-1).

6.3.2. Nitrogen cycling

The addition of ^{15}N as a stable isotope tracer to the aspen leaf litter allowed for a coarse assessment of the nitrogen cycling within the stand. Results indicate that the vegetation growing within the mesocosms was enriched in ^{15}N for both sampled roots and above-ground live vegetation (Table 6-2). The tracer material was incubated in the field across two growing seasons and after the first

growing season the live vegetation growing within the amended mesocosms indicated an enrichment of about 3 ‰ compared to the unamended pairs. Roots were enriched by about 1 ‰ after both the first and second growing seasons. Spruce samples were interesting as they indicated a greater enrichment of their roots with the addition of labelled litter compared to the Aspen samples (Table 6-2). Roots were coarsely subsampled from the soil and bulked for analysis. Vegetation growing within the mesocosms was also bulked and treated as one homogeneous pool although it consisted of a variety of plants, including mosses, herbs, grasses and even small shrubs. We acknowledge the limitations of bulking the vegetation as differences in species nitrogen utilization, biomass and local availability could cause variation in the isotopic signature and may account for some of the variability in the reported $\delta^{15}N$ of both the amended and unamended mesocosms. One mesocosm did not contain live vegetation and eight contained less than 0.5 g, therefore, combined with low overall replication, it was difficult to quantify treatment effect from the variability within the data. Yet, trends in the data were present with both live vegetation and roots increasing in $\delta^{15}N$ after the growing season compared to their respective, paired mesocosm.

6.3.3. Soil microbial community structure

Quantitative differences in the soil microbial biomass (as determined by the PLFA procedure) were not evident between Aspen and Spruce sites nor were they apparent between amended and control mesocosms (Table 6-3). Soil microbial biomass ranged from a low of 4.0 $\mu molg^{-1}$ in a Spruce initial sample to a high of 5.1 $\mu molg^{-1}$ in the 16 month Aspen amended mesocosm. The effect of sampling time was evident when the PLFA data were examined by NMDS ordination and tested for grouping patterns. Ordination on the PLFA data yielded a 3 dimensional solution with a stress of 12.1 after 53 iterations. The first axis only accounted for 6 % of the variation while the second and third represented 15 % and 66 % respectively; hence, the PLFA data are presented with respect to the second and third axes of the ordination in Figure 6-2. Separation of data along axis 3 was explained by changing concentrations of the 16:1ω11, and Sum 5

biomarkers from correlation to the secondary matrix of PLFA concentration (r^2= 0.60). Testing of the grouping variables (site, collection time, soil plot and treatment) was performed by MRPP with results presented in Table 4. Both site (Aspen vs Spruce) and collection time (initial vs 4 vs 16 months) were significant. To illustrate the importance of collection time on the soil microbial community structure in this experiment, Figure 6-2a was coded by color for collection time and by symbol shape for site. In this way the individual grouping of the three collection times becomes clear. Testing of the ordination for soil plot was to determine if differences were evident between the plots on the individual sites. While soil plot was significant, the differences were larger between sites than within a given site (Table 6-4). Figure 6-2b presents the same data as 6-2a but coded by treatment i.e., accounting for the addition of leaf litter. Treatment was also found to be a significant grouping factor of the ordination (Table 6-4), but the differences were not significant between Aspen paired mesocosms; there was insufficient data to determine differences for Spruce (n =2).

The structure of the soil microbial community was distinct between Aspen and Spruce sites after two growing seasons (MRPP; T=-3.73, A=0.22, $p<0.01$) but the addition of leaves appeared to drive the treated communities to a common, or more similar, structure (Figure 6-3). Correlations with the secondary matrix of PLFA concentration (r^2= 0.65) indicated that biomarkers of Sum 5 and cy19:0ω8c explained data variation along axis 2 with both increasing towards the top of the ordination and the Aspen samples. Unfortunately, with the loss of one Spruce plot during the first growing season, there was insufficient replication for statistical analysis on the grouping of the treated samples. However, data may be assessed qualitatively by examining the ordination patterns of the mesocosm pairs. To facilitate interpretation of the ordination, points were labelled with their plot identification (Aspen – A, B, C; Spruce – D, E). After 16 months of field incubation across two growing seasons, the treated Spruce mesocosms were both pushed away from the untreated mesocosms on a similar trajectory and were clustered towards the treated Aspen mesocosms. While significance cannot be ascertained, the results indicated that litter amended mesocosms may be

developing more similar microbial community structures compared to unamended mesocosms.

Our PLFA identification procedure, Sherlock Microbial Identification System, did not resolve between the two peaks of 18:2ω6,9c and *a*18:0. The PLFA 18:2ω6,9c is a recognized fungal biomarker while *a*18:0 is regarded as a biomarker for Gram positive bacteria (Ruess and Chamberlain 2010). The unresolved 18:2ω6,9c /*a*18:0 peak was found in all of our samples, along with 18:1ω9c which has been identified as a positively correlating fungal biomarker to 18:2ω6,9c (Frostegård et al. 2011) and a third marker, 18:3ω3,6,9c, was extracted from the majority of Aspen and Spruce soils and has been categorized as originating from higher fungi (Ruess and Chamberlain 2010). With the multiple fungal indicators present, we believe that it was the 18:2ω6,9c biomarker which was present in Sum 5.

6.3.4. Soil microbial community function

Determining which components of the microbial community utilized the added litter as a carbon source was assessed by compound specific analysis of the PLFA results. Those lipids which were enriched in the ^{13}C tracer indicated utilization of the added leaf litter input (Figure 6-4a and b). As noted in Figure 6-2 and 6-3 there were differences in soil microbial structure, which derived from different PLFAs being present in different samples i.e., not all samples have the same biomarkers. In addition, some biomarkers co-eluted during GC-C-IRMS analysis and could not be resolved from adjacent peaks while some biomarkers were not sufficiently abundant to be detected by the instrument. Therefore, of the 35 PLFAs extracted and characterized for community structure, 13 are reported here for their isotopic composition. These 13 biomarkers were generally sufficiently common and concentrated to allow for comparisons among treatments.

Compound specific analysis of the PLFA biomarkers indicated $\delta^{13}C$ enrichment with the addition of labelled aspen leaf litter (Figure 6-4). Unamended Aspen and Spruce mesocosm soil biomarkers ranged from $\delta^{13}C$ –34 to –22 ‰.

Enrichment in ^{13}C of the biomarkers occurred 4 months after the addition of the labelled litter to the Aspen samples, and further increased after 16 months. As with Aspen, all Spruce biomarkers were enriched in ^{13}C after 4 months; however, for Spruce, there was an apparent enrichment in only four biomarkers after 16 months. In addition to greater numbers of enriched biomarkers, Aspen biomarkers tended to be enriched more than Spruce biomarkers relative to their respective controls. Common between the two sites was a strong enrichment in the 10me18:0 PLFA. The three other biomarkers for Spruce samples which indicated enrichment were: *i*16:0i, *a* 17:0 and Sum 5 (18:2ω6,9c) (Figure 6-4b).

6.4. Discussion

Evidence of ^{13}C stable isotope tracer enrichment in the soil microbial community, due to the addition of labelled leaf litter, was from the shift in $\delta^{13}C$ signature of some individual PLFAs (Figure 6-4a and b). Increased quantities of ^{13}C in PLFAs from mesocosms amended with the labeled litter as compared to control mesocosms indicated that the soil microbial community readily utilized the vegetation input. Biomarker enrichment occurred in both Aspen and Spruce samples after one growing season. After a second growing season, Aspen biomarkers were further enriched while Spruce samples did not seem to change. Greater enrichment of the PLFA biomarkers in Aspen samples compared to those from the Spruce site could have been caused by several factors. First, a decreased rate of microbial processes due to cooler temperatures in the Spruce site (Figure 6-1). Sampling procedure for PLFA was 0-5 cm below the litter and fibric (LF) horizons of the forest floor, a forest floor which was deeper on the Spruce site (Table 6-1); therefore, one could expect it would take longer for inputs to the surface of the forest floor to move downwards to the sampling region. Finally rodent interference during the first growing season caused a loss of labelled material from most of the mesocosms on the Spruce site, which would therefore decrease the input of stable isotope tracers.

Of the Spruce biomarkers found within the labelled mesocosms, it was 10me18:0, 18:2ω6,9c and *a*17:0 which were greatly enriched compared to their

paired controls. Aspen mesocosms amended with the labelled litter were also greatly enriched in the 10me18:0 biomarker compared to their paired controls. This PLFA biomarker, 10me18:0, has been attributed to actinomycetes (Zelles 1999); filamentous bacteria common to aspen stands of western Canada (Jayasinghe and Parkinson 2008; Swallow et al. 2009). While 18:2ω6,9c has been defined as a fungal biomarker (Frostegård and Bååth 1996), the remaining PLFA biomarkers present are believed to represent the bacterial community (Ruess and Chamberlain 2010; Zelles 1999). Moore-Kucera and Dick (2008) also noted an enrichment of 18:2ω6,9c PLFA, along with several other PLFAs, after their field incubation of labelled needles and roots. In a separate study, PLFAs representing fungi, actinomycetes, and bacteria were all observed to be enriched relative to their controls 11 months following the addition of ^{13}C enriched poplar litter to the soil surface (Rubino et al. 2010). These results are in contrast to those reported by Kramer et al (2010) who reported that the addition of ^{14}C labelled organic matter to a constructed soil mesocosm was not significantly incorporated into PLFAs of the soil microbial community. As each study had slightly different conditions (litter input, soil microbial community studied and climate) these different results cannot be readily explained. For the organic matter inputs, Kramer et al (2010) collected vegetation in the study area (i.e., grown under natural environmental conditions) and litter for this study was grown under natural conditions but outside our study area while in other two studies (Moore-Kucera and Dick 2008; Rubino et al. 2010) the labelled plants were grown under more controlled conditions. The soil microbial community investigated in two studies was of the mineral soil (Kramer et al. 2010; Rubino et al. 2010) while our study and Moore-Kucera and Dick (2008) examined the community from within the forest floor to forest floor mineral interface. In terms of climate, one study under a Mediterranean climate (Rubino et al. 2010), two under temperate (Kramer et al. 2010; Moore-Kucera and Dick 2008) and this study in the boreal forest. Therefore the lack of incorporation of isotope tracers by the soil microbial community in the Kramer et al (2010) study compared to the other three cannot readily be explained except for, perhaps, differences between using ^{13}C and ^{14}C to trace carbon fluxes

in soils or functional differences within the soil microbial community investigated.

As expected, the soil microbial composition differed between the Aspen and Spruce sites (Figure 6-2 and Table 6-4). This clear difference between Aspen and Spruce soil microbial communities in the boreal forest of western Canada has been noted by several authors (Hahn and Quideau 2012; Hannam et al. 2006; Norris et al. 2013; Swallow et al. 2009). Our study was initiated and sampling occurred at the start of the growing season, and again after one and two growing seasons. Each sampling time was determined to be unique in terms of the soil microbial structure (Table 6-4); therefore, our study adds to the growing understanding of the importance of sampling time and the differences due to seasonality (e.g., Myers *et al.* 2001). Shifts in composition have previously been noted due to soil moisture content (Hahn and Quideau 2012) and soil temperature (Waldrop and Firestone 2004); both of which strongly relate to seasonal variation and therefore would be directly impacted by collection time.

Overall differences in soil microbial structure due to the addition of the labelled litter were not evident (Table 6-4). We expected that the PLFA signature of the Aspen samples would not be altered with the addition of aspen leaf litter, while the Spruce samples would become more similar to the Aspen samples with time. While, as expected, the Aspen soil microbial community structure did not change, we also could not determine changes in the Spruce community due to the loss of replication from rodent interference. Application of labelled poplar leaves to mineral soil in a poplar stand was also not observed to change the soil microbial biomass population (Rubino et al. 2010). Yet after 16 months of field incubation across two growing seasons (Figure 6-3), one could argue that the treated Spruce mesocosms were becoming more similar to the Aspen samples, in particular the Spruce D plot. Although this hints at our expectation of changing vegetation influence on changing soil microbial community composition this is of course speculative as the data lacked sufficient replication.

Instead of adding organic matter inputs to investigate changes to soil microbial communities, researchers might use reciprocal transfer studies which

transplant material from one location to another (often of different vegetation or climate). Reciprocal transfer studies of mineral soil have reported changes to soil microbial community structure changes which were attributed to water availability (Zumsteg *et al.* 2013) and vegetation influence (Bottomley *et al.* 2006). However, in a reciprocal transfer study of aspen and spruce forest floor no changes in the soil microbial community structure were evident after one year (Hannam *et al.* 2007). The authors hypothesized that the absence of a shift in the community was due to the short experimental time and high carbon content of the transplanted material. Combining a reciprocal transfer study of oak and grassland soils with the addition of a stable isotope labelled labile substrate in a short term incubation allowed determination of not only changes to the structure but also the function of the soil microbial communities (Waldrop and Firestone 2006). Our results, utilizing stable isotope enriched litter applied to the forest floor of Aspen and Spruce stands, illustrated the seasonal changes in the soil microbial community structure, for example the increased concentration of 18:2ω6,9c from the initial to the 16 month collection (Figure 6-2), and showed how different components of the community utilized the labelled litter, for example how a greater enrichment of 18:2ω6,9c and 10me18:0 occurred in the Spruce samples (Figure 6-4b).

Community composition differed between the Aspen and Spruce soils, yet their total biomass, as determined by total PLFAs, was comparable (Table 6-3). Previously Aspen and Spruce sites have been noted to show differences in soil microbial biomass for both their forest floors (Hannam et al. 2006) and underlying mineral soils (Norris et al. 2013). Compared to the mineral soil microbial biomass quantities reported by Norris et al (2013), using similar methodology, values reported here for the soil (0-5 cm below the fibric layer of the forest floor) were about ten times higher. Results were more similar to those of forest floor concentrations reported by Hannam et al (2006) but due to methodological differences they were not directly comparable.

Aspen and Spruce sites in this study had similar air temperatures 10 cm above their forest floor but Spruce was much cooler in temperature 15 cm into the

mineral soil (Figure 6-1). Differences in mineral soil temperatures were likely due to the insulation provided by the thicker forest floor on the Spruce site (Table 6-1). Aspen stands have previously been observed to have warmer soils (Macdonald and Fenniak 2007) and shallower forest floors (Hannam et al. 2006) than spruce dominated stands. In a compositional gradient from black spruce to aspen stands, a gradual decrease in forest floor thickness and soil moisture was observed (Laganière *et al.* 2009). Linked to warmer and drier conditions, greater amounts of litter decay were observed on aspen forest floors compared to spruce (Flanagan and Van Cleve 1983). However, the boreal climate overall decreases decomposition rates, versus the temperate or tropical climate, as illustrated by soil organic matter which, for the boreal, more closely resembles organic matter inputs (Vancampenhout *et al.* 2009). As decomposition is the limiting step in nutrient cycling of the boreal forest, the ability of the soil microbial community to change under shifting vegetation inputs, and make available nutrients within the forest floor, is critical for ecosystem health.

Of the nutrients required for plant growth, it is nitrogen which is often limiting in the western boreal forest (Vitousek and Howarth 1991). With the differences in biomolecular composition between Aspen and Spruce organic matter inputs (Preston et al. 2000) and their forest floor (Hannam *et al.* 2005), we were interested in knowing if nitrogen cycling would be impacted on the Spruce site after the addition of aspen leaf litter. The addition of ^{15}N as a stable isotope tracer to the aspen leaf litter allowed for a general assessment of the nutrient cycling within the stands. Results showed that ^{15}N from the addition of labelled aspen leaf litter was incorporated in the growing vegetation on both Aspen and Spruce sites after the first and second growing season (Table 6-2). Not only the above-ground live vegetation but also the coarse subsampling of roots was enriched in ^{15}N after one and two growing seasons (Table 6-2). Differences between the paired treated and untreated mesocosms were difficult to determine due to: variability in the quantity of vegetation present, proximity of vegetation to added leaves, uniformity of enrichment in the leaves, overall enrichment level of the leaves and the possible differences in the utilization pathway of nitrogen

uptake. These results were in agreement with a previous study of aspen and spruce soil microbial function (Norris et al. 2013) which indicated redundancy within the community to labile organic matter cycling on mineral soil. Despite their qualitative nature, these results indicate that the nitrogen tracer, used here to assess nitrogen release from recent litter inputs was readily available in both stand types.

6.5. Conclusion

It has been demonstrated that aspen and spruce stand types have distinctive characteristics in terms of, for example, understory vegetation (Kembel and Dale 2006), spiders (Pinzon *et al.* 2011) and forest floor microbial communities (Hannam et al. 2006). However, a previous reciprocal transfer study to determine if the soil microbial community would shift in composition with changes in organic matter inputs found no change in the community after one year (Hannam *et al.* 2007). This experiment, while limited both in stand level and site level replication, demonstrates that with identical leaf litter inputs, the microbial community structure of the two stands may become more similar while also remaining active in nitrogen cycling after two growing seasons. This indicates that the macromolecular chemistry of the litter input does have a significant role in determining the composition of the soil microbial community.

Table 6-1: General Aspen and Spruce site characteristics.

Site	Classification	Canopy	Location	Stand Age	Forest Floor	Soil Texture (%)			Textural class	pH	TN	TOC	^{13}C
				(years)	(cm)	Sand	Silt	Clay			(%)	(%)	(atom %)
Aspen	Gray Luvisol	aspen	N 56 57.5 W 111 38.9	>70	7	35	55	10	silt loam	4	0.07	1.20	1.0752
Spruce	Gray Luvisol	spruce	N 56 56.6 W 111 44.3	>70	19.5	30	56	14	silt loam	5	0.07	1.09	1.0756

Table 6-2: $\delta^{15}N$ values of live above-ground vegetation and below-ground root samples from Aspen and Spruce sites across a 16 month field incubation study with mesocosms ammended with stable isotope ^{13}C and ^{15}N enriched aspen leaves and unammended controls. p values determined by a paired t-test. Dots indicated insufficient data.

		Aspen			Spruce		
	months	control	leaves	p	control	leaves	p
Live	0	-2.0 (0.5)	-3.1 (0.7)	.	0.2 .	-1.6 (1.6)	0.95
	4	-0.7 (0.4)	3.2 (2.4)	0.10	-0.8 (1.4)	1.9 (0.6)	0.13
	12	-2.2 (1.3)	1.7 (2.3)	0.16	-1.4 (1.0)	-1.8 (2.0)	0.89
	16	0.2 .	9.5 (9.1)	.	-1.8 (0.9)	1.1 (5.3)	0.52
Roots	0	-2.7 (0.2)	-2.4 (1.4)	0.71	-2.5 (0.6)	-3.7 (0.5)	0.02
	4	-2.0 (0.7)	-1.7 (0.4)	0.26	-3.3 (0.9)	-2.4 (0.2)	0.45
	12	-1.9 (0.2)	-2.1 (1.2)	0.83	-3.3 (1.7)	-2.1 (0.8)	0.32
	16	-0.8 (1.8)	0.0 (0.9)	0.64	-2.3 (0.2)	-0.8 (1.0)	0.33

Table 6-3: PLFA quantities (µmolg^{-1}) from Aspen and Spruce sites across a 16 month field incubation study with mesocosms ammended with stable isotope ^{13}C and ^{15}N enriched aspen leaves and unammended controls.

	Aspen		Spruce	
months	control	leaves	control	leaves
0	4.1 (0.1)	4.7 (0.4)	4.6 (0.4)	4.0 (0.9)
4	4.2 (0.9)	4.6 (0.8)	4.4 (1.0)	4.2 (0.9)
16	4.8 (0.5)	5.1 (0.4)	5.0 (1.1)	4.6 (0.3)

Table 6-4: MRPP results on NMS ordination proportional PLFA data from Aspen and Spruce sites across a 16 month field incubation study with mesocosms ammended with stable isotope 13C and 15N enriched aspen leaves and unammended controls (Figure 2). Dots indicated insufficient data.

	T	A	*p*
Group (α=0.05)			
Site	-3.17	0.06	<0.01
Time	-3.58	0.22	<0.0001
Soil	-3.66	0.18	<0.01
Treatment	-3.69	0.31	<0.0001
Pairwise Comparison			
Time (Bonferroni adjusted α=0.02)			
initial vs 4 months	-1.88	0.05	<0.05
initial vs 16 months	-8.28	0.26	<0.0001
4 vs 16 months	-6.01	0.19	<0.001
Soil (Bonferroni adjusted α=0.01)			
Aspen- plot A vs B	-0.91	0.05	0.2
Aspen- plot A vs C	-0.32	0.02	0.3
Aspen- plot B vs C	0.76	-0.05	0.8
Spruce- plot D vs E	-1.99	0.11	0.04
Treatment of control vs leaves (Bonferroni adjusted α=0.008)			
Aspen- initial	0.77	-0.1	0.8
Aspen-4 months	1.31	-0.14	0.9
Aspen-16 months	0.43	-0.03	0.7
Spruce-initial	1.05	-0.13	0.9
Spruce-4 months	-1.22	0.3	.
Spruce-16 months	0.34	-0.1	.

Figures

Figure 6-1: Daily average air and soil temperature values across the second growing season (June 1 – October 1, 2011) in the Aspen and Spruce study sites.

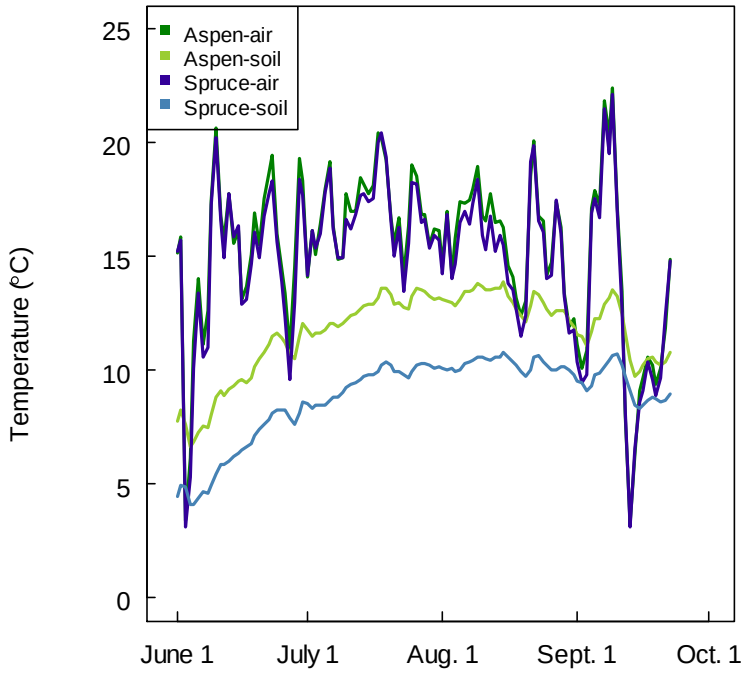
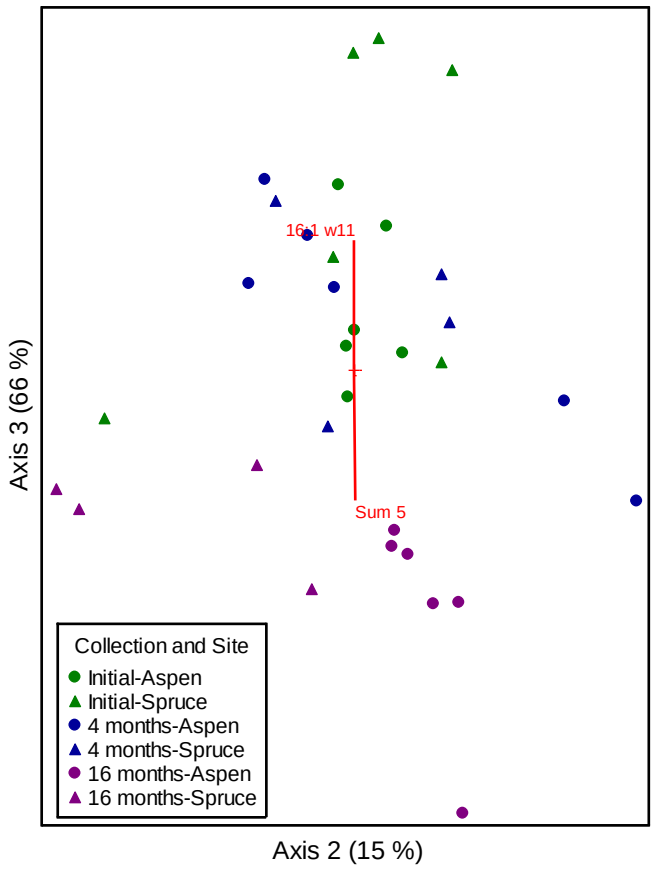


Figure 6-2: Microbial community structure as presented by an ordination of Aspen and Spruce soil extracted PLFA biomarkers from a leaf litter amended mesocosm 16 month field experiment. Ordination (a) and (b) are identical but for categorical identification of the data. The first (a) is presented to highlight the influence of sample collection time and site while the second (b) illustrates treatment results.

a.



b.

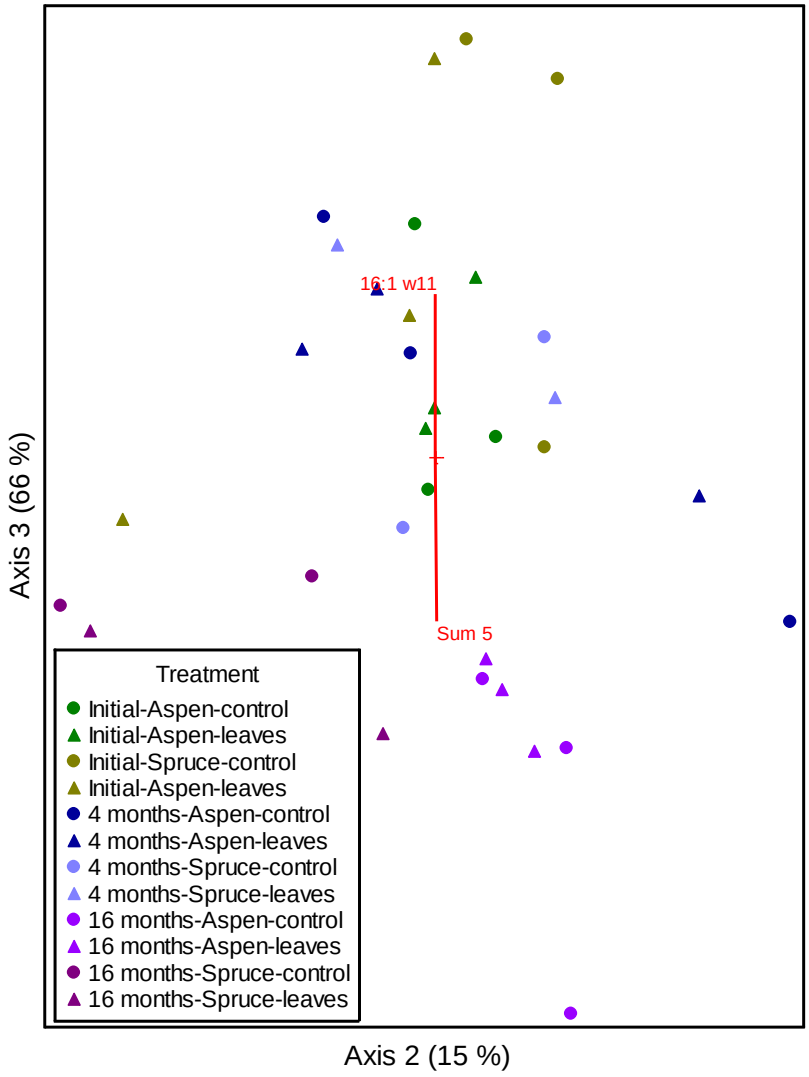

16:1 w11
Sum 5
Axis 3 (66 %)
Axis 2 (15 %)
Treatment
Initial-Aspen-control
Initial-Aspen-leaves
Initial-Spruce-control
Initial-Aspen-leaves
4 months-Aspen-control
4 months-Aspen-leaves
4 months-Spruce-control
4 months-Spruce-leaves
16 months-Aspen-control
16 months-Aspen-leaves
16 months-Spruce-control
16 months-Spruce-leaves

Figure 6-3: Microbial community structure determined by soil extracted PLFA biomarkers after a 16 month leaf litter amended mesocosm field experiment on Aspen and Spruce sites. Letters adjacent to the points indicate which plots the paired samples were from (Aspen = A, B, C and Spruce = D, E).

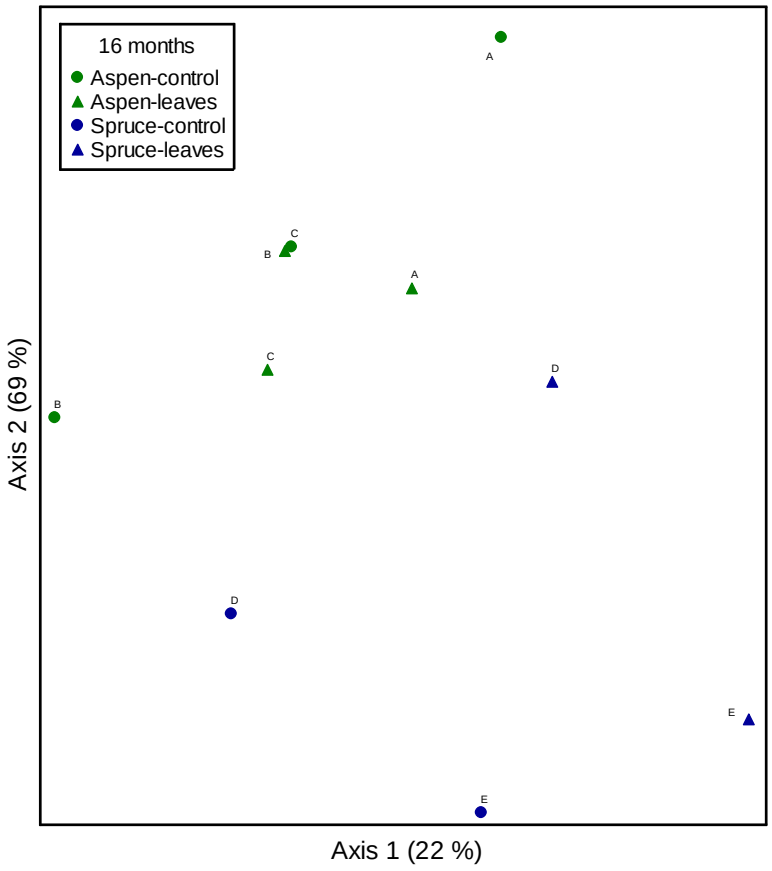
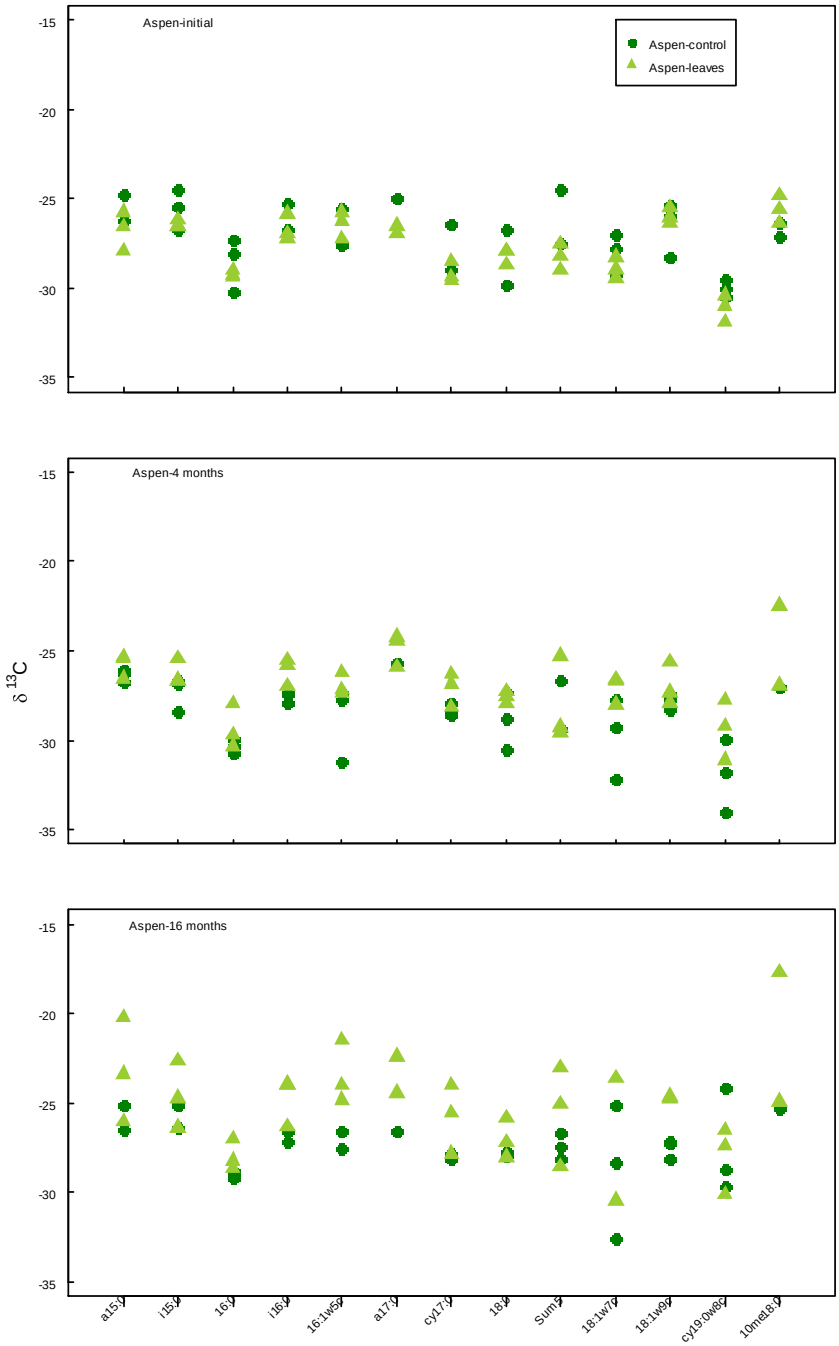
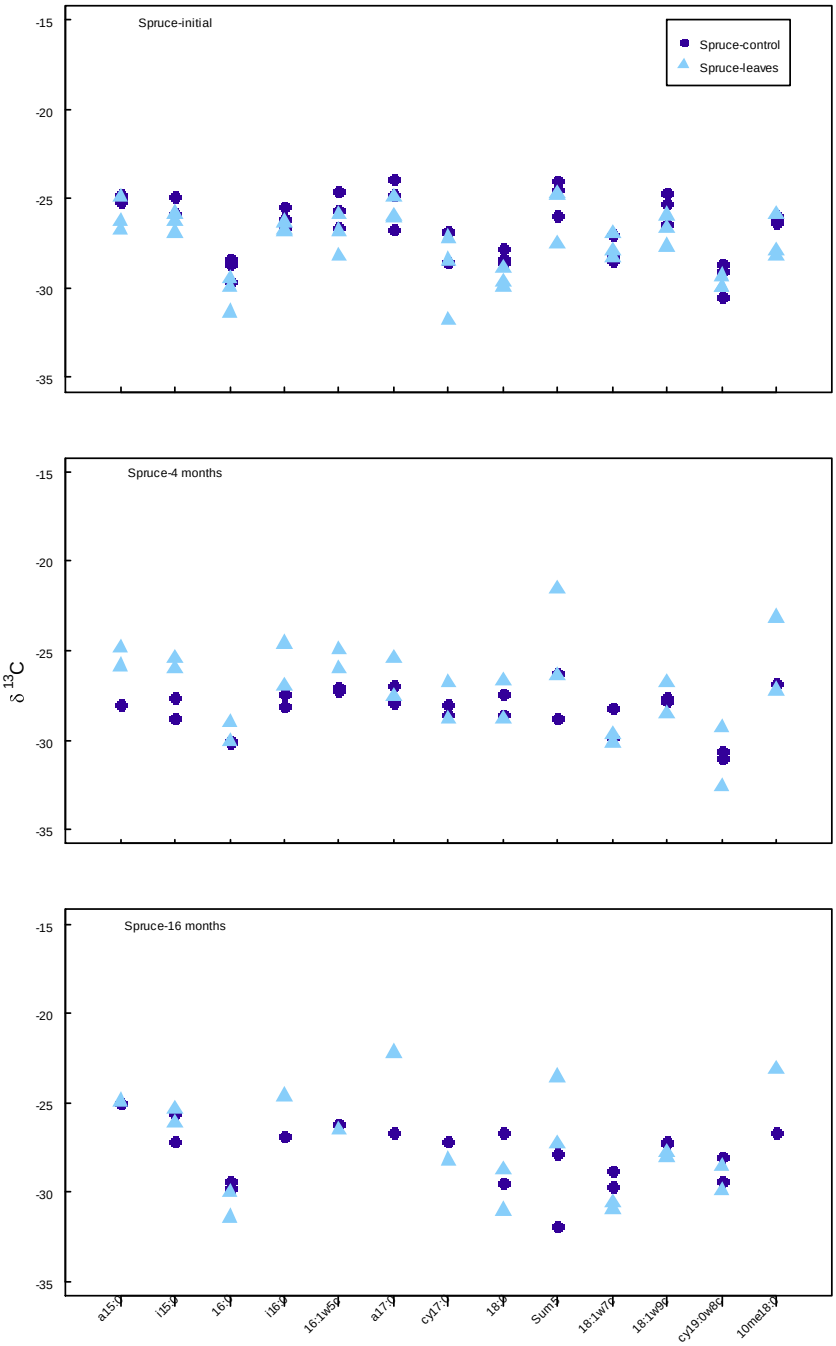


Figure 6-4: Isotopic ratio compound specific analysis of soil PLFA biomarkers from Aspen (a) or Spruce (b) across three collection times from a 16 month field incubation experiment with control or stable isotope enriched aspen leaf litter amended mesocosms.

a.



b.


Spruce-initial
Spruce-control
Spruce-leaves
Spruce-4 months
Spruce-16 months
δ 13C
-15
-20
-25
-30
-35
a15:0
i15:0
16:0
i16:0
16:1w5c
a17:0
cy17:0
18:0
Sum1
18:1w7c
18:1w9c
cy19:0w8c
10me18:0

References

Amelung, W., Brodowski, S., Sandhage-Hofmann, A., Bol, R. 2008. Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter. Advances in Agronomy 100, 155-250.

Baldock, J.A., Oades, J.M., Nelson, P.N., Skene, T.M., Golchin, A., Clarke, P. 1997. Assessing the extent of decomposition of natural organic materials using solid-state ^{13}C NMR spectroscopy. Aust. J. Soil Res. 35, 1061 - 1083.

Bird, J.A., Herman, D.J., Firestone, M.K. 2011. Rhizosphere priming of soil organic matter by bacterial groups in a grassland soil. Soil Biol. Biochem. 43, 718-725.

Bligh, E.G., Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

Boschker, H.T.S., Nold, S.C., Wellsbury, P., Bos, D., de Graff, W., Pel, R., Parkes, R.J., Cappendberg, T.E. 1998. Direct linking of microbial populations to specific biogeochemical processes by ^{13}C-labelling of biomarkers. Nature 392, 801-805.

Bottomley, P.J., Yarwood, R.R., Kageyama, S.A., Waterstripe, K.E., Williams, M.A., Cromack Jr, K., Myrold, D.D. 2006. Responses of soil bacterial and fungal communities to reciprocal transfers of soil between adjacent coniferous forest and meadow vegetation in the Cascade Mountains of Oregon. Plant Soil 289, 35-45.

Brant, J.B., Sulzman, E.W., Myrold, D.D. 2006. Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. Soil Biol. Biochem. 38, 2219-2232.

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

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., Tu, K.P. 2002. Stable isotopes in plant ecology. Annu. Rev. Ecol. Syst. 33, 507-559.

Donaldson, J.R., Kruger, E.L., Lindroth, R.L. 2006. Competition- and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (*Populus tremuloides*). New Phytol. 169, 561-570.

Dungait, J.A.J., Kemmitt, S.J., Michallon, L., Guo, S., Wen, Q., Brookes, P.C., Evershed, R.P. 2011. Variable responses of the soil microbial biomass to trace concentrations of ^{13}C-labelled glucose, using ^{13}C-PLFA analysis. Eur. J. Soil Sci. 62, 117-126.

Environment Canada 2013. Canadian climate normals 1971-2000, Fort McMurray.

Flanagan, P.W., Van Cleve, K. 1983. Nutrient cycling in relation to decomposition and organic-matterr quality in taiga ecosystems. Can. J. For. Res. 13, 795-817.

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

Frostegård, Å., Tunlid, A., Bååth, E. 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. J. Microbiol. Methods 14, 151-163.

Frostegård, Å., Tunlid, A., Bååth, E. 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43, 1621-1625.

Garcia-Pausas, J., Paterson, E. 2011. Microbial community abundance and structure are determinants of soil organic mineralisation in the presence of labile carbon. Soil Biol. Biochem. 43, 1705-1713.

Glaser, B. 2005. Compound-specific stable-isotope ($\delta^{13}C$) analysis in soil science. J. Plant Nutr. Soil Sci. 168, 633-648.

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

Hahn, A.S. 2012. Soil microbial communities in early ecosystems. University of Alberta.

Hahn, A.S., Quideau, S.A. 2012. Long-term effects of organic amendments on the recovery of plant and soil microbial communities following disturbance in the Canadian boreal forest. Plant Soil, 1-14.

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

Hannam, K.D., Quideau, S.A., Kishchuk, B.E. 2007. The microbial communities of aspen and spruce forest floors are resistant to changes in litter inputs and microclimate. Appl. Soil Ecol. 35, 635-674.

Hannam, K.D., Quideau, S.A., Kishchuk, B.E., Oh, S.-W., Wasylishen, R.E. 2005. Forest-floor chemical properties are altered by clear-cutting in boreal

mixedwood forest stands dominated by trembling aspen and white spruce. Can. J. For. Res. 35, 2457-2468.

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

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

Kembel, S.W., Dale, M.R.T. 2006. Within-stand spatial structure and relation of boreal canopy and understorey vegetation. Journal of Vegetation Science 17, 783-790.

Kögel-Knabner, I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol. Biochem. 34, 139-162.

Kramer, C., Trumbore, S., Fröberg, M., Cisneros Dozal, L.M., Zhang, D., Xu, X., Santos, G.M., Hanson, P.J. 2010. Recent (<4 year old) leaf litter is not a major source of microbial carbon in a temperate forest mineral soil. Soil Biol. Biochem. 42, 1028-1037.

Laganière, J., Paré, D., Bradley, R.L. 2009. Linking the abundance of aspen with soil fauna communities and rates of belowground processes within single stands fo mixed aspen-black spruce. Appl. Soil Ecol. 41, 19-28.

Legendre, P., Gallagher, E.D. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271-280.

Macdonald, S.E., Fenniak, T.E. 2007. Understory plant communities of boreal mixedwood forests in western Canada: Natural patterns and response to variable-retention harvesting. For. Ecol. Manage. 242, 34-48.

Maxfield, P.J., Evershed, R.P. 2011. Phospholipid fatty acid stable isotope probing techniques in microbial ecology, in: Murrell, J.C., Whiteley, A.S. (Eds.) Stable isotope probing and related technologies. ASM Press, Washington, DC.

McCune, B., Grace, J.G. 2002. 'Analysis of ecological communities.' (MJM Software Design: Gleneden Beach, OR).

Moore-Kucera, J., Dick, R.P. 2008. Application of ^{13}C-labeled litter and root materials for *in situ* decomposition studies using phospholipid fatty acids. Soil Biol. Biochem. 40, 2485-2493.

Mummey, D.L., Clarke, J.T., Cole, C.A., O'Connor, B.G., Gannon, J.E., Ramsey, P.W. 2010. Spatial analysis reveals differences in soil microbial community interactions between adjacent coniferous forest and clearcut ecosystems. Soil Biol. Biochem. 42, 1138-1147.

Myers, R.T., Zak, D.R., White, D.C., Peacock, A. 2001. Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Sci. Soc. Am. J. 65, 359-367.

Norris, C.E., Quideau, S.A., Bhatti, J.S., Wasylishen, R.E. 2011. Soil carbon stabilization in jack pine stands along the Boreal Forest Transect Case Study. Glob. Change Biol. 17, 480-494.

Norris, C.E., Quideau, S.A., Macey, D.E. 2013. Processing of ^{13}C glucose in mineral soil from aspen, spruce and novel ecosystems in the Athabasca Oil Sands Region. Appl. Soil Ecol. 71, 24-32.

Pinzon, J., Spence, J.R., Langor, D.W. 2011. Spider assemblages in the overstory, understory, and ground layers of managed stands in the western boreal mixedwood forest of Canada. Environmental Entomology 40, 797-808.

Preston, C.M., Bhatti, J.S., Flanagan, L.B., Norris, C. 2006. Stocks, chemistry, and sensitivity to climate change of dead organic matter along the Canadian Boreal Forest Transect Case Study. Clim. Change 74, 223-251.

Preston, C.M., Trofymow, J.A., Canadian Intersite Decomposition Experiment Working Group 2000. Variability in litter quality and its relationship to litter decay in Canadian forests. Can. J. Bot. 78, 1269-1287.

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

Quideau, S.A., Chadwick, O.A., Graham, R.C., Wood, H.B. 1996. Base cation biogeochemistry and weathering under oak and pine: a controlled long-term experiment. Biogeochemistry 35, 377-398.

Quideau, S.A., Chadwick, O.A., Trumbore, S.E., Johnson-Maynard, J.L., Graham, R.C., Anderson, M.A. 2001. Vegetation control on soil organic matter dynamics. Org. Geochem. 32, 247-252.

Quideau, S.A., Graham, R.C., Chadwick, O.A., Wood, H.B. 1998. Organic carbon sequestration under chaparral and pine after four decades of soil development. Geoderma 83, 227 - 242.

Rubino, M., Dungait, J.A.J., et al. 2010. Carbon input belowground is the major C flux contributing to leaf litter mass loss: Evidences from a ^{13}C labelled-leaf litter experiment. Soil Biol. Biochem. 42, 1009-1016.

Ruess, L., Chamberlain, P.M. 2010. The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biol. Biochem. 42, 1898-1910.

Soil Classification Working Group 1998. The Canadian System of Soil Classification, in: Agriculture and Agri-Food Canada- Research Branch (Eds.). NRC Research Press. pp. 53 - 59.

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

Ushio, M., Wagai, R., Balser, T.C., Kitayama, K. 2008. Variations in the soil microbial community composition of a tropical montane forest ecosystem: Does tree species matter? Soil Biol. Biochem. 40, 2699-2702.

Vancampenhout, K., Wouters, K., De Vos, B., Buurman, P., Swennen, R., Deckers, J. 2009. Differences in chemical composition of soil organic matter in natural ecosystems from different climatic regions - A pyrolysis-GC/MS study. Soil Biol. Biochem. 41, 568-579.

Vitousek, P.M., Howarth, R.W. 1991. Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry 13, 87-115.

Waldrop, M.P., Firestone, M.K. 2004. Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia 138, 275-284.

Waldrop, M.P., Firestone, M.K. 2006. Response of microbial community composition and function to soil climate change. Microbial Ecology 52, 716-724.

White, D.C., Ringelberg, D.B. 1998. Signature lipid biomarker analysis, in: Burlage, R.S., Atlas, R., Shahl, D., Geesey, G., Sayler, G. (Eds.) Techniques in microbial ecology. Oxford University Press, New York, NY.

Williams, R.J., Hallgren, S.W., Wilson, G.W.T. 2012. Frequency of prescribed burning in an upland oak forest determines soil and litter properties and alters soil microbial community. For. Ecol. Manage. 265, 241-247.

Zelles, L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol. Fert. Soils 29, 111-129.

Zelles, L., Bai, Q.Y. 1993. Fractionation of fatty acids derived from soil lipids by solid phase extraction and their quantitative analysis by GC-MS. Soil Biol. Biochem. 25, 495-507.

Ziegler, S.E., White, P.M., Wolf, D.C., Thoma, G.J. 2005. Tracking the fate and recycling of ^{13}C-labeled glucose in soil. Soil Sci. 170, 767-778.

Zumsteg, A., Stierli, B., Zeyer, J., Frey, B. 2013. Bacterial and fungal community responses to reciprocal soil transfer along a temperature and soil moisture gradient in a glacier forefield. Soil Biol. Biochem. 61, 121-132.

Chapter 7. Summary

7.1. Objectives

The specific objectives of this study were to determine if:

1. soil organic matter from novel ecosystems begins to resemble that of natural ecosystems with time since reclamation

2. a labile substrate, ^{13}C glucose, is decomposed via the same pathways in a peat mineral mixture as in natural soils,

3. the accumulation of labeled isotope (^{13}C or ^{15}N) varies both within the structure (leaves, stems, or roots) and within the macromolecular chemistry of an aspen seedling,

4. the addition of ^{15}N labelled leaf litter to the forest floor is utilized as a nutrient source for growing vegetation on constructed and natural soils, and

5. the addition of organic matter to the forest floor may shift the composition and function of the soil microbial community.

The objective summaries, or abstracts, were submitted with individual chapters as part of the journal publication process. Reference to their respective publication may be found at the top of the individual chapters. The summaries are presented together below in an effort to provide a concise overarching view of the entire project.

7.2. Biomarkers of ecosystem development

Novel ecosystem development is occurring within the western boreal forest of Canada due to land reclamation following oil sands surface mining. Sphagnum peat is the primary organic amendment used to reconstruct soil in the novel ecosystems. We hypothesised that ecosystem recovery would be indicated by an increasing similarity in the biomolecular characteristics of novel soil organic matter (SOM) derived from peat to those of natural boreal ecosystems. We evaluated the use of the homologous series of long chain ($\geq C_{21}$) *n*-alkanes with odd/even predominance to monitor the re-establishment of boreal forest on

these anthropogenic soils. The lipids were extracted from dominant vegetation inputs and SOM from a series of natural and novel ecosystem reference plots. Twice the concentration of *n*-alkanes was extracted from natural than from novel ecosystem SOM ($p<0.01$). We observed unique *n*-alkane signatures for the source vegetation; e.g. peat material was dominated by C_{31}, and aspen (*Populus tremuloides* Michx.) leaves by C_{25}. As determined by Non-metric Multidimensional Scaling, *n*-alkane distribution differed between the two systems ($p<0.001$) and reflected the dominant vegetation input, i.e. peat or tree species. Our results indicate that further research is required to clarify the influence of vegetation or disturbance on the signature of *n*-alkanes in SOM; however, the use of *n*-alkanes as biomarkers of novel ecosystem development is a promising application.

7.3. Processing of labile organic matter by novel and natural ecosystem soils

Microbial composition is known, on similar soil types, to vary based on differing organic matter inputs, or stand composition. Fine-textured luvisolic soils, which dominate the upland boreal forests of Western Canada, support a canopy cover of aspen (*Populus tremuloides* Michx.), white spruce (*Picea glauca* (Moench) Voss) or a mixture of the two. These soils then reflect different belowground biogeochemical processing of organic matter. Novel, anthropogenic soils formed from a combination of peat litter and fine textured mineral soil, are now also a part of the landscape in the western boreal. This study set out to determine if a simple labelled compound (^{13}C glucose) was processed differently by soils from the two dominant stand types (aspen and spruce) and from an anthropogenic (newly reclaimed) site. Results indicate that while all three soils rapidly incorporated and respired the labelled carbon, each maintained a distinct microbial community structure (as evidenced by phospholipid fatty acid analysis) throughout the 300 hour experiment. Therefore soils with different microbial communities from varied organic matter inputs decompose organic carbon by different processes, even in the case of simple labile compounds.

7.4. Macromolecular stable isotope enrichment of leaf litter

Enriching plant tissues with ^{13}C and ^{15}N isotopes has provided long-lasting, non-reactive tracers to quantify rates of terrestrial elemental fluxes (e.g., soil organic matter decomposition). However, the molecular location and level of isotope enrichment may differ among plant tissues. This factor is central to the integrity and interpretation of tracer data, but is seldom considered in experiments. We propose a rapid, non-destructive method to quantify molecular isotope allocation using solid-state ^{13}C and ^{15}N nuclear magnetic resonance spectroscopy. With this method, we tracked and quantified the fate of multiple pulses of $^{13}CO_2(g)$ and $K^{15}NO_3(l)$ in boreal tree seedling roots and leaves as a function of time. Results show that initial preferential ^{13}C carbohydrate enrichment in the leaves was followed by redistribution to more complex compounds after seven days. While ^{13}C allocation within the roots was uniform across molecules, ^{15}N results indicate an initial enrichment of amine molecules after two hours.

7.5. Organic matter nutrient cycling on novel and natural ecosystem soils

Soil provides the foundation for life and, with landscape level reclamation a reality in many regions, it plays a vital role in possible reclamation success. This study compared reclaimed, harvested and undisturbed aspen forest sites for nutrient cycling between soil and live vegetation over a 16 month field incubation following the addition of ^{15}N labeled aspen (*Populus tremuloides* Michx.) leaf litter to the soil surface. Soil moisture and microbial biomass were higher on the undisturbed site; however, these properties improved on all sites. Cycling of ^{15}N was similar across all sites, indicating leaf litter nutrients were readily available regardless of individual site conditions. From these results we propose a more appropriate analogue to assess the trajectory of reclamation success is a disturbed rather than an undisturbed site. In addition, these results not only indicate improved soil properties with increased leaf litter input but also immediate nitrogen cycling on reclamation stands.

7.6. Vegetation influence on soil microbial community structure

Above-ground vegetation inputs are a major source of carbon for the soil microbial community, with the amount and type of input believed to shape both microbial structure and function. This study set to examine if this is true. We utilized double stable isotope labelled aspen (*Populus tremuloides* Michx.) leaves as an amendment to the forest floor of aspen and spruce (*Picea glauca* (Moench) Voss) stands in a 16 month paired mesocosm field experiment. The leaves were enriched in ^{15}N as to provide a tracer to assess N cycling in the stands, while the ^{13}C tracer quantified the active segment of the microbial community though compound-specific stable isotope analysis of phospholipid fatty acids (PLFA). Nitrogen cycling from the leaf litter occurred on both aspen and spruce sites as evidenced by ^{15}N enrichment of live above-ground vegetation and roots. Microbial community structure remained distinct between the aspen and spruce soils, as determined by PLFA biomarkers, although collection time was an important community driver. Compound-specific analysis of the PLFA biomarkers indicated that a wide range of the aspen soil microbial community was involved in decomposition of the leaf litter, while in the spruce soil, two specific PLFAs, 10me18:0 and 18:2ω6,9c, biomarkers attributed to actinomycetes and fungi respectively, were the most enriched in ^{13}C. While these results were qualitative, they demonstrated active N cycling on both stands and indicate at the influence of vegetation inputs on soil microbial community structure and function.

7.7. Project limitations and future research needs

Much of the work presented here was proof-of-concept; we provided results which we believe supports conceptual ideas. We acknowledge that much of this work was limited in scope with few (to no) replications of treatments on site or in the laboratory and at the wider ecological stand level. With the large amount of within-stand variability experienced, and with no assessment of the landscape level variability, our ability to make firm conclusions from the data is hindered. Despite these limitations, results enhance our understanding of organic matter cycling in the western boreal forest, and they point us in a direction of

further exploration. For example: 1. the contribution of under-story vegetation biomarkers to soil organic matter formation and how that may reflect ecosystem development is a promising research avenue that deserves further investigation, 2. redundancy was observed within the soil microbial community in terms of how it utilized simple labile carbon sources; however, the more specialized groups which degrade more complex compounds merit examination, as well as how this may translate to soil organic matter development at the landscape level, 3. there is a need to further our understanding of the potential importance and ramification of the specific atoms that are labelled during tracer experiments, and their relevance to biogeochemical studies, 4. valuable research may be found in investigations on increasing seedling stocking density of reclaimed stands and the impacts this has on improving soil organic matter quality, accelerating the re-establishment of biogeochemical processes, and potentially enhancing reclamation success, and 5. the environmental conditions responsible for, and the implications of shifting soil microbial community composition require further investigation, while the potential of using organic amendments to manipulate soil microbial composition needs to be better defined.

7.8. Implications for reclamation

Soil is the foundation of life. Improving soil organic matter quantity and quality is known to improve soil quality. Reclamation success can be defined in part using the land capability system (Cumulative Environmental Management Association 2006). In all cases, ecosystems need to become self-sustaining in terms of fundamental processes. This relies on functioning biogeochemical cycles, in particular soil nutrient cycling. Assessment of novel ecosystem status in terms of these cycles is difficult. Three key results from this project may improve our understanding of what is needed for forest reclamation: 1. Comparison of response variables among similar stand types (Chapter 2), 2. Comparison of response variables with other land disturbances, e.g., harvest (Chapter 4), and 3. Evaluation of the potentially profound effects that organic matter inputs have on soil quality (Chapter 4 & 5).

Due to the complexity of forest ecosystems, which include multiple pools of organic matter turning over on different time scales, and both biotic and abiotic factors operating in tandem, being able to isolate the different processes driving carbon and nitrogen cycles is challenging. Such intricacy requires a multi-faceted investigative approach to determine how the ecosystem functions. This project set out in part to determine if reclaimed stands on novel ecosystems could be compared to natural ecosystems in terms of their carbon and nitrogen cycles. Using *n*-alkanes as vegetation biomarkers provided a means to not only differentiate between novel and natural ecosystems, but also among different stand types within these ecosystems (Norris et al. 2013a). This approach would therefore be useful in monitoring the evolution of organic matter within the Oil Sands novel ecosystems. Combining an assessment of *n*-alkane biomarkers with characterization of the macromolecular fingerprint of soil organic matter by NMR and other methods of characterizing organic matter, e.g., lignin monomers, would create an unparalleled means of quantifying soil organic matter development.

The use of multiple investigative tools and techniques used here indicate a way of quantifying carbon and nitrogen cycles. Soil fractionation techniques can help describe and quantify the distribution of organic matter among different soil carbon pools. Using the PLFA technique allows for a structural fingerprint of the microbial community which is associated with a particular forest floor or mineral soil. Each of these methods is valuable in itself, but combined with stable isotope enriched organic matter they provide an especially powerful means of tracing the fluxes of carbon or nitrogen from added litter input to different organic matter pools. In particular, ^{13}C tracer was successfully followed into the microbial biomass of different natural and reconstructed soils after the incorporation of a ^{13}C-labeled labile substrate (glucose) (Norris et al. 2013c). Therefore, this unique approach is very valuable as it not only allows for identification of the microbial community but also the functional role of the microorganisms.

Organic matter is composed of both labile (e.g., glucose) and complex compounds therefore there was also the need to follow the fate of more complex organic compounds that may be more relevant to the soil environment than

glucose. Labelled organic matter was therefore generated by the pulse-chase technique to aspen seedlings (Norris et al. 2012). Pulse labeling is cheaper and presents fewer technical difficulties than continuous labeling. However, in addition to quantifying the overall enrichment level, it is crucial to characterize the biopolymers that are enriched in order to have a true understanding of the subsequent decomposition flux measurements. Analysis of the enriched organic matter by NMR provides unequaled biopolymer description.

Once this labeled litter was produced, it was utilized in field incubations to follow carbon and nitrogen fluxes within different stand types. One of our original objectives was to quantitatively determine the amount of nitrogen in above-ground vegetation coming from the current soil organic matter (which may include a peat amendment) and the amount deriving from new litter inputs. We hypothesized that the proportion of nitrogen originating from the litter versus the soil would be less on reclaimed stands of novel ecosystems than on natural stands. Within the reclaimed stands, we hypothesized that a greater proportion of the nitrogen in the above-ground vegetation on younger stands would derive from the peat amendment than on older reclaimed stands. Results from this project could not fully meet this objective—not in a quantitative manner (Norris et al. 2013b). Quantification was hampered by both uncontrolled and controllable factors but derived from insufficient replication within each site. Uncontrolled interference, rodents, destroyed valuable instrumentation and samples on one site; increased replication on this site may have mitigated some of these damages. Reducing the number of treatments overall by eliminating aspen vs. spruce, reclamation age or type of disturbance and increasing the replication of treatment sites would have provided the necessary increases in replication and been beneficial to the project overall.

We had hoped, when this project was established, to quantitatively determine nitrogen fluxes from the soil organic matter to above-ground vegetation. While the concept was proven, these techniques are both field and laboratory intensive and, as such, ecosystem variability was underestimated and quantification of fluxes could not be determined. In addition, due to obvious time

constraints linked to a PhD program, a greenhouse incubation experiment was not undertaken. Such an experiment, i.e.; tree seedlings growing under controlled conditions in natural and reconstructed soils with labelled leaf litter added, would have been the logical link between the laboratory and field incubation experiments performed here. The lack of a greenhouse experiment is a missing link and there is definite value in conducting such an experiment in the future. Yet, if conducted with a reduced number of stand types and an increased number of replicates, this multi-faceted field approach of determining the biogeochemical cycling of organic matter looks promising.

7.9. Conclusions

Novel ecosystems, comprised of planted tree stands on constructed soils, thus far have been shown to be different in terms of their key soil response variables (e.g., soil microbial biomass, soil organic matter composition, soil nitrogen content) when compared to undisturbed natural ecosystems in the Athabasca Oil Sands Region. This suggests that there is also a difference in terms of central ecosystem processes. Previous research has indicated that with novel ecosystem development a shift in soil response variables also occurs. The results presented here further this concept; novel ecosystems change with time. But determining which variables change and when they begin to resemble natural ecosystems was the greater aim of this study. Results from Chapter 3 and 5 indicate that nitrogen is readily cycled from organic matter inputs. Results from Chapter 6 indicate that with a major change in organic matter inputs, the soil microbial structure may shift in composition, although it still maintains active nitrogen cycling. Cumulatively, results presented here hint at the possibility that novel ecosystems will function and be self-sustaining in terms of central ecosystem processes. In addition, results point towards the importance of organic matter inputs to soil and how changes in the macromolecular composition of the organic matter lead to changes in the carbon and nitrogen cycling of the western boreal forest.

References

Cumulative Environmental Management Association 2006. Land capability classification system for forest ecosystems in the oil sands p. 53. (Alberta Environment.

Norris, C.E., Dungait, J.A.J., Joynes, A., Quideau, S.A. 2013a. Biomarkers of novel ecosystem development in boreal forest soil. Org. Geochem., In press.

Norris, C.E., Quideau, S.A., Landhäusser, S.M., Bernard, G.M., Wasylishen, R.E. 2012. Tracking stable isotope enrichment in tree seedlings with solid-state NMR spectroscopy. Scientific Reports 2, 719.

Norris, C.E., Quideau, S.A., Landhäusser, S.M., Drozdowski, B. 2013b. Improving forest soils during reclamation. Ecological Engineering, submitted 2 June 2013.

Norris, C.E., Quideau, S.A., Macey, D.E. 2013c. Processing of ^{13}C glucose in mineral soil from aspen, spruce and novel ecosystems in the Athabasca Oil Sands Region. Appl. Soil Ecol. 71, 24-32.