



# Processing of $^{13}\text{C}$ glucose in mineral soil from aspen, spruce and novel ecosystems in the Athabasca Oil Sands Region



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## ABSTRACT

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 labeled compound ( $^{13}\text{C}$  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 respiration the labeled 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.

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## 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<sup>2</sup> 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 (Mumme et al., 2010), fire (Williams et al., 2012), and in surface mine reclamation areas (Dimitriu et al., 2010; Mumme 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; Mumme et al., 2002a), which in some cases were found to be

**Abbreviations:** AOSR, Athabasca Oil Sands Region; PLFA, phospholipid fatty acid; DOC, dissolved organic carbon; NMDS, non-metric multidimensional scaling; MRPP, multi-response permutation procedures.

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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 ( $^{13}\text{C}$ ) 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  $^{13}\text{C}$  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  $^{13}\text{C}$  labeled glucose during a laboratory incubation experiment. We expected that the movement of  $^{13}\text{C}$  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.

## 2. Materials and methods

### 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.

### 2.2. Laboratory analysis

#### 2.2.1. 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 min (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 thermocouple detector (Costech Analytical Technologies Inc., Valencia, USA), and for  $^{13}\text{C}$  and  $^{15}\text{N}$  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  $\delta$ -notation, part per thousand variations from the standard Pee Dee Belemnite, and as atom %.

#### 2.2.2. 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- $^{13}\text{C}$  and 0.5 mg of L-alanine- $^{15}\text{N}$  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 h) and at 5 sampling points during the incubation (22, 46, 70, 142, and 310 h;  $n=3$ ). One vial from each unit immediately underwent soil fractionation, one was frozen at  $-20^{\circ}\text{C}$  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).

### 2.2.3. 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, 445, 685, and 140 h). For each measurement, the amount of CO<sub>2</sub> 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 445 to 685 h (except 18.5 and 27 h which were sealed for 1 and 5 h 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<sub>2</sub> 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 <sup>13</sup>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<sub>2</sub> and mg CO<sub>2</sub>-<sup>13</sup>C) was calculated as the sum of respiration from each sampling time.

### 2.2.4. Phospholipid fatty acid analysis

Freeze dried soil samples (200 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 0.33 μm 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<sup>-1</sup> 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<sup>-1</sup> dry soil) of all reported PLFAs. The PLFA nomenclature used follows standard formatting (Maxfield and Evershed, 2011).

### 2.2.5. 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 h 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.

### 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  $\alpha$  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. Results

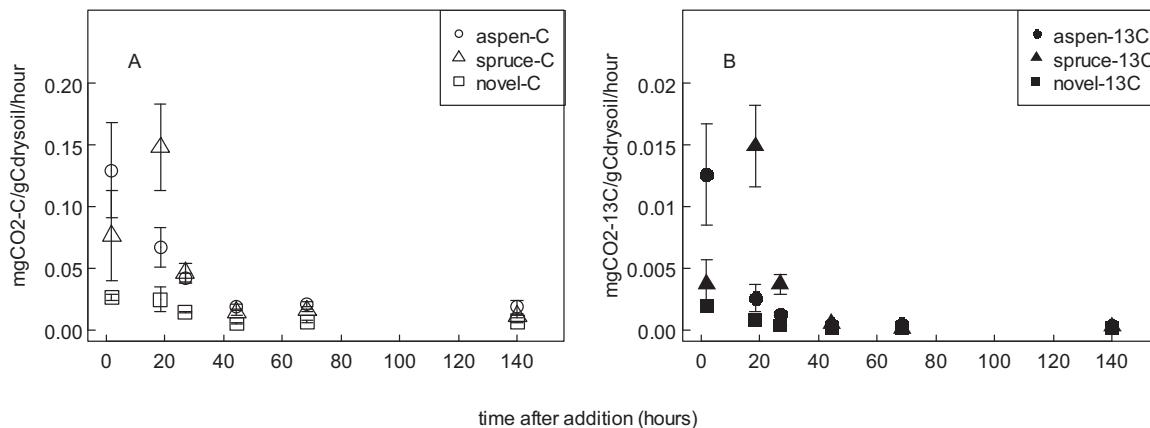
### 3.1. General soil characteristics

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 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

**Table 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	$^{13}\text{C}$ (atom %)
	Canadian	FAO					Sand	Silt	Clay	pH	(%)	(%)		
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



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

carbon and  $^{13}\text{C}$  atom %, the novel soil initially contained more  $^{13}\text{C}$  in its incubation vials compared to the aspen and spruce soils (0.019 g vs 0.004 g). Thus, the addition of  $^{13}\text{C}$  labeled glucose only represented an isotope addition of 2% for the novel soil, while this corresponded to 9 and 10% for aspen and spruce, respectively.

### 3.2. Soil respiration

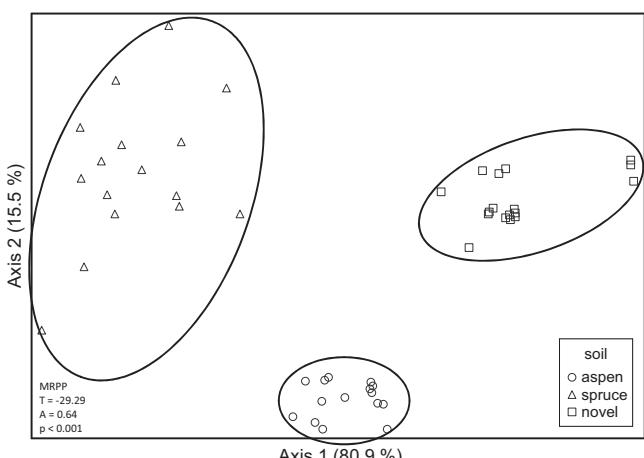
Total respiration by overall soil mass was greater for the novel soil than for either spruce or aspen for both total mg CO<sub>2</sub>-C and CO<sub>2</sub>- $^{13}\text{C}$  (Table 2), as could be expected from the greater quantity of total carbon and  $^{13}\text{C}$  in this soil. However, total respiration 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 2). This was true for both total carbon and  $^{13}\text{C}$ . 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 labeled substrate, and then steadily declining for both total CO<sub>2</sub>-C and CO<sub>2</sub>- $^{13}\text{C}$  (Fig. 1). Interestingly, aspen and spruce diverged in the timing of their peak respiration rates, with the greatest activity occurring 2 h after substrate addition for aspen and 18 h for spruce. The novel soil samples were notable for their lower respiration during the entire incubation experiment for both  $^{13}\text{C}$  and overall CO<sub>2</sub>.

### 3.3. Phospholipid fatty acid analysis

Total soil microbial biomass as determined by PLFA analysis differed among the three soil types (Table 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 (Fig. 2). The NMDS on the data of 36 PLFAs resulted in a

two dimensional ordination solution (stress of 783 after 92 iterations), where subsequent MRPP testing showed that the three soil types were separate ( $p < 0.001$ ,  $T = -2929$ ,  $A = 0.64$ ). 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 toward the novel soil (16:1ω5c, 16:1ω9c, and 17:1ω8c), while one increased toward 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

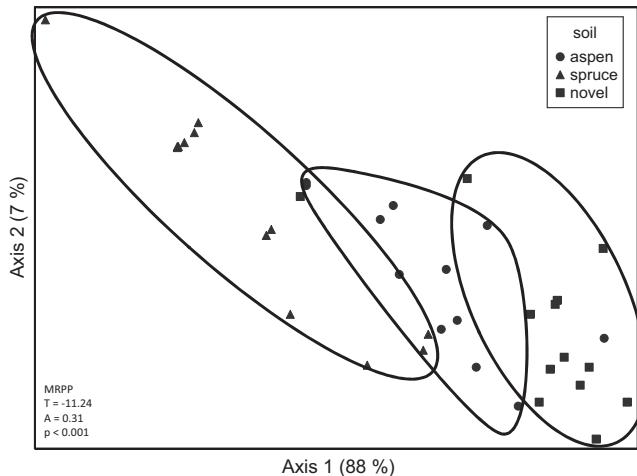


**Fig. 2.** NMDS ordination of PLFA profiles in three mineral soils from the western boreal forest (aspen, spruce and novel) incubated with  $^{13}\text{C}$ -glucose. Grouping based on soil type and MRPP analysis is highlighted with circles.

**Table 2**

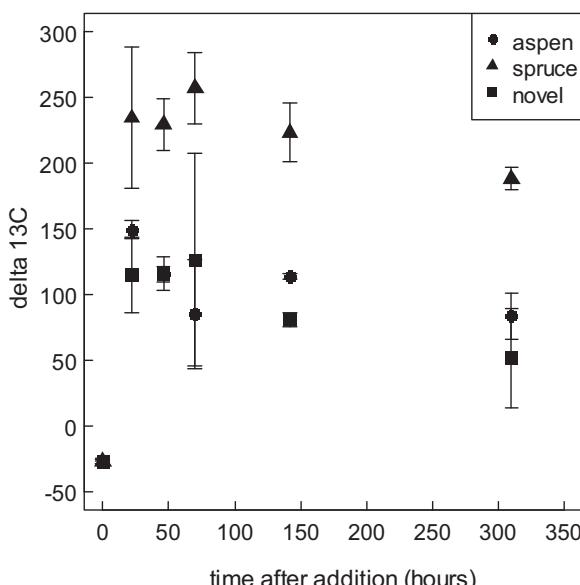
Soil microbial response to addition of  $^{13}\text{C}$ -glucose in a 310 hour incubation. Numbers in parentheses represent one standard deviation from the mean ( $n=3$ ). Statistical differences between the soils are represented by different letters with alpha = 0.05.

Soils	Total respiration				Total microbial biomass ( $\text{nmol g}^{-1}$ dry soil) at specific sampling times (h)						
	mg $\text{CO}_2\text{-C}$	mg $\text{CO}_2\text{-}^{13}\text{C}$	mg $\text{CO}_2\text{-C g}^{-1}\text{C}$	mg $\text{CO}_2\text{-}^{13}\text{C g}^{-1}\text{C}$	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) a	744 (85) a	661 (225) a	666 (49) a	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) b	274 (23) b	222 (20) b	205 (13) b	200 (7) b	
Novel	4.1 (0.3) a	0.095 (0.006) a	0.78 (0.05) c	0.018 (0.001) a	321 (22) b	459 (70) b	328 (33) b	457 (47) b	356 (50) a	338 (30) a	



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

all 36 PLFAs (data not shown). The same separation was evident in the ordination of  $\delta^{13}\text{C}$  PLFA values (Fig. 4) as in the ordination of nmol% (Fig. 3). 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



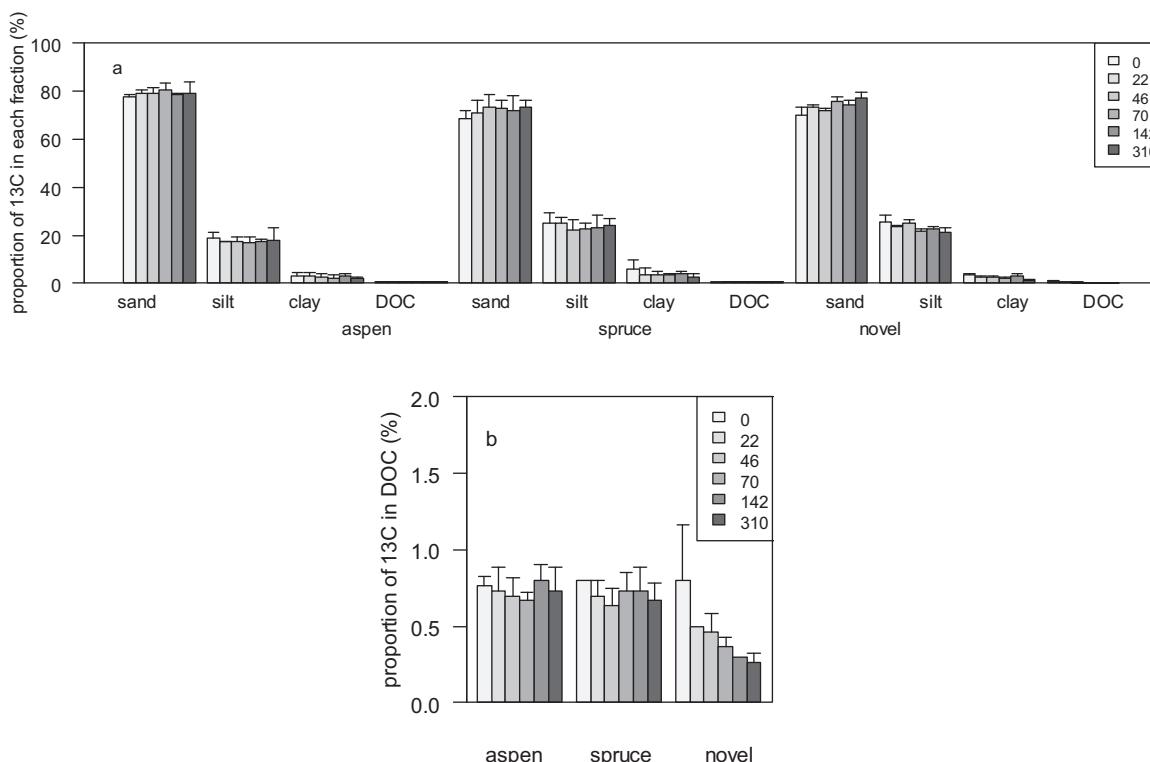
**Fig. 4.**  $\delta^{13}\text{C}$  enrichment of the 18:1 $\omega$ 7c PLFA in three mineral soils from the western boreal forest (aspen, spruce and novel) incubated with  $^{13}\text{C}$ -glucose. Error bars reflect one standard deviation from the mean ( $n=3$ ).

separate ( $T = -11.24$ ,  $A = 0.31$ ,  $p < 0.001$ ) 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 16:1 $\omega$ 5c and 18:2 $\omega$ 6,9c/a18 sum feature 5 (indicator value  $> 55\%$ ,  $p \leq 0.001$ ). Incorporation of  $^{13}\text{C}$ , as evidenced by the enrichment of a representative PLFA 18:1 $\omega$ 7c (Fig. 5), indicated differences among soil types in the processing of  $^{13}\text{C}$  labeled 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 18:2 $\omega$ 6,9c and a18:0. The PLFA 18:2 $\omega$ 6,9c is a recognized fungal biomarker while a18:0 is regarded as a biomarker for Gram positive bacteria (Ruess and Chamberlain, 2010). The unresolved 18:2 $\omega$ 6,9c/a18:0 peak was found in all of our samples and we believe that both lipids were present in varying proportions. The PLFA 18:1 $\omega$ 9c, 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, 18:3 $\omega$ 3,6,9c, was extracted from both aspen and spruce soils but not the novel soil. Therefore, due to ambiguous assignment of 18:2 $\omega$ 6,9c/a18:0 peak, we hesitate to calculate fungal:bacterial ratios. We suspect that when 18:2 $\omega$ 6,9c/a18:0 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.

### 3.4. 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  $^{13}\text{C}$ , with a decreasing proportion of the total isotope with decreasing particle size (Table 3 and Fig. 5). All soil fractions were enriched immediately after the addition of the labeled glucose substrate and, once enriched above background levels, the  $^{13}\text{C}$  isotopic signature remained elevated (Fig. 6). The greatest variation in  $^{13}\text{C}$  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,  $^{13}\text{C}$  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  $^{13}\text{C}$  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 (Fig. 5).



**Fig. 5.** (a) Proportion of <sup>13</sup>C in four soil fractions from three mineral soils from the western boreal forest (aspen, spruce and novel) incubated with <sup>13</sup>C-glucose. Time (h) 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.

**Table 3**

Initial and final total organic carbon contents (mg) for the soil fractions from the <sup>13</sup>C-glucose addition incubation. Numbers in parentheses represent one standard deviation from the mean ( $n=3$ ).

Soil	Time	Sand <sup>a</sup>	Silt <sup>b</sup>	Clay <sup>c</sup>	DOC <sup>d</sup>	Sum
<i>Total organic carbon (mg)</i>						
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 (50)	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)
<i>Total <sup>13</sup>C organic carbon (mg)</i>						
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)

<sup>a</sup> Sand 53–2000 µm.

<sup>b</sup> Silt 2–53 µm.

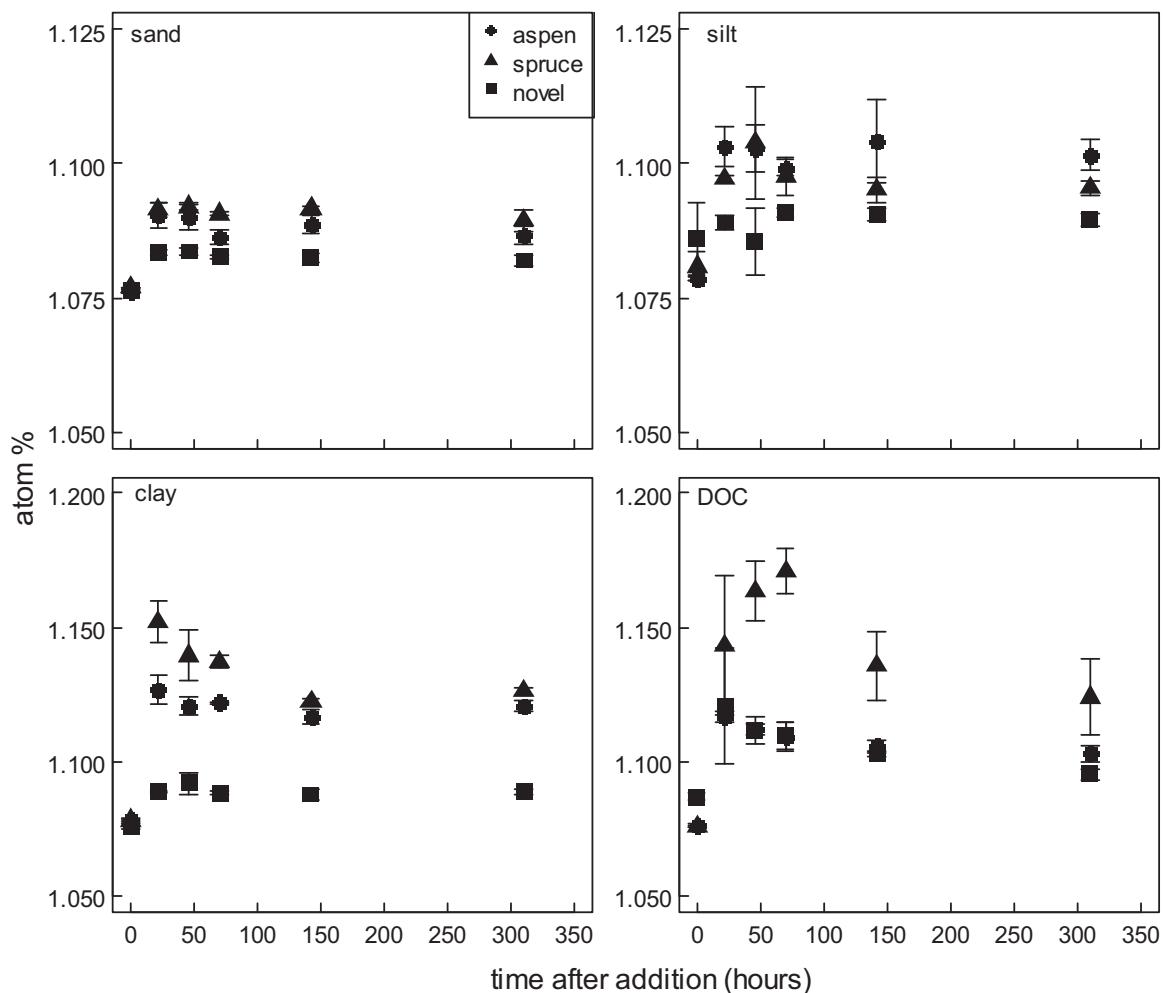
<sup>c</sup> Clay <2 µm.

<sup>d</sup> Dissolved organic carbon.

#### 4. Discussion

Processing of a simple labeled compound (<sup>13</sup>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 (11 and 12%) 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 (58 and 0.33%; Table 1). The pH was also higher in the novel soil (6), compared to aspen (5) and spruce (4). Differences in pH and



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

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 labeled substrate enriched all of the soil fractions in  $^{13}\text{C}$ , with the  $^{13}\text{C}$  in all fractions remaining above background levels for the duration of the incubation (Fig. 6). Of the four fractions, the largest proportional increase in  $^{13}\text{C}$  was observed in sand-sized fractions (Fig. 5). Of particular note was that both the clay and DOC fractions from the spruce soil had the highest levels of absolute enrichment (Fig. 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  $^{14}\text{C}$  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  $^{13}\text{C}$  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  $^{13}\text{C}$  in the sand fraction.

Further differences among mineral soils were observed in the microbial community composition (Fig. 2) and total biomass (Table 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 (Fig. 2 and Table 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 (Fig. 1 and Table 2). The largest total amounts of carbon and  $^{13}\text{C}$  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  $^{13}\text{C}$  than the novel soil. While the microbial communities that incorporated the isotope were different (Fig. 3), there was greater commonality in the microorganisms that assimilated the glucose than in the overall communities (Fig. 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 – Figs. 2 and 3), lower total microbial biomass (Table 2), and higher  $^{13}\text{C}$  enrichment in the DOC and clay fractions (Fig. 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 (Fig. 1). However, spruce soil microorganisms were highly efficient at incorporating  $^{13}\text{C}$  (Fig. 4), not necessarily for increased biomass (Table 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 (Fig. 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 18:1 $\omega$ 7c, commonly assigned to Gram negative bacteria (Dungait et al., 2011), that we observed in our study (Fig. 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 labeled 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  $^{13}\text{C}$ -glucose as a tracer we were able to determine that microorganisms incorporating the labeled 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.

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