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Processing of ^{13}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}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 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^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

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}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 labeled 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.

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}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 %.

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-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 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 °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).

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