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Microbial utilization of double-labeled aspen litter in boreal aspen and spruce soils

Charlotte E. Norris [a, *], Sylvie A. Quideau [a], Se-Woung Oh [b]

[a] Renewable Resources, University of Alberta, 442 Earth Sciences Building, Edmonton, AB, T6G 2E3, Canada
[b] Department of Chemistry, Mokpo National University, Muan, Chonnam, 58554, Republic of Korea

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ABSTRACT

Aspen (*Populus tremuloides* Michx.) and white spruce (*Picea glauca* (Moench) Voss) are archetypal species of the boreal forest of western Canada. With the many new and varied environmental pressures (e.g., fire, harvesting, climate change) on, this boreal forest biome, we were interested in understanding the importance of potential changes in organic matter inputs to its soil microbial community structure and function. Double -labeled (^{15}N and ^{13}C) aspen leaf litter was utilized as an amendment to the forest floor of two stands, an aspen and a spruce, in a 16-month paired field experiment, where amended mesocosms were compared to control mesocosms with no litter added. Nitrogen mineralization from the aspen leaf litter occurred within both stand types, as evidenced by higher $\delta^{15}N$ values in roots and live aboveground vegetation. In term of carbon fluxes, stable isotope ^{13}C analysis of the forest floor microbial phospholipid fatty acid (PLFAs) indicated a slight initial enrichment within all amended mesocosms, likely due to leaching of ^{13}C enriched solubles from the labeled aspen litter. Within the aspen stand, continued ^{13}C enrichment of most PLFAs demonstrated that incorporation of the litter-derived carbon by a wide range of microorganisms was maintained through both growing seasons. Response of the spruce microbial community differed from that of aspen. Its overall structural composition did not alter in response to aspen litter addition; yet, $\delta^{13}C$ values of several PLFA biomarkers decreased at the end of the first growing season, suggesting that priming stimulated mineralization of a ^{13}C-depleted pool. These results indicate that while nitrogen mineralization was maintained, the deep forest floor carbon stocks of spruce stands may be vulnerable to increased decomposition rates with changing organic matter input.

1. Introduction

Climate change is posing a threat to historical patterns of succession in the boreal forest where disproportionate temperature increases in northern regions (Christensen et al., 2007) may directly affect ecological succession. On the fine textured upland soils of the western Canadian boreal forest, succession is characterized by a relay from the initial pioneer species of aspen (*Populus tremuloides* Michx.) to shade tolerant white spruce (*Picea glauca* (Moench) Voss) emerging to dominate the canopy following aspen mortality (Bergeron et al., 2014). In North America the boreal forest extends across the continent below the permafrost zone with deciduous dominated stands in the east and coniferous stands in the west. Research in the coniferous dominated boreal forest of North America may help inform how climate change could affect the Canadian western boreal forest. For example, there has been a reported decline in the growth of white spruce trees in Alaska, USA due to climate change (Soja et al., 2007). In addition, the predicted increase in number and size of forest fires in the region (Flannigan et al., 2009) could promote deciduous stands as was found for a number of decades following severe burning in Alaska (Beck et al., 2011) and an increase in fire frequency promoted deciduous dominated stands even when conifers are present prior to the fire in the Yukon, Canada (Johnstone and Chapin III, 2006). This led to the conclusion that there will be a shift towards a more deciduous dominated landscape in the boreal forest (Beck et al., 2011). This is important as a new state of equilibrium for the boreal forest may fundamentally alter several key ecosystem processes such as the biogeochemical cycling of carbon and nitrogen.

Soils from the boreal forest typically contain large surficial organic matter accumulation in their forest floors and therefore large stocks of nutrients may become susceptible to loss following

* Corresponding author.
E-mail address: charlotte.norris@ualberta.ca (C.E. Norris).

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disturbance. For example, disturbance may include partial loss due to forest fires or increased rates of decomposition of the forest floor from increased atmospheric temperatures. Aspen and white spruce trees, two archetypal species of the Canadian boreal forest, produce very different litters with a greater proportion of more complex aromatic biopolymers in spruce litter than in aspen (Preston and Trofymow, 2000; Hannam et al., 2005). Biopolymers are known to have variable rates of decomposition in the soil environment based on both their chemical structure and the surrounding environmental conditions (Quideau et al., 2000; Podrebarac et al., 2016). Furthermore, there appears to be a home field advantage (HFA) where microbial communities are better adapted and decompose litter at a faster rate in ecosystems that have a plant composition similar to the one litter is derived from (Gholz et al., 2000); e.g., leaf litter decomposes faster in a deciduous than a coniferous forest stand. Differences in decomposition rates are particularly evident with greater differences in litter quality (Ayres et al., 2009). Therefore, a shift in soil organic matter inputs from white spruce to aspen litter could alter spruce forest floor characteristics and possibly slow-down biogeochemical processes such as decomposition and mineralization.

Different soil microbial communities exist under aspen and white spruce (Hannam et al., 2004, 2006). Resolution of microbial community structures by Hannam et al. (2006) was achieved by extracting and characterizing soil phospholipid fatty acids (PLFAs); a technique which has also demonstrated how microbial communities vary across the landscape (Grayston and Prescott, 2005; Ushio et al., 2008), and within a particular location following fire (Williams et al., 2012) or harvest (Mummey et al., 2010). Combining stable isotope tracers to compound specific analysis of PLFA biomarkers provides a means to probe various influences (e.g., vegetation, climate or disturbance type) on fundamental soil processes such as carbon and nitrogen biogeochemical fluxes. This experimental approach has been used to investigate soil microbial response to ^{13}C-glucose addition in terms of recycling of the label (Ziegler et al., 2005), carbon fluxes in different soil types (Brant et al., 2006; Norris et al., 2013), and priming effects (Garcia-Pausas and Paterson, 2011). However, there are few cases (e.g., Moore-Kucera and Dick, 2008; Rubino et al., 2010) of field scale studies utilizing complex labeled substrates to investigate soil microbial dynamics.

With the many current and future environmental pressures (e.g.; harvesting, mining and global climate change) which could shift the western boreal forest to an earlier successional stage, we were interested in understanding the importance of changing litter inputs to the soil microbial community structure and function of the climax forest type. Previously we reported that while both aspen and spruce soil microorganisms utilized ^{13}C labeled glucose, their respective community composition remained unique as determined in a laboratory incubation with stable isotope probing of microbial PLFAs (Norris et al., 2013). Here, we were interested in building upon our understanding of aspen and spruce soil microbial dynamics by utilizing a complex organic matter source; i.e., double-labeled (^{15}N, ^{13}C) aspen leaf litter. Further, we chose to conduct our incubation experiment in the field for more representative conditions. Specifically we were interested in tracing soil carbon and nitrogen fluxes following the addition of aspen leaf litter to the forest floor of a spruce dominated stand. We hypothesized that the composition of the microbial community in the spruce forest floor would change with this shift in litter input. To test this hypothesis we established a field incubation on a representative spruce forest where we amended mesocosms with double-labeled aspen leaf litter and compared them to paired control mesocosms with no litter addition. Labelling leaf litter with ^{15}N provided a stable isotope tracer to quantify nitrogen fluxes within the system, while ^{13}C provided a means of assessing the active microbial community by compound-specific stable isotope analysis of chosen PLFA biomarkers.

Lastly, we conducted a comparable field incubation in an adjacent aspen forest, where we hypothesized that the soil microbial community would not change with the addition of aspen litter. Further, following the HFA hypothesis, we expected that the aspen microbial community would be able to incorporate the labeled litter more readily than in the case of the spruce forest.

2. Methods

2.1. Organic matter 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, Canada. Double labelling of leaves was accomplished through 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 concurrently with the ^{13}C labelling from July 10 to July 31. For ^{13}C labelling, 100 ml of 99.9% ^{13}C CO_2 gas was applied to selected tree branches enveloped in a Mylar tubular bag (VacPac®, Baltimore MD), with a fixed 36 cm diameter and a variable length adjusted based on the branch length. Trees were left to take up the ^{13}C-enriched CO_2 for 30 min, 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.

2.2. Field sites

The sites chosen for our field incubation experiment consisted of two mature boreal forest stands (greater than 70 years old) located in the same continuous boreal forest landscape from northern Alberta. A canopy of trembling aspen (Aspen) dominated the first site, and the second site supported primarily white spruce (Spruce). Both of these sites were included in earlier studies examining soil microbial communities in the area (Hahn and Quideau, 2012; Norris et al., 2013). The sites were located within 3 km of each other (Aspen N56 57.5 W111 38.9 and Spruce N56 56.6 W111 44.3) to minimize climatic variations between them. There is an average of 69 frost-free days, a mean annual temperature of 0.7 °C and mean annual precipitation of 455 mm in the area (Environment Canada, 2013). Both stands were growing on fine-textured Gray Luvisol soils, which supported a wide diversity of understory vegetation, as surveyed by Hahn and Quideau (2012).

Our overall experimental design consisted of a paired incubation experiment, where mesocosms amended with aspen litter were compared to control mesocosms with no litter added. Three replicate plots (roughly 5 m by 5 m) were established at least 10–15 m apart at the Aspen and Spruce sites, a scale at which microbial variables are spatially independent (Das Gupta et al., 2015). The importance of canopy cover and distance to tree trunks in influencing soil microbial communities was reported in previous research conducted in the same geographical area (Sorenson, 2011). Therefore, to minimize the confounding effect of tree canopies, we consistently established our mesocosms for the incubation experiment within 1 m of tree trunks. Each replicate plot included three healthy dominant trees (see Fig. 1 for schematic diagram of an Aspen plot). One set of paired mesocosms was placed under the canopy of each of these three trees, within 1 m of the tree trunk. In addition, the paired mesocosms were installed within 1 m of each other. Each mesocosm was delineated by a cylinder constructed of SDR 35 PVC plastic (diameter of 20 cm, length of 11 cm), which was inserted approximately 7 cm into the ground. In May