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Shifting patterns of microbial N-metabolism across seasons in upland Alaskan tundra soils

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ABSTRACT

In the arctic tundra, N controls the productivity and composition of tundra plant communities. In the Alaskan tundra, microbes only ever appear to be N-limited in *Eriophorum vaginatum*-dominated tussock tundra, and that only during the summer growing season; during winter microbes continue to respire but show no evidence of N-limitation. What drives the shifts in microbial N-limitation and how are they developed by the metabolic pathways involved in processing available soil organic compounds? To answer these questions, we collected soils from tussock and shrub tundra during four seasons and incubated them with ^{13}C-labeled glucose, glutamate, and protein; then we measured ^{13}C in CO_2, microbial biomass, and specific phospholipid fatty acids (PLFAs). By analyzing ^{13}C-PLFAs, we were able to assess whether different groups of microbes processed the substrates differently, and to assess changes in N-use brought on by the Arctic winter. Monomers were metabolized during both seasons. In tussock tundra, glutamate-C was assimilated into PLFAs more extensively in winter than summer, suggesting glutamate was used as a C-source during winter but as a N-source during summer. In shrub soils, the flow of C from glutamate to PLFAs tracked with glucose year-round suggesting that the communities were primarily using glutamate as a C source. These results parallel biogeochemical evidence showing shifts in N availability and limitation. Protein metabolism was negligible in winter in soils other than tussocks. In summer, protein was broken down and all communities incorporated C; however, fungi did not assimilate protein-C at all while Gram-positive bacteria appeared to be proteolytic specialists. The different patterns of metabolism of C vs. N containing compounds across microbial groups regulate the dynamics of both soil communities and of soil carbon and nitrogen in tundra soils. As such, the flow of ^{13}C into different biomarker PLFAs provided a lens to evaluate the shifting dynamics of microbial communities and of the resource environment in which they find themselves.

1. Introduction

How soil microorganisms metabolize different organic molecules is fundamental in driving ecosystem function and is increasingly central to our understanding of ecosystem dynamics. For example, microbial carbon use efficiency (how much of a substrate is synthesized into biomass vs. respired to CO_2) has emerged as a critical term in global biogeochemical models (Geyer et al., 2016; Wieder et al., 2013). Yet the significance of microbial metabolic pathways and controls is not limited to just regulating C sequestration for the whole ecosystem. Rather, how microbes process specific N-containing compounds can both reflect and control whether microbes are primarily limited by C or N (Geisseler et al., 2010; Manzoni and Porporato, 2007). Shifting patterns of metabolism may therefore reflect how microbial systems respond to, and control environmental and vegetation changes in a range of ecosystems, perhaps most notably those that experience strong shifts over space or time (diurnal, seasonal, or even interannual).

One biome where both spatial and temporal shifts affect the microbial C/N stoichiometry that ties together plant and soil processes is the arctic tundra (Melle et al., 2015; Sistla et al., 2014). Nitrogen is a critical limiting resource that controls both the productivity and composition of tundra plant communities (Jonasson et al., 1999; Shaver et al., 1986). As vegetation becomes increasingly dominated by the deciduous shrubs *Betula nana* and *Salix* spp., N appears to become progressively less limiting (Weintraub and Schimel, 2005a), apparently as a result of a positive feedback between soil microbes and plants that produce high quality litter

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(Hobbie, 1996). A major factor that appears to limit tundra plant access to N is competition with soil microbes (Jonasson et al., 1999; Schimel and Chapin, 1996) which can themselves be N-limited (Sistla et al., 2012; but see Melle et al., 2015). Yet, in the Alaskan Arctic, microbes only ever appear to be N-limited in *Eriophorum vaginatum*-dominated tussock tundra (Weintraub and Schimel, 2005a), and that only during the summer growing season (Schimel et al., 2004; Sturm et al., 2005); during winter microbes continue to respire but show no evidence of N-limitation (Giblin et al., 1991; Schimel et al., 2004). Rather, bioavailable C appears to become limiting when soils freeze, leading to net mineralization (Buckeridge and Grogan, 2008; Giblin et al., 1991; Miller et al., 2007; Schimel et al., 2004).

The shifts in the degree of overall microbial N limitation with season and plant community raises questions of what drives these shifts and of how they are developed by the metabolic pathways involved in processing available soil organic compounds. The shifting balance of C vs. N availability across vegetation type and seasons will regulate how microbes process N-containing substrates (Schimel et al., 2004) and ultimately therefore should structure microbial communities, and the fate of organic molecules in these systems, as well as how much N is available to support plant growth.

Most of the N in soil enters as plant proteins (Kögel-Knabner, 2006; Weintraub and Schimel, 2005b). However, for microbes to access this N, they must depolymerize the protein, generally by extracellular proteases (Lipson et al., 2001). During the growing season, extracellular enzymes function effectively (Weintraub and Schimel, 2005b). However, when soils are frozen, as they are for nine months of the Arctic year, these enzymes are less effective (Wallenstein et al., 2009). First, enzymes and their breakdown products must diffuse through the water films that survive in frozen soil (Watanabe and Mizoguchi, 2002). Second, the catalytic capacity of the enzymes is reduced (Feller, 2003; Feller and Gerday, 2003; Wallenstein et al., 2009). Once a protein is fragmented, however, microbes must still take up the released amino acids and metabolize them.

Microbes metabolizing amino acids face an allocation dilemma: target it as a C source or as an N source? The relative availability of inorganic N and soluble C may dictate the outcome of this decision. When microbes are N-limited, they are more likely to use amino acids as an N-source either by directly incorporating them into protein, or by using one amino acid as an amino-group donor in a transamination reaction to allow them to produce different amino acids (Moat et al., 2002). If microbes are C-limited (N-saturated) they may use oxidative deamination to mineralize the N to NH_4^+ (Moat et al., 2002). Both transamination and oxidative deamination leave an α-keto acid which can either be used as a C-skeleton for biosynthesis of other cell constituents or catabolized and respired.

Measurements of bulk soil processes (e.g. mineralization/immobilization) suggest overall patterns of C vs. N availability and limitation, but they cannot give deeper insight into the specific metabolism occurring or how different organisms may be experiencing their local resource environments. Microorganisms have distinct life histories and vary in their abilities to use particular substrates (Fierer and Lennon, 2011; Philippot et al., 2010; Schimel and Schaeffer, 2012). How different groups of microbes vary their metabolic pathways with changing substrate and environmental conditions will likely influence both the fate of specific substrates and of the microbes using them (Baumann et al., 2012; Schimel and Schaeffer, 2012). Thus, both the composition of the microbial community, and the functioning of the ecosystem may reflect the nature of specific microbial allocation patterns, patterns that shift seasonally in the Arctic as soils freeze and thaw.

Our objective was to determine how specific groups of microbes allocate N-containing substrates throughout the year in tussock vs. shrub tundra. Do microbes use the C from amino acids to fuel energy generation or growth? How does the N-status of the system shift substrate partitioning? How do different groups of microbes use proteins and their amino acid breakdown products—are there consistent patterns of metabolism across microbial community members and soil types in the tundra?

To answer these questions, we collected soils from both tussock and shrub tundra during four seasons throughout the year and incubated them with ^{13}C-labeled glucose, glutamate, and protein; then we measured ^{13}C in respired CO_2, microbial biomass, and specific phospholipid fatty acids (PLFAs). By analyzing ^{13}C-PLFAs, we were able to assess whether different groups of microbes processed the substrates differently, and thus to assess how sensitive patterns of N-use are to plant and microbial community composition and to the dramatic changes brought on by the Arctic winter.

2. Materials & methods

2.1. Sample collection, handling & processing

Soils were collected from two tundra ecosystem types, tussock and shrub, near the Toolik Field Station, Alaska (68° 38′ N, 149° 39' W) in May, June, August and November 2005. These sampling times represented late winter, spring, summer and early winter respectively.

Soil in tussock tundra is classified as a Typic Aquiturbel and was collected from beneath tussock (TU) mounds or from the intertussock (IT) zones. Tussock mounds are formed from dead *Eriophorum vaginatum* roots while intertussock zones are comprised of mosses and shrub seedlings (*Betula nana* & others). Shrub tundra soil is classified as an Aquic Umbrothel and forms beneath a mixture of willow (*Salix* spp.) and dwarf birch (*B. nana*) shrubs. In shrub tundra, soil was collected from the surface organic layer.

When soils were frozen, cores (8 cm diameter, 20 cm deep) were drilled using a SIPRE ice auger (Jon's Machine Shop, Fairbanks, Alaska). Thawed growing season samples were cut by hand using a serrated knife. Winter samples were kept frozen at all times by processing soil either outside in Alaska during the winter or by being shipped to California on dry ice and then processed in a walk-in freezer. Obvious plant material was removed from the tops of cores using a power saw. Core sections were then smashed by hand with a hammer into pieces approximately 1 cm or smaller, and were then ground in a stainless steel Waring blender. Smashing and blending is the frozen-soil equivalent to sieving mineral soil through a 5 mm sieve. This level of homogenization was required to ensure substrates could be added uniformly. Thawed samples from spring and summer were treated similarly to the winter cores: most living plant material was cut off with a knife, cores were cut into small pieces which were then pulsed through the blender to homogenize them.

2.2. Incubations

Individual cores were too small to provide enough soil for all necessary lab replicates in all experiments. Thus, soil from eight field replicates was bulked for use in the incubations. This may create effective pseudoreplication at the field scale, but allows maximal sensitivity for comparing the physiological patterns across substrate treatments, which is the focus of this study.

Incubation experiments were conducted on 6 g (dry weight equivalent) of soil in 1 L canning jars. Treatments were prepared in quadruplicate and consisted of 100 μg C g^{-1} soil added in aqueous solutions of glucose or L-glutamic acid (glutamate) or 24 μg C g^{-1} soil of SH3 domain protein (Cambridge Isotope Laboratories, Inc.,