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Do shifts in life strategies explain microbial community responses to increasing nitrogen in tundra soil?

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Subarctic tundra soils store large quantities of the global organic carbon (C) pool as the decomposition of plant litter and soil organic matter is limited by low temperatures and limiting nutrients. Mechanisms that drive organic matter decomposition are still poorly understood due to our limited knowledge of microbial communities and their responses to changing conditions. In subarctic tundra large grazers, in particular reindeer, exert a strong effect on vegetation and nutrient availability causing drastic nutrient pulses in the soils located along the migratory routes. Here we studied the effect of increased nitrogen (N) availability on microbial community structure and activities by laboratory incubations of soil collected from two sites with contrasting grazing intensities. We hypothesized that heavily grazed soil experiencing nutrient pulses harbor more copiotrophic taxa that are able to respond positively to increases in available N leading to increased enzyme activities and respiration. Contrary to our hypothesis, there were only minor differences in the microbial community composition between the lightly and heavily grazed soils. N amendment shifted the bacterial community composition drastically, but the changes were similar at both grazing intensities. The relative abundance of diverse *Actinobacteria* and *Rhodanobacter*-affiliated *Gammaproteobacteria* increased in the N amended microcosms, while the abundance of *Acidobacteria, Alphaproteobacteria, Deltaproteobacteria, Verrucomicrobia* and *Bacteroidetes* decreased. Contrary to our hypotheses, increased N availability decreased respiration and microbial biomass at both grazing intensities, while increased N availability had little influence on the extracellular enzyme activities. We propose that similar to what has been reported in other systems, elevated N availability suppressed microbial respiration and biomass by favoring copiotrophic species with faster growth rates and with limited capabilities to decompose recalcitrant organic matter. Similar responses in soils from contrasting vegetation types, soil organic matter (SOM) quality and N availabilities in response to grazing intensity indicate that nutrient pulses may have a strong direct impact on the microbial communities. Responses detected using laboratory incubations are likely amplified in the field where the direct effect of increased N availability is combined with increase in labile C through changes in plant production and species composition.

1. Introduction

As subarctic tundra soils store a significant proportion of the global soil C stock, there is a considerable interest in understanding how the C stored in these systems will respond to changes in environmental conditions. In addition to low temperatures as a factor that limits primary production and soil microbial activity, the role of N availability for soil C decomposition is considered to be a key factor in the responses of soil C stocks to climate change (Hobbie et al., 2002; Robinson, 2002; Mack et al., 2004). Accelerated SOM decomposition and nutrient mineralization in response to climate warming may lead to increased nutrient availability, which can in turn further increase decomposition rates. However, the role of increased N availability on soil microbial biomass and

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activities remains under dispute due to the fact that there are two contrasting lines of evidence regarding the direction of the N effect on soil microbial biomass and activities. The first line of evidence suggests a negative effect of increased N, as fertilization experiments across biomes have indicated that increased N availability reduces microbial biomass and activities (Treseder, 2008; Ramirez et al., 2010, 2012). The negative effect of N enrichment on respiration has been attributed to inhibitory effects of added N on ligninolytic enzymes (e.g. Sinsabaugh, 2010), reduced need of the microorganisms to decompose organic matter for N acquisition (Craine et al., 2007), and switching of the microbial community from overflow metabolism to utilization of C for growth (Schimel and Weintraub, 2003). In overflow metabolism microorganisms respire the excess C that cannot be utilized for growth due to nutrient limitations. Thus adding N to nitrogen limited systems may reduce respiration as it allows the microbes to utilize the excess C for growth (Schimel and Weintraub, 2003). The "nitrogen mining hypothesis" (Craine et al., 2007) suggests that SOM decomposition may be driven by N limitation whereby microbes utilize complex organic matter as a N source rather than a C source.

Especially in Arctic tundra, another line of evidence suggests a positive effect of increased N. In these extremely N-limited tundra soils, low N availability may limit the production of extracellular enzymes and, hence, increased N availability may accelerate SOM decomposition by enhancing microbial enzyme production (Schimel and Weintraub, 2003; Wallenstein et al., 2009; Sistla et al., 2012), especially enzymes that hydrolyze C-rich compounds (Koyama et al., 2013; Stark et al., 2014). Long-term fertilization experiments in Alaskan tundra have shown increased C losses due to increased N availability in the soil organic layer (Mack et al., 2004), suggesting that increased N availability subjects centuries old SOM to microbial decomposition (Nowinski et al., 2008).

The divergent results on the effects of N on microbial biomass and activities reflect the close and complex inter-linkages between soil N and C cycles. The mechanisms by which microbial communities influence the nutrient cycles are still poorly understood, but the community structure is considered to be a key determinant of the functions (Fontaine et al., 2003; Fierer et al., 2012; Chen et al., 2014; Leff et al., 2015). Both field and laboratory experiments have identified major shifts in microbial community structure after fertilization treatment (Nemergut et al., 2008; Ramirez et al., 2010, 2012; Fierer et al., 2012; Koyama et al., 2014; Leff et al., 2015) which are considered to reflect concurrent changes in microbial biomass (Fierer et al., 2012), enzymatic activities (Koyama et al., 2014) or soil respiration (Ramirez et al., 2010, 2012). It has been suggested that N availability is an important determinant for the dominant life strategy of the soil microbial community (Fontaine et al., 2003; Fierer et al., 2012; Chen et al., 2014; Leff et al., 2015). The priming effect theory predicts that under N limitations, microbial communities are dominated by slow growing K-strategists (oligotrophic species) that are able to mine nutrients in SOM whereas under abundant N concentrations, microbial communities are dominated by fast-growing r-strategists (copiotrophic species) that utilize mineral N and more labile C (Fontaine et al., 2003; Chen et al., 2014). A decrease in respiration and enzyme activities through increased N availability has been linked to a shift in the microbial community to more copiotrophic species which have higher growth rates and carbon use efficiencies, and utilize simpler C sources with reduced need for extracellular enzyme production (Fierer et al., 2012; Ramirez et al., 2012; Leff et al., 2015). The abundance of *Actinobacteria, Proteobacteria* and *Bacteroidetes* has been shown to increase with increased N availability and these are suggested to represent copiotrophic taxa while members of the *Acidobacteria* and *Verrucomicrobia* have been considered to represent oligotrophic taxa (Fierer et al., 2007, 2012; Ramirez et al., 2012; Leff et al., 2015).

Investigations on the effects of increased N availability on soil microbial activity and community composition have considered mainly steady increases in soil N availability due to long-term fertilization, while little attention has been paid to plant–animal interactions, which may result in high temporal and spatial variation in soil N availability. Large migratory grazers have a substantial effect on vegetation across the world's biomes, with important effects on soil C storage as well as N availability (Tanentzap and Coomes, 2012). In subarctic tundra, grazing by reindeer (*Rangifer tarandus* L., same species as caribou in the northern parts of the American continent) causes important changes in vegetation (Zamin and Grogan 2013; Bernes et al., 2015), N availability (Olofsson et al., 2004), and soil microclimate (Stark et al., 2015), but grazing also leaves significant temporal variation in soil N. Reindeer migrate annually between summer and winter ranges depending on the availability of suitable forage, and in sites located along the migration routes reindeer herds cause substantial N pulses with drastically increasing N concentrations repeated each year (Stark and Väisänen, 2014). Migratory grazing is often associated with a shift in the dominant vegetation from evergreen and deciduous dwarf shrubs to a grassland that is associated with enhanced plant productivity, soil N availability, and microbial respiration (Olofsson et al., 2004). Tundra systems along migration routes thus differ from the surrounding systems in several ways; while lightly grazed systems experience continuously low N concentrations and are dominated by slow-growing dwarf shrub plant species, systems along the migration route experience drastic N pulses each year during the reindeer migration and are dominated by graminoids. This difference in the natural patterns of soil N concentrations could provide interesting opportunities for investigating the soil microbial responses to increasing N, and offer novel insights into the capacity of microbial communities for adapting to different patterns of soil nutrient availability.

Here, we hypothesized that – resulting from community-level adaptations to prevailing N levels – soil microbial responses to increasing N availability depend on natural patterns in soil N concentrations. Understanding this relationship was expected to improve our understanding on the basic mechanisms by which microbial communities influence tundra soil C and N cycles. We tested the hypothesis through laboratory incubations with experimental N additions to soils from two sites with contrasting reindeer grazing intensities. In a lightly grazed area, the access of reindeer during migration is prevented by a fence built in the 1960's, the vegetation is dominated by dwarf shrubs and the soil contains low N concentrations with little temporal variation (Stark and Väisänen, 2014). In a heavily grazed area, reindeer migration causes a drastic N pulse every year, the vegetation is dominated by graminoids and the soil shows higher N concentrations as well as extreme temporal variation during the growing season. We hypothesized that parallel to shifts in vegetation and soil N, grazing has induced shifts in the microbial community composition towards higher functional adaptation of the soil microbial community to sudden increases in N. More specifically, we predicted that bacterial communities and decomposition under lightly grazed N-poor soils are driven by more oligotrophic, slow growing K-strategist bacteria that utilize organic N by decomposing the recalcitrant C pools (a.k.a nitrogen mining). Consequently increased N availability triggers only weak responses in the microbial activities. On the other hand, we predicted that bacterial communities in the heavily grazed soils with higher N availability are dominated by more copiotrophic r-strategist species that respond more strongly to increased nutrient availability which in turn is detected as increased respiration, enzyme activities for C acquisition and significant shifts in microbial community structure reflecting growth of the faster growing r-strategist taxa.