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Detecting microbial N-limitation in tussock tundra soil: Implications for Arctic soil organic carbon cycling

Seeta A. Sistla*, Shinichi Asao [1], Joshua P. Schimel

Department of Ecology, Evolution and Marine Biology, University of California Santa Barbara, Santa Barbara, CA 93108, USA

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ABSTRACT

More than a third of the global soil organic carbon (SOC) pool is estimated to be stored in northern latitudes. While the primary regulators of microbially-mediated decomposition in physically unprotected organic soils are typically attributed to abiotic factors (e.g. temperature and moisture), in extremely nutrient-poor environments such as the Alaskan Arctic tussock tundra, evidence from field studies suggests that low N-availability may also strongly limit microbial growth, and thus the rate of SOC decomposition. However, there have been few direct tests of microbial nutrient-limitation, particularly in Arctic systems. We predicted that during the Arctic summer growing season, when both plants and microbes are competing for mineralized nutrients, N-availability in tussock tundra soil is so low that it will limit microbial biomass production, and thus decomposition potential. We tested this prediction by adding N and C to tussock tundra organic soil and tracking microbial responses to these additions. We used a combination of approaches to identify microbial N-limitation, including changes in microbial biomass, C-mineralization, substrate use efficiency, and extracellular enzyme activity. The Arctic soil's microbial community demonstrated strong signals of N-limitation, with N-addition increasing all aspects of decomposition tested, including extracellular enzyme activity, the rate-limiting step in decomposition. The corresponding C-addition experiment did not similarly influence the microbial activity of the tundra soil. These results suggest that tundra SOC decomposition is at least seasonally constrained by N-availability through microbial N-limitation. Therefore, explicitly including N as a regulator of microbial growth in this N-poor system is critical to accurately modeling the effects of climatic warming on Arctic SOC decomposition rates.

1. Introduction

Slow decomposition rates in permafrost soils is a major driver in the development of a soil organic carbon (SOC) pool that is estimated to be 1672 Pg—more than twice as much carbon (C) as is in the atmosphere (Schlesinger and Andrews, 2000; Tarnocai et al., 2009). In Arctic tundra organic soils, although the soil organic matter (SOM) is not physically protected (Weintraub and Schimel, 2003), C accumulates and microbial biomass accounts for only 2–4% of the total C (Cheng and Virginia, 1993; Jonasson et al., 1996). The slow decomposition of C-rich material is promoted by the biome's cold climate, which supports the buildup of SOC and concomitant low bio-availability of nitrogen (N), producing a highly N-limited plant community (Shaver and Chapin, 1980, 1986; Chapin and Shaver, 1989; Giblin et al., 1991). Climate models unequivocally predict that the Arctic will significantly warm during this century (Moritz et al., 2002; Solomon et al., 2007), however, projecting the extent to which warming will alter net Arctic C balance depends upon mechanistically understanding how N-availability regulates tundra SOM decomposition (Mack et al., 2004; Weintraub and Schimel, 2005; Sturm et al., 2008).

Despite the recognition of the intensity of N-limitation to plants in tundra systems (Giblin et al., 1991; Shaver and Chapin, 1986), there have been relatively few direct tests of the potential for microbial nutrient-limitation to limit tundra SOM decomposition (e.g. Jonasson et al., 1996; Mack et al., 2004; Churchland et al., 2010; Lavoie et al., 2011). While N-addition has been demonstrated to drive a wide array of effects on C turnover in a variety of systems, ranging from enhancement to the inhibition of decomposition (e.g. Craine et al., 2007; Cusack et al., 2010), several studies have identified N as a potential regulator of tundra SOC decomposition. This includes both modeling work (Moorhead and Reynolds, 1993) and empirical experiments showing that N-additions to tundra organic soils can

* Corresponding author. Tel.: +1 518 331 8309; fax: +1 805 893 5885.
E-mail addresses: sistla@lifesci.ucsb.edu, seetasistla@gmail.com (S.A. Sistla).
[1] Current address: Graduate Degree Program in Ecology, Natural Resources Ecology Laboratory, Colorado State University, Fort Collins, CO 80523-1499, USA.

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promote microbial N-immobilization, enhance microbial activity and potential SOC loss (Jonasson et al., 1996; Mack et al., 2004; Churchland et al., 2010; Lavoie et al., 2011). These studies suggest that it is not simply temperature that limits decomposition in this system. To break down plant polymers, microorganisms rely on extracellular enzymes, which are N-rich; when N is limiting, microbial investment in enzymes can control the dynamics of the decomposer system (Schimel and Weintraub, 2003). If N-limitation is extreme enough, it is possible that microbes would lack adequate N to both make the enzymes needed for decomposition and the biomass needed for survival and growth.

Most tundra SOM research has historically focused on temperature (e.g. McKane et al., 1997; Rustad et al., 2001; Walker et al., 2006; Oberbauer et al., 2007; Schuur et al., 2008), SOM chemistry (Giblin et al., 1991; Weintraub and Schimel, 2003), or the interaction between soil chemistry and temperature as a primary regulators of tundra decomposition (Giblin et al., 1991; Nadelhoffer et al., 1991; Hobbie et al., 2000, 2001; Shaver et al., 2006). While these studies have identified site-driven differences in SOM C chemistry (likely driven by differences in chemistry quality of plant litter inputs) as a primary predictor SOM C turnover rates at broad spatial scales, several long-term soil incubation studies have demonstrated that despite substantial and relatively constant rates of C loss for a variety tundra organic soils, SOM C chemistry remains relatively unchanged, with decomposition of the major chemical fractions being proportional to lignin degradation (Weintraub and Schimel, 2003; Shaver et al., 2006). These findings suggest that tundra SOM consists of a large pool of potentially mineralizable C, with all SOM fractions relatively equally available to microbial attack, despite being in later stages of decomposition (Weintraub and Schimel, 2003; Shaver et al., 2006). However, these studies are not conclusive about the mechanism limiting decomposition of this potentially available material.

Given that nutrient addition experiments suggest microbial productivity in the organic horizon may be at least seasonally N-limited (Moorhead and Reynolds, 1993; Jonasson et al., 1996; Mack et al., 2004; Churchland et al., 2010; Lavoie et al., 2011), and that field studies reveal substantial *in situ* net N-immobilization during the growing season (Giblin et al., 1991), microbial N-limitation appears to be a likely potential regulator of Arctic SOM decomposition. This effect contrasts to N-addition studies in less N-limited soils, where increased N availability is hypothesized to decrease mineralization of recalcitrant N, while not affecting the mineralization rate of more labile N sources (Craine et al., 2007; Janssens et al., 2010).

If N limits tundra organic soil's microbial growth and enzyme synthesis, and hence directly controls decomposition and C-cycling, it would require rethinking biogeochemical models of tundra ecosystems (McKane et al., 1997). Yet, microbial N-limitation is difficult to demonstrate because it is challenging to measure microbial growth in soil and to separate biomass growth from changes in metabolism. It is important to conclusively establish the mechanism by which N controls C-storage in tundra ecosystems (e.g. Sullivan et al., 2007) before reconstructing our paradigms about tundra soil processes.

One challenge in assessing N-limitation to decomposers is that under moderate N-limitation, enzyme production (and thus decomposition) may not be significantly restricted, but cellular growth may be. Under extreme N-limitation, however, microbes may not have adequate N to synthesize the needed enzymes, limiting decomposition itself (Schimel and Weintraub, 2003). Microbial dynamics may therefore be constrained by C, either as quantity (availability) (Waksman and Stevens, 1929; Tate III, 1995) or quality (recalcitrance) (Fontaine et al., 2003); or by N, as a resource for synthesizing either cellular biomass or extracellular enzymes. Limited extracellular enzyme production may drive secondary C-limitation regardless of the potential C supply (Fontaine et al., 2003; Schimel and Weintraub, 2003).

Separating these different mechanisms requires more sophisticated analyses than simply measuring changes in respiration with C or N-additions. For example, if microbial growth is moderately N-limited, adding C can stimulate microbial respiration without affecting growth, while adding N can reduce respiration but increase growth (Schimel and Weintraub, 2003). If N-limitation is severe however, we hypothesize that adding N would increase extracellular enzyme production and stimulate both respiration and microbial growth. Understanding the nature of microbial responses to additions of limiting resources requires distinguishing the responses of enzyme production, shifts in cellular C-allocation, and overall respiration. Misrepresenting the responses to added resources on microbial processes could misestimate the impact of changing nutrient availability on decomposition dynamics.

To determine whether microbial activity is N limited in an Arctic tundra soil, and to assess the intensity and mechanisms of response to limitation, we performed a laboratory incubation study on soil from an Alaskan tussock tundra site. To ensure that the observed responses to N-addition were due to N as a limiting resource (and not C quality), we contrasted the responses of labile N- and C-addition to soil microbial processes. We used two different concentrations of C and N, and assessed thier effects on: respiration, substrate-use efficiency (SUE), cellulase activity, and microbial biomass, which are key indices of the functioning of the soil's microbial community. We hypothesized that summer-collected tussock tundra organic horizon soil is highly N-limited from the microbial perspective, such that adding a labile N source to them will stimulate decomposition by increasing the community's SUE, thereby shifting C allocation towards greater growth and extracellular enzyme synthesis. We postulated that this effect would increase with greater N substrate concentration. In contrast, we hypothesized that additional C would not increase the tundra soil's microbial growth or SUE, although C-mineralization may be stimulated by microbial processing of newly available highly labile C.

2. Materials and methods

2.1. Site description

Experimental soils were collected from moist acidic tundra (MAT) near Toolik Lake, Alaska, USA (68°38′N, 149°34′W). MAT forms on old glacial surfaces (>11,000 years BP). The tussock-forming sedge *Eriophorum vaginatum* L. drives the formation of regular vegetation patterns, with deciduous shrubs, forbs, and herbaceous plants growing between the tussocks. The vegetation in the experimental area is similar to MAT across the Alaskan North Slope, northern Canada, and eastern Siberia (Oechel et al., 1993). There is approximately 0.9×10^6 km^2 of MAT tussock tundra worldwide (Oechel et al., 1993).

MAT soil is classified as coarse-loamy, mixed, acidic, gelic Typic Aquiturbels (Romanovsky et al., 2011). Mean air temperature during the June–August growing season is 9 °C, with a mean annual temperature of −8.5 °C and total precipitation is on average 350 mm (Deslippe and Simard, 2011). In the growing season, soil temperatures rapidly decline with depth, ranging from 10 to 20 °C at the surface to 0 °C at the bottom of the seasonally thawed active layer, which varies from 30 to 60 cm depth in late July (Shaver et al., 2006).

2.2. Soil collection and initial storage

Eight field replicate soil samples were taken from MAT tundra in July 2002, along random points of arbitrarily laid transects following the methods of Weintraub and Schimel (2003). We

defined our target tussock tundra organic soil material as decomposed organic matter consisting primarily of decaying roots, collected directly underneath *E. vaginatum* plants. Any loose green litter surface material and tussock thatch (standing dead tussock shoots) were removed, and decomposed organic soil was collected by hand cutting approximately a 10 cm^{-2} area with a serrated knife to approximately 5 cm depth beneath the standing thatch and surface material. Immediately following collection, soils were hand-sorted to remove debris and live plant material, including visible live roots (*E. vaginatum* have characteristic white roots that are easily distinguishable from surrounding SOM). The field replicates were homogenized by hand mixing, and the soils were shipped on ice packs to the University of California at Santa Barbara, where our experiments and analyses were conducted.

The tussock tundra organic horizon soils were maintained in the dark at 4 °C prior to the C and N-addition experiment, which is within the approximately 0–20 °C soil field temperature range during the growing season for these soils (Shaver et al., 2006). Soil water content (0.158 g dry g^{-1} wet weight) was measured gravimetrically from subsamples of the homogenized soil. Additional soil subsamples were dried to a constant mass at 100 °C and ground to a fine powder, and soil C and N concentrations were determined using a Fisons NA1500 C/N analyzer. The soils were 48% C and 0.74% N, with a C:N of 64. Sub-samples (20 g) of the homogenized field-collected soil were used for all resource addition experiments.

2.3. Resource addition experiments

To determine the amount of N and C to be added, C-mineralization rates were assessed in a pilot study to estimate the amount of C the microbial community could process over a two-week period ($n = 3$). We assumed that 20% of the respired C–CO_2 from the pilot study was incorporated into biomass (Holland and Coleman, 1987) and that microbial biomass has an average C:N of 10 (Cleveland and Liptzin, 2007), such that amount of C used to build microbial biomass over the two week incubation required N equivalent to approximately 2% of the total C respired. Therefore, low N treatment was equivalent to 2% of 14 days basal respiration-C as determined from the pilot study (107 μg N g^{-1} soil; approximately doubling the N that would be consumed during a 2 week incubation), and high N treatment was equivalent to five-times the low N treatment (535 μg N g^{-1} soil). Inorganic N was added drop-wise as a 5 mL aqueous $(NH_4)_2SO_4$ solution.

The C-addition treatment consisted of two levels (low and high) of cellulose $(C_6H_{10}O_5)_n$ to contrast with the effects of the analogous N-addition treatment. Cellulose was added as a powder into the soil subsamples and mixed with 2.5 mL of Milli-Q water that was added drop-wise. The low C treatment was equivalent to 14 days basal respiration-C as determined from the pilot study (5.4 mg C g^{-1} soil), and high C treatment was equivalent to five times the low C treatment (27 mg C g^{-1} soil). Separate water amended controls ($n = 3$) were tracked for the C and N-additions. Following the N- and C-amendments, soils were incubated for 14 days at 20 °C, which is within the upper range of *in situ* surface organic horizon soil temperatures during this period (Shaver et al., 2006), in 0.9 L Mason jars fitted with septa.

2.4. Microbial responses to resource additions

In order to distinguish changes in microbial C use in response to nutrient addition, several physiological responses to the N-additions were characterized, including effects on: C-mineralization, succinate SUE, cellulase extracellular enzyme activity, microbial biomass and dissolved inorganic N (DIN). Changes in C-mineralization, SUE, cellulase extracellular enzyme activity, and DIN in response to C-addition were also characterized. Assays of microbial response to the resource pulses were completed on three analytical replicate samples of each soil type, at either control, low, or high N- or C-addition.

2.4.1. C-mineralization

Cumulative C-mineralization (CO_2 accumulation) was measured over the course of the incubation (14 days). Headspace air samples were drawn from the incubation vessel and injected into an infrared gas analyzer (IRGA, LI-COR 6252, Lincoln NB) at the beginning and end of the incubation period. Cumulative C-mineralization was calculated as the increase in total amount of CO_2 in the vessel per dry mass of soil.

2.4.2. Microbial biomass: substrate induced respiration (SIR)

Microbial biomass was estimated by substrate induced respiration (SIR) only for the N-addition samples. Samples were first amended with autolyzed yeast extract (a 10 mL solution of 3 g yeast extract dissolved into 250 mL Milli-Q H_2O) in airtight test tubes. Headspace CO_2 concentrations were then monitored at 3 time points over a four hour incubation to assure linearity of changing CO_2 concentrations over time. The respiration rate (μg C–CO_2 g^{-1} soil hr^{-1}) over the four hour incubation period is an index of the microbial biomass.

2.4.3. Microbial substrate use efficiency

Substrate use efficiency (SUE) is the efficiency with which organisms build biomass C relative to C respired following substrate uptake, with SUE measured as the ratio of ^{14}C labeled substrate incorporated into biomass over ^{14}C labeled substrate respired. Following the incubation period, SUE was assessed using a ^{14}C labeling method ($n = 3$; consisting of 3 chloroform fumigated and 3 non-fumigated). Each of the soil samples was incubated for 3h with 2,3-^{14}C succinic acid (200 μL, to provide approximately 500 Bq). To compare ^{14}C respired with ^{14}C incorporated into biomass, $^{14}CO_2$ was trapped with a 0.5 mL 2 N NaOH trap and assayed using a liquid scintillation counter. Biomass incorporated ^{14}C was measured by fumigating the samples with $CHCl_3$ and comparing them with non-fumigated samples. Both samples were then extracted with 0.5 M K_2SO_4, and acidified to assay for $^{14}CO_2$. Substrate use efficiency is measured as the ratio of ^{14}C microbial biomass flush relative to respired ^{14}C. Total ^{14}C recovery was calculated for the samples, and ranged between 85 ± 4% for the C-addition and 88 ± 6% for the N-addition.

2.4.4. Cellulase potential

Cellulase activity was assayed as an index of available C-flow to microbes through the decomposition of cellulose, a relatively labile SOC component. Tussock tundra organic horizon soils largely consist of decomposed plant detritus. When incubated for up to one year, despite losing 25% of their total C, tussock organic soil's lignin-cellulose index remained largely unchanged (Weintraub and Schimel, 2003), indicating that lignin decomposition is closely tied to cellulose decay in this soil. Increased substrate availability tends to be correlated with increased extracellular enzyme activity (Sinsabaugh et al., 2008). However, if extracellular enzyme synthesis is N-limited in tussock tundra soils, we would not expect cellulase activity to be stimulated by the presence of additional cellulose substrate in the C-addition experiments.

Total potential cellulase activity was measured as described by Hope and Burns (1987). This method adds the substrate, cellulose, to soil samples to saturate cellulase activity and then measures the concentration of the resulting product, glucose. Crystalline powder cellulose was added to a slurry of 2 g (wet weight) of soil with 0.1 M sodium acetate buffer adjusted to pH

5.5. Sodium azide was added to the buffer to inhibit the microbial uptake of the product. The slurry was then incubated for 16 h at 20 °C and centrifuged to stop the enzymatic reaction. Glucose concentration in the supernatant was measured using a 96-well-plate version of the Nelson-Somogyi colorimetric method (Spiro, 1966), with anhydrous D-glucose as the standard. In a tall 96 well plate, 1 mL of supernatant was mixed with 1 mL of Somogyi-copper solution and heated in a boiling water bath for 20 min. After cooling, 1 mL of Nelson-molybdate solution was added. The mixture was then diluted with 3 mL of water and aliquots were transferred to clear bottom 96 well plate. Absorbance was measured at wavelength of 520 nm. The enzyme activity is expressed as μg of D-glucose equivalent produced per dry gram of soil per hour.

2.4.5. Dissolved inorganic N (DIN)

A sub-sample of each soil type was extracted with 0.5 M K_2SO_4 at the beginning and end of the incubation period to quantify extractable inorganic N. The extracts were analyzed on a Lachat autoanalyzer for NH_4^+ using diffusion method (Lachat method #31-107-06-5-A, Milwaukee, WI), and NO_3^- using a Griess-Ilovsay reaction after cadmium reduction (Lachat Method #12-107-04-1-B, Milwaukee, WI).

2.5. Statistical analyses

For each microbial response to substrate addition, replicate samples were tested for normality. An ANOVA was used to compare means at $\alpha = 0.05$. If a significant difference was identified among means, Dunnett's test comparing each experimental mean with the control mean was used *a posteriori* to identify groups that significantly differed from each other at a family wide error rate of $\alpha = 0.05$ using JMP statistical software (JMP Version 7. SAS Institute Inc., 2007). In all figures, * denotes $p \leq 0.05$, ** denotes $p \leq 0.01$, *** denotes $p \leq 0.001$.

3. Results

3.1. C-mineralization

N-addition stimulated C-mineralization only in the N-poor tundra soil (ANOVA; $F_{2,8} = 151.74$, $p < 0.0001$). High N-addition increased C-mineralization by 144% (Dunnett's $p < 0.0001$), while low N-addition increased mineralization by 133% (Dunnett's $p = 0.04$). The C-addition did not significantly increase C-mineralization in the tussock tundra soil (ANOVA $F_{2,8} = 0.6$, $p = 0.58$; Fig. 1).

3.2. Cellulase activity

N-addition increased the tundra soil's cellulase activity (ANOVA; $F_{2,8} = 18.18$, $p = 0.0028$) to 184% of control (Dunnett's $p = 0.015$). Low N-addition slightly (but not significantly, Dunnett's $p = 0.14$) increased cellulase activity to 129% of the control soil. In contrast to N-addition, the soil's cellulase activity was not significantly affected by C-addition (ANOVA $F_{2,8} = 1.9$ $p = 0.23$; Fig. 2).

3.3. Substrate use efficiency

High N-addition increased the tundra soil's succinate SUE (ANOVA; $F_{2,8} = 12.81$, $p = 0.0068$) to 158% of control (Dunnett's $p = 0.004$), while the low N-addition increase in SUE to 126% of the control was marginally significant (Dunnett's $p = 0.11$). Tussock tundra soil did not significantly increase succinate SUE in response to C-addition (ANOVA $F_{2,8} = 1.93$ $p = 0.23$; Fig. 3).

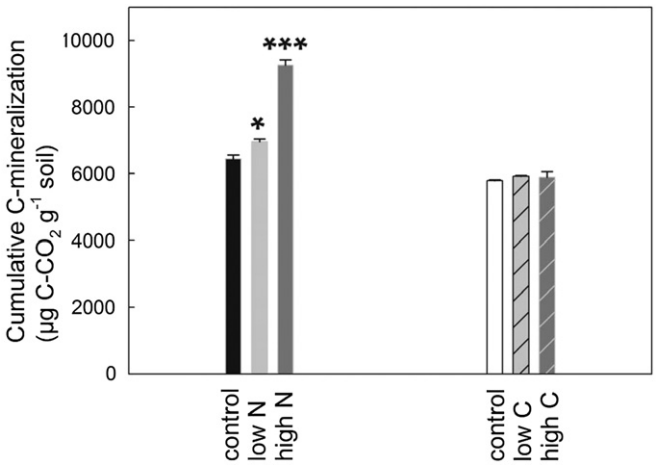

Fig. 1. Cumulative C-mineralization over 2 week incubation period with N and C addition; means ± SE ($n = 3$); asterisks above bars denote significance from respective control: $^*P < 0.05$; $^{***}P < 0.001$.

3.4. Microbial biomass

N-addition stimulated the growth of the tundra soil's microbial biomass (ANOVA; $F_{2,8} = 8.26$, $p = 0.02$). N-addition increased the tundra soil's SIR to 175% ($p = 0.01$) and 148% ($p = 0.08$) of control for the high and low N-additions (Fig. 4).

3.5. Total dissolved inorganic N

N-addition significantly increased total dissolved inorganic N (DIN) (ANOVA; $F_{2,8} = 42.35$, $p < 0.0003$). This increase was significant for the high N-addition (Dunnett's $p = 0.0003$), but the low N-addition had no significant effect on the tundra soil's DIN (Dunnett's $p = 0.81$). Neither C-addition treatment significantly affected DIN (ANOVA; $F_{2,8} = 1.22$, $p = 0.36$; Fig. 5).

4. Discussion

Distinguishing between microbial resource limitation and other factors that affect decomposition rates increases our ability to predict how changing environmental conditions may regulate Arctic SOC dynamics and interrelated ecosystem feedbacks (Hobbie et al., 2000). Given the potential for significant changes in Arctic

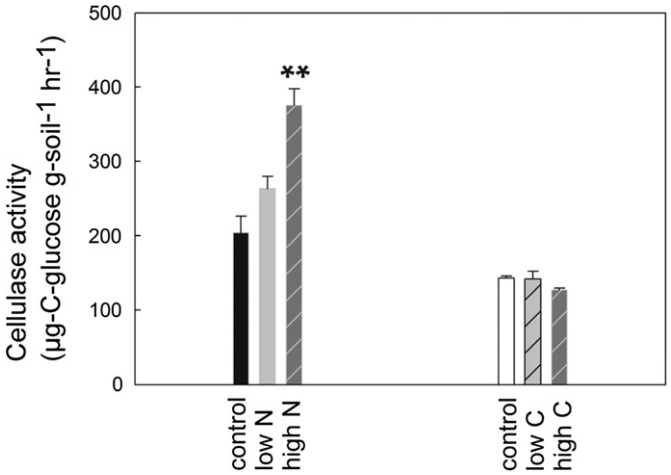

Fig. 2. Potential cellulase activity following 2 week incubation with N and C addition; means ± SE ($n = 3$). $^{**}P < 0.01$.

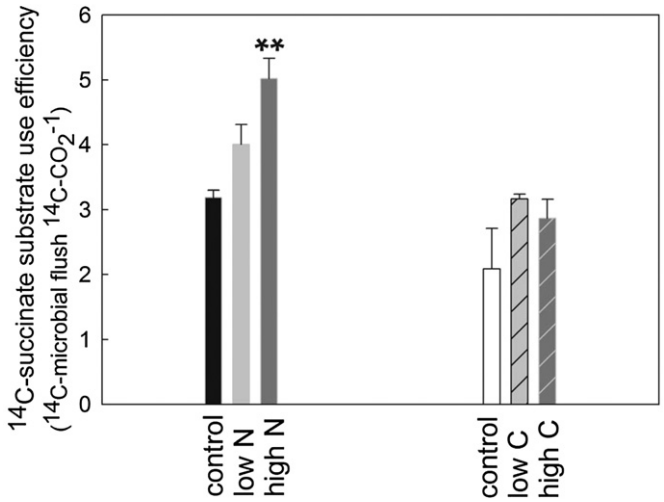

Fig. 3. Microbial 2,3-^{14}C succinate substrate use efficiency following 2 week incubation with N and C addition; means ± SE ($n = 3$). $^{**}P < 0.01$.

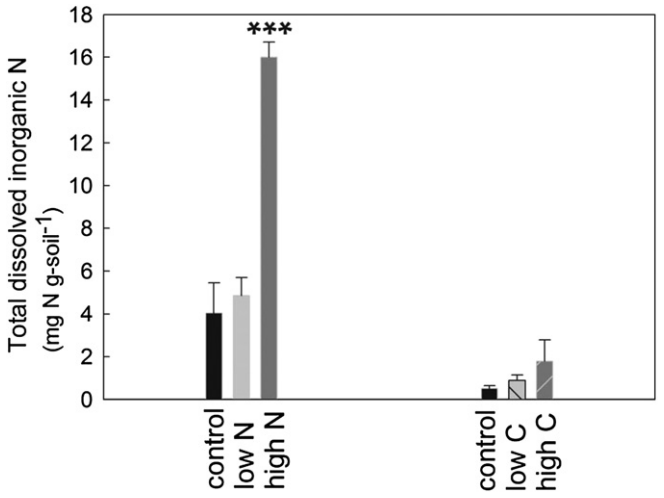

Fig. 5. Total dissolved inorganic N following 2 week incubation period with N and C addition; means ± SE ($n = 3$). $^{***}P < 0.001$.

soils' nutrient availability driven by seasonal climate patterns (Buckeridge and Grogan, 2008) and warming (Chapin III et al., 2005; Sturm et al., 2008), the ability to characterize nutrient limitation as a constraint on microbial growth is critical in this system. In the case of moist acidic tussock tundra organic soils, gaps between the microbial drivers of decomposition and predicted SOM dynamics led to a significant underestimation of the potential for the rate of SOC decomposition to accelerate with nutrient addition. This phenomenon was clearly illustrated by the dissonance between modeled tundra C storage dynamics in response to increase N-availability (McKane et al., 1997) and a tundra field study where fertilization decreased the system's net C storage due to accelerated SOC decomposition, despite substantial increases in plant biomass (Mack et al., 2004).

We predicted that during the Arctic summer growing season, tussock tundra soil was so N-limited that N-availability could limit not only plant growth (Shaver and Chapin III, 1986, 1991), but both microbial biomass production and extracellular enzyme synthesis; our results supported that hypothesis. The N-addition experiments demonstrated that in extremely nutrient-poor environments such as summer-collected Arctic tussock tundra soils, all components of decomposition and microbial growth appear to be N-limited, including the ability to break down and respire C substrates. The high N-addition strongly increased the tundra organic soil's active microbial biomass, extracellular enzyme activity, C-mineralization and SUE, indicating that N limits not only biomass production but also enzyme synthesis this system during the growing season. Furthermore, only the high N-addition stimulated increased DIN concentrations in the tundra soil, suggesting strong microbial N immobilization potential in these soils during the incubation period, similar to field observations (Giblin et al., 1991).

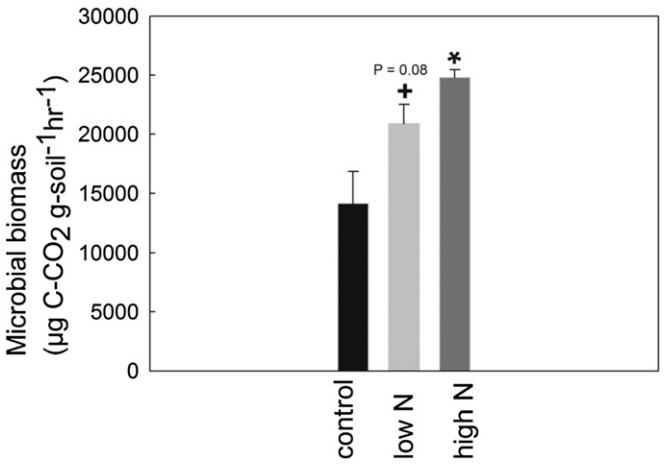

Fig. 4. Microbial biomass as assayed by substrate induced respiration following 2 week incubation with N addition; means ± SE ($n = 3$). $^{*}P < 0.05$; $+ P < 0.1$.

The C-addition experiments provided a secondary line of evidence supporting the conclusion that tussock tundra soil's microbial community is extremely N-limited during the growing season. C-addition had no significant effect on any aspect of the tussock tundra soil microbial metabolism measured, even at an incubation temperature at the high end of the average summer soil temperature (Shaver et al., 2006). C-addition caused no detectable increase in cellulase activity in response to added cellulose, suggesting that greater substrate availability (Sinsabaugh et al., 2008) was not sufficient to stimulate enzyme activity in this system. Additionally, the lack of increased C-mineralization with the addition of a labile C substrate suggests that the tussock tundra soil microbial community was C-saturated at the extracellular enzyme level (Schimel and Weintraub, 2003). This effect contrasted with a stimulation in C-mineralization observed with cellulose addition in a variety of non-tundra soils tested (data not shown).

In contrast to the labile C-addition, N-addition increased C-mineralization in the tussock tundra organic soil, reflecting the increase in microbial biomass and enzyme production observed. These results may add a mechanistic underpinning to studies from the same system suggesting that long-term fertilization drives substantial mineralization of soil C (Mack et al., 2004). Our results also parallel the findings of a study by Lavoie et al. (2011), who found that N-addition stimulated tussock tundra organic soils C-mineralization when incubated at 15 °C. However, the same study reported that N-addition suppressed tussock tundra organic soil's C-mineralization in a 4 °C incubation. Similarly, Shaver et al. (2006) reported no effect of a long-term *in situ* field N-addition on SOC mineralization in a multi-year incubation experiment. The cause of these contrasting results is uncertain, but may be due to freezing the soils for storage prior to initiating their incubation studies, which can drive substantial N mineralization and a decline in total microbial biomass in previously unfrozen tussock tundra organic soil (Schaeffer and Sistla, personal communication), affecting microbial N-demand and decomposition potential.

The increase in C-mineralization observed with N-addition was slightly counterbalanced by altered SUE, which also increased. Tussock tundra microbes were able to make more enzymes and so increase C-flow, but they were also able to capture more of that C and use it to synthesize additional biomass. When microbes were given needed N, they were able to rapidly shift C-allocation to biosynthesis and growth pathways. Therefore, the rate of C-mineralization observed in the un-manipulated tundra soil most likely included some 'waste' metabolism – C that is respired without building additional biomass – suggesting that soil C-mineralization dynamics may be challenging to interpret in N-limited systems where waste metabolism is a significant contributor to total soil respiration.

This study did not consider other metrics of SOM quality recognized as important determinants in biogeochemical cycling (Oades, 1983; Schimel and Bennett, 2004). Variation in physical structure of soils may influence the potential for microbial response to resource addition through physical processes that diminish enzyme catalytic activity or restrict access to substrate. Such factors may cause substrate desorption (Allison, 2006), rather than enzymatically-regulated depolymerization, to be the critical rate limiting step in certain soils' decomposition. Furthermore, studies of tundra mineral soils' response to N-addition suggest that nutrient limitation is not a primary control on decomposition in more recalcitrant mineral soils, where increased N availability suppressed C-mineralization in a long term incubation study, potentially through the stabilization of that soil C pool (Lavoie et al., 2011). However, because our study was designed to evaluate whether the tussock tundra soil's microbial community is N-limited in its ability to decompose rather than the *rate* of SOC breakdown, the robustness of our results suggests that organic tussock tundra soil decomposition is strongly N-limited during the growing season and that the substrate addition experiments provided an appropriate assessment of microbial resource limitation.

Our N-addition experiment suggests that in extremely nutrient-poor environments such as tussock tundra soils during the Arctic summer – when *in situ* microbes are immobilizing N (Giblin et al., 1991) – all aspects of the microbial system are N-limited, including: growth, C-allocation and the synthesis of extracellular enzymes themselves, the direct catalyst of SOM decomposition. While several recent studies have explored the direct effects of long-term warming on microbial community structure (Deslippe et al., 2005, 2011; Fujimura et al., 2008; Walker et al., 2008; Lamb et al., 2011), none have directly tested the impact of warming on microbial nutrient-limitation status. The experimental framework presented here for characterizing microbial nutrient limitation may therefore be particularly useful in characterizing changes in Arctic nutrient cycling that are postulated to be driven by seasonal temperature dynamics, or by long-term manipulative field studies, such as warming-driven changes in soil nutrient availability through altered litter inputs and plant nutrient uptake dynamics. Additionally, the explicit incorporation of microbial nutrient limitation as a regulator of decomposition in global change models (Knorr et al., 2005; Davidson and Janssens, 2006) may significantly influence their greenhouse gas emissions projections, particularly in areas of recognized uncertainty, such as Arctic soil C pools.

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