

Increased Above- and Belowground Plant Input Can Both Trigger Microbial Nitrogen Mining in Subarctic Tundra Soils

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ABSTRACT

Low nitrogen (N) availability in the Arctic and Subarctic constrains plant productivity, resulting in low litter inputs to soil. Increased N availability and litter inputs as a result of climate change, therefore, have the potential to impact the functioning of these ecosystems. We examined plant and microbial responses to chronic inorganic N ($5 \text{ g m}^{-2} \text{ year}^{-1}$) and/or litter ($90 \text{ g m}^{-2} \text{ year}^{-1}$), supplied during three growing seasons. We also compared the response to more extreme additions, where the total cumulative additions of N (that is, 15 g m^{-2}) and litter (that is, 270 g m^{-2}) were concentrated into a single growth season. Plant productivity was stimulated by N additions and was higher in the extreme addition plots than those with chronic annual additions. Microbial commu-

nity structure also differed between the chronic and extreme plots, and there was a significant relationship between plant and microbial community structures. Despite differences in microbial structure, the field treatments had no effect on microbial growth or soil C mineralization. However, gross N mineralization was higher in the N addition plots. This led to a lower ratio of soil C mineralization to gross N mineralization, indicating microbial targeting of N-rich organic matter (“microbial N-mining”), likely driven by the increased belowground C-inputs due to stimulated plant productivity. Surprisingly, aboveground litter addition also decreased ratio of soil C mineralization to gross N mineralization. Together, these results suggest that elevated N availability will induce strong responses in tundra ecosystems by promoting plant productivity, driving changes in above- and belowground community structures, and accelerating gross N mineralization. In contrast, increased litter inputs will have subtle effects, primarily altering the ratio between C and N derived from soil organic matter.

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Key words: Plant-soil-microbial interactions; Nitrogen fertilization; Carbon and nitrogen cycling; Subarctic tundra ecosystem; Microbial structure and

function; Bacterial and fungal growth; Microbial N-mining; Decomposition; Shrubification; Biogeochemistry.

HIGHLIGHTS

- Elevated N availability impacted above- and belowground communities and functions.
- N and litter additions both induced a shift towards microbial use of N-rich organic matter.
- Extreme litter addition reduced microbial carbon use efficiency.

INTRODUCTION

Arctic and subarctic ecosystems are characterized by low nitrogen (N) availability, which together with cool temperatures results in low rates of plant productivity and associated plant inputs to soil (Chapin 1983; Hobbie and others 2002). Increases in the availability of N, whether due to an acceleration of N mineralization caused by climate warming (Hobbie and others 2002; Jiang and others 2016; Salazar and others 2020) or N deposition (Kühnel and others 2011), therefore has the potential to stimulate plant productivity and impact the activities of decomposer microorganisms belowground. Plant growth and composition is also changing across the Arctic due to climate warming, with higher plant productivity (“arctic greening”) and an increased abundance of deciduous shrubs (“shrubification”) resulting in increased leaf-litter inputs to soil (Hobbie and Chapin 1998; Sistla and others 2013; Myers-Smith and others 2019; Nielsen and others 2019). Understanding how changes in the supply of inorganic N and litter inputs impacts the functioning of arctic and subarctic systems is therefore crucial to predict the response of these ecosystems to future global change.

Although there is much evidence that elevated N availability stimulates plant growth in high-latitude systems (Shaver and Chapin 1980; Atkin 1996; Mack and others 2004), the effect on belowground decomposer microorganisms remains unclear. In one study of arctic tundra soils, inorganic N addition increased microbial biomass, enzyme activities and soil carbon (C) mineralization (Sistla and others 2012). However, in many other cases, the addition of inorganic N has been shown to inhibit microorganisms (Treseder 2008; Ramirez and others 2010; Rousk and others 2011; Kristensen and

others 2018), including in a subarctic forest where high levels of inorganic N reduced microbial respiration and enzyme activities (Sanden and others 2020). As such, elevated N availability could lead to increased C sequestration (Pregitzer and others 2008).

In contrast with the negative effects of inorganic N on microorganisms (Treseder 2008; Ramirez and others 2010; Rousk and others 2011; Kristensen and others 2018; Sanden and others 2020), increased N availability may have a positive effect indirectly, by stimulating plant productivity which in turn provides organic matter to fuel microbial activities (Wardle 2002; Mack and others 2004). Indeed, results from a long-term nutrient addition experiment in the Swedish Subarctic revealed higher rates of bacterial growth in fertilized soils, while fungal growth remained unaffected (Hicks and others 2020a). A shift towards more bacterially dominated decomposition in more productive ecosystems is often associated with faster soil C turnover and a high potential for nutrient loss (Moore and others 2003; Wardle and others 2004), potentially leading to reduced C sequestration.

Increased N availability may also influence rates of N mineralization derived from soil organic matter (SOM). In N-poor ecosystems, depolymerization of N-containing compounds by extracellular enzymes is rate-limiting for the production of bioavailable N (Schimel and Bennett 2004). As N availability increases, however, microorganisms may be able to increase the synthesis of N-acquiring enzymes (Weintraub and Schimel 2003), resulting in enhanced rates of N depolymerization and mineralization (Wild and others 2015). In two laboratory-based studies of subarctic soils, increased N availability and inputs of labile organic matter simulating root exudates reduced the ratio of soil C mineralization to gross N mineralization, suggesting that these changes in resource supply induced the microbial use of more N-rich components of SOM (“selective microbial N-mining”; Kristensen and others 2018; Hicks and others 2020b). However, to date, no studies have observed this phenomenon under field conditions.

Leaf litter provides a supply of high-quality C to soil microorganisms, especially during the initial phase of decomposition where soluble C compounds are leached to the soil (Ibrahima and others 1995). However, owing to the high C content, the C/N ratio of leaf litter is generally higher than that of microbial biomass and SOM (Cleveland and Liptzin 2007; Strickland and Rousk 2010), meaning that soil microorganisms must immobilize additional N from soil in order to use the C and N

contained within the litter (Parton and others 2007). As such, C-rich litter inputs might induce or exacerbate N limitation in already N-poor soils, leading to reduced plant productivity and lower rates of microbial activity. Litter inputs have also been shown to increase the dominance of fungal decomposers (Rousk and Frey 2015; Rousk and others 2016). This change in microbial community structure could impact the fate of C within soil due to differences in microbial carbon use efficiency (CUE)—the proportion of C used for growth relative to that consumed—between fungi and bacteria. For example, fungi are often associated with slow but more efficient C use (Six and others 2006), and thus a greater potential for C sequestration (Liang and others 2017).

In addition to chronic changes in soil N availability and litter inputs as a result of gradual climate warming (Myers-Smith and others 2019; Nielsen and others 2019; Salazar and others 2020), episodic events also occur in high-latitude systems, resulting in more dramatic changes in resource availability. Heatwaves during the growing season have been shown to support periods of high plant productivity and decomposition (Marchland and others 2005), leading to large inputs of litter and pronounced increases in soil N availability. For example, a doubling of NH_4^+ availability was reported in soils during a summer heatwave in the high-arctic (Zona and others 2014). Atmospheric N deposition in the Arctic has also been shown to be dominated by episodic deposition events (Kühnel and others 2011), further highlighting a need to assess the responsiveness of high-latitude systems to more extreme changes in litter and inorganic N availability, as well as to chronic changes.

In this study, we set out to examine the above- and belowground responses to altered resource availability in a Swedish subarctic heath (Figure 1). Our specific aims were (1) to assess the effect of increased N availability and litter inputs on soil C and N cycling and (2) to determine whether the response to gradual changes in resource availability differed from the response to more extreme changes. We expected that the addition of inorganic N to these N-poor soils would increase plant productivity and that this would drive changes in microbial activity belowground, including higher rates of bacterial growth and more bacterially dominated decomposition (Moore and others 2003; Wardle and others 2004), together with a shift towards microbial use of more N-rich SOM (Schimel and Bennett 2004; Hicks and others 2020b). In contrast, we expected that the addition of litter with a high C/N ratio would lead to high N immobilization by

microbes (Parton and others 2007), exacerbating N-limitation to plants and reducing productivity. As a result, in the litter addition plots, we expected more fungal-dominated C use (Wardle and others 2004), and a higher microbial CUE (Six and others 2006). Finally, we expected to observe more pronounced above- and belowground responses to the extreme N and litter additions than the chronic addition treatments.

MATERIALS AND METHODS

Field Experiment

The experimental site is located in a tundra heath, above the treeline near Abisko in northern Sweden ($68^\circ 19' \text{ N}$, $18^\circ 50' \text{ E}$). The mean annual temperature is 0.2° C and the total annual precipitation is 340 mm (30-year mean 1986–2015; Abisko Scientific Research Station 2016), with the growing season lasting from June to early September. The vegetation is dominated by dwarf shrubs and graminoids, together with scattered forbs and mosses (see “Results” section for more details). Soils have formed on base-rich mica schists and are classed as Histosols (IUSS World Reference Base for Soils 2006). The depth of the organic layer is about 5–20 cm and the pH is close to neutral (Table 1).

Experimental field treatments were established at the site in June 2017 (“chronic treatments”), with two additional field treatments established in June 2019 (“extreme treatments”). The chronic treatments included (1) control, (2) N addition (+N), (3) litter addition (+L) and (4) combined N and litter addition (+NL), whereas the extreme treatments included (5) high N addition (+XN) and (6) high litter addition (+XL). All field treatments were established in $1 \times 1 \text{ m}$ experimental squares, with six replicates. For the chronic N addition treatment, NH_4Cl equivalent to 5 g N m^{-2} was applied annually at the start of each growing season in June. This was chosen as an environmentally relevant level of N addition, reflecting the inorganic N release from high-latitude soils in response to a moderate $< 3^\circ \text{ C}$ climate warming scenario (Mack and others 2004). The NH_4Cl was applied in 5 L water, followed by an additional 5 L water addition to avoid the build-up of NH_4Cl salts on the vegetation surface. For the chronic litter addition treatment, 90 g m^{-2} dried mountain birch litter (*Betula pubescens*; C/N ratio 37.5) collected from a nearby forest during the autumn was added annually in June. This litter addition rate corresponded to the annual litter fall in a nearby open birch forest (Bylund and Nordell 2001), thus

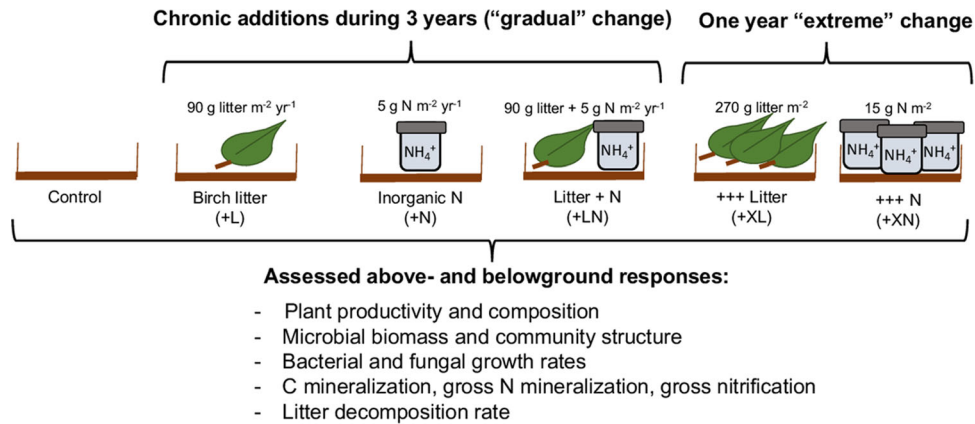


Figure 1. Schematic overview of the field experiment and measurements conducted. Field treatments included: control, chronic N addition over three years (+N), chronic litter addition over three years (+L), combined chronic N and litter additions over three years (+NL), extreme N addition over one growing season (+XN) and extreme litter addition over one growing season (+XL).

Table 1. Physiochemical Properties of Soils Sampled from a Subarctic Tundra Heath in Response to Inorganic N and Litter Addition Field Treatments.

	Control	+N	+L	+NL	+XN	+XL
Water content (g H ₂ O g ⁻¹ dwt)	2.1 (0.2)	1.9 (0.3)	1.9 (0.2)	2.0 (0.3)	1.7 (0.1)	1.7 (0.1)
Soil organic matter (g g ⁻¹ dwt)	0.75 (0.03)	0.66 (0.08)	0.63 (0.08)	0.67 (0.09)	0.77 (0.03)	0.76 (0.05)
Soil pH	6.6 (0.03) ^a	6.7 (0.05) ^a	6.5 (0.15) ^{ab}	6.6 (0.09) ^a	6.3 (0.20) ^b	6.5 (0.03) ^{ab}
Electrical conductivity (μS cm ⁻¹)	65 (5) ^b	81(8) ^{ab}	67 (17) ^b	78 (11) ^{ab}	111 (12) ^a	66 (7) ^{ab}
Soil C/N	20.2 (0.9)	18.1 (0.4)	19.2 (1.3)	20.3 (0.9)	21.5 (1.6)	22.2 (1.3)
Extractable NH ₄ -N (μg N g ⁻¹ SOM)	93.3 (23.5) ^{abc}	174.3 (23.4) ^{ab}	83.6 (23.1) ^{bc}	123.6 (20.3) ^{abc}	187.9 (25.7) ^a	76.0 (19.0) ^c
Extractable NO ₃ -N (μg N g ⁻¹ SOM)	7.1 (2.8)	4.5 (0.9)	5.8 (1.0)	3.9 (2.4)	1.8 (0.3)	2.2 (0.6)
Extractable PO ₄ -P (μg P g ⁻¹ SOM)	6.8 (0.6)	8.4 (1.8)	8.1 (1.5)	9.2 (2.0)	6.7 (0.8)	6.2 (1.4)

Treatments include control, chronic N addition for three years; +N, chronic litter addition for three years; +L, combined chronic N and litter additions for three years; +NL, extreme N addition for one growing season; +XN and extreme litter addition for one growing season; +XL; see Materials and Methods for further description. Data represent mean (SE; n = 6).

Lowercase letters denote significant difference according to Tukey's HSD pairwise comparisons.

reflecting the increase in litter inputs expected as a result of future arctic greening and shrubification (Myers-Smith and others 2019; Nielsen and others 2019). We used litter from *Betula pubescens*, rather than the dominant shrub *Betula nana*, due to the quantity of litter required to apply the field treatments. However, the leaves of *Betula pubescens* and *Betula nana* have a very similar C/N ratio (Metcalf and others 2019), and crucially, both plants are of the same functional type (that is, deciduous), which has previously been shown to be the strongest driver of differences in decomposition dynamics among different litter types in Arctic and

subarctic ecosystems (Hobbie 1996; Parker and others 2018). For the chronic combined N and litter addition treatment, 5 g N m⁻² and 90 g m⁻² dried birch litter were added together each year. For the extreme treatments, three-times the annual inorganic N addition (that is, 15 g N m⁻²) or litter addition (that is, 270 g m⁻² dried birch litter) were applied as a single addition in June 2019. Thus, these plots received the same N or litter additions as the chronic treatment plots, but concentrated in a single growth season, simulating a pulse of elevated inorganic N or litter input which may occur as a result of, for example, a summer heatwave

(Marchland and others 2005; Zona and others 2014). Soils from an additional six replicated plots without N or litter treatments, served as a control. All plots including the control received 10 L water during the treatment application, to match the water supplied with the inorganic N addition.

Plant Productivity and Composition

Plant productivity and community composition were measured in mid-August 2019. The normalized difference vegetation index (NDVI)—which is an estimate of total photosynthetic capacity (or greenness)—was used as a proxy for plant productivity (Mänd and others 2010; Walker and others 2012). The NDVI was determined by using a SpectroSense2 hand-held pole (Skye Instruments Ltd.), comparing the incident and reflected light in the red and near-infrared spectral regions (categorically different values for green vegetation and other surfaces). In the case of the litter-treatment subplots, the dead plant material on the surface reduced the NDVI signal from the native vegetation. In order to account for this effect, we first estimated the area covered by the added litter in each subplot by using Photoshop, and subtracted its influence on the NDVI by means of the NDVI-LAI relationship from Street and others (2007), where the LAI (leaf area index) was re-defined as the green leaf area per unit of litter-free ground surface.

Plant species composition (relative abundance) was assessed by conducting a frequency analysis of plants occurring within each subplot (identified species grouped by plant functional type are provided in Table 2). As described by Graglia and others (2001), a 0.5 × 0.5 m frame, divided into 100 squares, was placed in the centre of each subplot. Vascular plants were identified to the species level, and the number of squares within which each species appeared was counted and recorded. Although mosses and lichens also appeared within the plots (< 30% ground cover), we focused on vascular plants due to their close link with the belowground microbial community via their root systems.

Litter Decomposition Rates

Bags containing green and rooibos tea were buried (ca. 5 cm depth) in June 2019 (immediately before the annual treatment application), with three replicates in each plot (control, +N, +L and +NL treatments only). The teabags were retrieved in mid-August 2019, after 58 days incubation in the field. After retrieval, the teabags were cleaned of

adhering soil particles and roots before being dried at 70 °C for 48 h to determine the % mass loss. The decomposition rate (k) and stabilization factor (S) were then calculated according to Keuskamp and others (2013).

Soil Sampling

Soils were sampled in mid-August 2019, two months after the annual treatment application in June. Soils were collected from 3 to 5 randomized cores from each plot, including only the O-horizon to a depth of about 5 cm. After removing stones and visible roots, the soils from each plot were sieved (< 4 mm), before being stored at 4 °C until assessment, which occurred within two weeks of sampling.

Soil Physiochemistry

Fresh soil subsamples were used to measure gravimetric soil moisture content (105 °C for 24 h) and SOM content through loss on ignition (600 °C for 12 h). Soil pH and electrical conductivity were determined in a 1:5 (w:v) soil/water extraction (5.0 g soil + 25 mL H₂O) followed by measurement using an electrode. Inorganic NH₄-N and NO₃-N were extracted from 5.0 g soil subsamples by shaking for 2 h with 0.2 M KCl before filtering and measuring spectrophotometrically using flow injection analysis. Inorganic PO₄-P was extracted from 5.0 g soil subsamples by shaking for 1 min in a 1:10 (w:v) soil/solution ratio with Bray 1 extractant (0.03 M NH₄F + 0.025 M HCl), before filtering and measuring using flow injection analysis.

Microbial Community Structure

Microbial phospholipid fatty acid (PLFA) composition was determined from 0.50 g frozen subsamples, according to Frostegård and others (1993) with modifications (Cruz-Paredes and others 2017). An internal standard (methyl nonadecanoate fatty acid 19:0) was added before the methylation step for quantification. The derived fatty acid methyl esters were quantified on a gas chromatograph with a flame ionization detector. Bacterial (i14:0, i15:0, a15:0, br16:0, i16:0, 16:1 ω 9, 16:1 ω 7, 10Me16:0, i17:0, a17:0, 17:1 ω 8, cy17:0, 10Me17:0, 18:1 ω 7, 10Me18:0 and cy19:0) and fungal-specific (18:2 ω 6,9) PLFAs were used to estimate the relative abundance of these functional groups (Frostegård and Bååth 1996; Ruess and Chamberlain 2010). The total concentration of PLFAs (14:0, 15:0, 16:1 ω 5, 16:0, 17:0, 18:1 ω 9,

Table 2. Vascular Plant Composition (% Relative Abundance, Grouped by Plant Functional Type) in Response to Inorganic N and Litter Addition Field Treatments.

Species	Functional type	Control	+N	+L	+NL	+XN	+XL
<i>Carex parallella</i>	Graminoid	8.2 (4.2)	9.5 (4.6)	10.5 (3.1)	8.2 (6.5)	7.9 (1.9)	7.2 (3.5)
<i>Carex vaginata</i>	Graminoid	15.6 (3.9)	17.0 (4.1)	9.6 (3.0)	22.8 (6.7)	5.8 (2.8)	4.2 (3.2)
<i>Carex rupestris</i>	Graminoid	11.1 (2.8)	9.7 (8.1)	6.3 (2.5)	12.9 (4.8)	1.6 (0.9)	2.5 (1.3)
<i>Festuca ovina</i>	Graminoid	0	0.1 (0.1)	0.9 (0.5)	1.6 (0.5)	0	0
Total graminoid		34.9 (5.3) ^{ab}	36.4 (8.3) ^{ab}	27.3 (4.0) ^{ab}	45.4 (10.6) ^a	15.2 (3.2) ^{ab}	13.9 (3.3) ^b
<i>Arctostaphylos alpina</i>	Forb	2.5 (1.5)	0	0.6 (0.6)	0.4 (0.4)	1.5 (1.3)	3.8 (2.5)
<i>Astragalus alpinus</i>	Forb	0.1 (0.1)	0	1.4 (1.1)	0.5 (0.5)	7.8 (3.8)	6.7 (1.7)
<i>Astragalus frigidus</i>	Forb	0.5 (0.4)	0.3 (0.3)	0.2 (0.1)	0.3 (0.2)	0.5 (0.5)	1.2 (1.2)
<i>Bartsia alpina</i>	Forb	0	0.6 (0.6)	0.1 (0.1)	0.5 (0.4)	0.2 (0.1)	1.1 (0.7)
<i>Dryas octopetala</i>	Forb	2.9 (1.0)	3.0 (2.1)	3.9 (1.7)	4.2 (1.3)	9.9 (5.1)	15.3 (5.3)
<i>Polygonum viviparum</i>	Forb	4.5 (1.5)	2.1 (0.8)	2.9 (1.4)	2.6 (0.8)	3.3 (2.0)	1.1 (0.6)
<i>Saxifraga oppositifolia</i>	Forb	0.5 (0.3)	0	0.7 (0.7)	0.4 (0.4)	0.2 (0.1)	0.1 (0.1)
<i>Silene acaulis</i>	Forb	1.6 (1.1)	0.9 (0.9)	1.7 (1.4)	0.2 (0.2)	0.4 (0.2)	0
<i>Tofieldia pusilla</i>	Forb	1.7 (1.5)	1.4 (1.1)	1.7 (1.6)	0.4 (0.2)	0.4 (0.3)	0.1 (0.1)
Total forb		14.3 (1.3)	8.2 (3.2)	13.2 (3.7)	9.4 (1.6)	24.2 (9.3)	29.3 (4.8)
<i>Andromeda polifolia</i>	Evergreen	4.6 (0.4)	3.8 (1.9)	7.2 (2.0)	3.6 (1.7)	1.5 (0.6)	2.3 (0.8)
<i>Empetrum hermaphroditum</i>	Evergreen	11.1 (5.3)	10.0 (4.7)	12.3 (7.5)	14.2 (8.0)	15.3 (7.9)	15.4 (6.9)
<i>Cassiope tetragona</i>	Evergreen	0	0	0	0	0.4 (0.4)	0
<i>Phyllodoce caerulea</i>	Evergreen	0.2 (0.2)	0	0.1 (0.1)	0	0	0
<i>Rhododendron lapponicum</i>	Evergreen	3.3 (1.8)	3.4 (1.4)	9.5 (2.7)	5.5 (4.4)	9.6 (5.5)	8.9 (3.8)
Total evergreen shrub		19.2 (5.7)	17.3 (6.7)	29.1 (6.3)	23.3 (8.9)	26.8 (7.6)	26.5 (7.1)
<i>Betula nana</i>	Deciduous	10.6 (4.7)	11.3 (3.5)	7.9 (4.6)	5.7 (2.1)	12.5 (5.0)	13.7 (5.8)
<i>Salix reticulata</i>	Deciduous	1.1 (0.5)	0.4 (0.4)	1.2 (0.6)	0.4 (0.2)	1.4 (0.6)	0.9 (0.4)
<i>Salix myrsinifolia</i>	Deciduous	0.4 (0.2)	9.2 (3.4)	2.9 (2.6)	4.0 (2.4)	4.0 (2.7)	2.7 (1.6)
<i>Salix lapponicum</i>	Deciduous	1.8 (0.7)	0.5 (0.5)	2.3 (1.5)	1.5 (1.2)	0.4 (0.4)	0.2 (0.2)
<i>Vaccinium uliginosum</i>	Deciduous	16.3 (4.0)	14.2 (2.5)	10.9 (1.8)	8.9 (3.0)	9.1 (3.3)	9.9 (3.7)
Total deciduous shrub		30.3 (4.7)	35.6 (7.2)	25.2 (6.9)	20.5 (4.5)	27.4 (7.9)	27.5 (4.8)
<i>Equisetum scirpoides</i>	Fern	1.4 (0.4)	2.5 (1.2)	5.1 (1.4)	1.5 (0.8)	6.4 (2.3)	2.8 (1.4)
Total fern		1.4 (0.4)	2.5 (1.2)	5.1 (1.4)	1.5 (0.8)	6.4 (2.3)	2.8 (1.4)

Data represent mean (SE; n = 6).

Lowercase letters denote significant difference according to Tukey's HSD pairwise comparisons.

18:1, 18:0 and 19:1, in addition to those listed above) was used as a measure of total microbial abundance.

Respiration and Microbial Biomass

Soil C mineralization (that is, the production of respired CO₂) was measured using 0.50 g soil in 20 ml glass vials. The headspace was purged with pressurized air before the vial was closed with a crimp lid. Vials were incubated for 24 h at 16 °C—matching a typical soil temperature during the peak growing season at the site (Rousk and Michelsen 2017)—before the headspace CO₂ concentration was analysed using a gas chromatograph equipped with a methanizer and flame ionization detector. The CO₂ concentration of the pressurized

air was also determined in empty vials and subtracted to determine the rate of soil C mineralization.

Substrate induced respiration was measured as a proxy for microbial biomass. Briefly, 12 mg 4:1 glucose/talcum was vigorously mixed into 0.50 g soil (corresponding to 8 mg glucose-C g⁻¹ soil). After 30 min, vials were purged with pressurized air and incubated at 22 °C for 2 h before the concentration of CO₂ was determined (as described above). Substrate induced respiration was used to estimate microbial biomass C, where 1 µg CO₂ at 22 °C corresponds to 20 µg microbial biomass C (Anderson and Domsch 1978).

Bacterial and Fungal Growth

Bacterial growth was determined by measuring the rate of ^3H -Leucine (Leu) incorporation in extracted bacteria (Bååth and others 2001; Rousk and others 2009). For this, 0.50 g soil was mixed with 20 ml demineralized water, vortexed for 3 min and centrifuged (10 min at 1000 g). The resulting bacterial suspension was incubated at 16 °C, with 2 μl 1-[4,5- ^3H]-Leucine (5.7 TBq mmol $^{-1}$, PerkinElmer, USA) and unlabelled Leu with a final concentration of 275 nM Leu in the bacterial suspension. Bacterial growth was terminated after 2 h at 16 °C by adding 75 μl of 100% trichloroacetic acid. Centrifugation and washing were performed as described by Bååth and others (2001). Scintillation cocktail (Ultima Gold; PerkinElmer, USA) was added and the radioactivity was measured using a liquid scintillation counter. The amount of leucine incorporated into extracted bacteria (pmol Leu g $^{-1}$ SOM h $^{-1}$) was used to estimate the bacterial growth rate (in units pmol TdR g $^{-1}$ SOM h $^{-1}$), from which the rate of bacterial-C production ($\mu\text{g C g}^{-1}$ SOM h $^{-1}$) was calculated according to Soares and Rousk (2019).

Fungal growth and biomass (ergosterol concentration) was measured using the acetate-in-ergosterol incorporation method (Newell and Fallon 1991) adapted for soil (Bååth 2001; Rousk and others 2009). For this, 0.50 g soil was mixed with 20 μl of ^{14}C -acetate solution ([1- ^{14}C] acetic acid, sodium salt, 2.07 GBq mmol $^{-1}$, Perkin Elmer) and unlabelled sodium acetate, resulting in a final acetate concentration of 220 μM in the soil slurry. Samples were incubated at 16 °C for 4 h before formalin was added to terminate growth. Ergosterol and incorporated acetate were measured according to Rousk and Bååth (2007). Ergosterol concentrations were estimated from the UV absorbance at 282 nm compared with external standards. The rate of acetate incorporation into ergosterol (pmol g $^{-1}$ SOM h $^{-1}$) was converted into fungal-C production ($\mu\text{g C g}^{-1}$ SOM h $^{-1}$) based on the empirical relationship determined by Soares and Rousk (2019). Microbial CUE was estimated as the ratio between total microbial growth (bacterial + fungal C production) to the total microbial C use (total microbial growth + respiration).

Gross N Mineralization and Nitrification

Gross N mineralization (that is, the production of ammonium) and gross nitrification rates (that is, the production of nitrate) were determined using

the ^{15}N pool-dilution method. In this method, $^{15}\text{NH}_4^+$ is added to soil and the rate at which the atom% ^{15}N enrichment of the NH_4^+ pool declines is used to estimate gross N mineralization, as the mineralization of soil organic ^{14}N to $^{14}\text{NH}_4^+$ dilutes the $^{15}\text{NH}_4^+$ pool. For each soil, two subsamples (each 5.0 g) were weighed into 100 ml plastic pots, to which 115 μl NH_4Cl (45 $\mu\text{g N ml}^{-1}$, enriched to 5 atom% ^{15}N) was administered. The soils were vigorously mixed before being lidded and incubated at 16 °C without light. One set of subsamples were extracted 1 h after ^{15}N addition (T_0), while the second set was treated identically after 24-h incubation (T_1). At each time point, soil NH_4^+ and NO_3^- were extracted using 50 mL 1 M KCl solution. After shaking for 1 h, the extracts were filtered through GF/F-filter paper and the filtered extracts were collected in a new set of plastic pots. The NH_4^+ and NO_3^- were then isolated from the extract by diffusion to acidified glass fibre traps consisting of a filter disc prepared with 10 μl 2.5 M KHSO_4 enclosed in a strip of PTFE-tape, according to standard procedures (IAEA 2001). First, 0.2 g MgO was added along with an acidified trap and the pots were shaken for five days. After this, the trap was removed and a further 0.2 g MgO was added to the pots which were left unlidded overnight, before 0.2 g Devarda's alloy was added along with another acidified trap. After shaking for a further five days the trap was removed. The filter discs were removed from all traps and dried in a desiccator before being placed in tin cups for analysis. The amount of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ was determined by isotope-ratio mass-spectrometry (IRMS), and the $^{15}\text{N}/^{14}\text{N}$ -content of the glass fibre traps were measured with a Flash 2000 elemental analyser coupled to a Delta V plus via the ConFlow interface (Thermo Fisher Scientific, Germany). Gross N mineralization, gross NH_4^+ consumption and gross nitrification rates were estimated based on differences in the concentration and ^{15}N content of $\text{NH}_4^+\text{-N}$ between T_0 and T_1 and $\text{NO}_3^-\text{-N}$ between T_0 and T_1 (see Bengtson and others 2005 for equations).

Data Analysis

The effect of field treatments on soil physiochemical properties, plant productivity, the relative abundance of plant functional types, microbial process rates and microbial biomass variables were assessed by one-way analysis of variance (ANOVA). Prior to analysis, where necessary, dependent

variables were first log-transformed to meet the assumptions (homogeneity of variance) of ANOVA. Pairwise comparisons of significant effects were conducted using Tukey's HSD post hoc tests, with significant differences identified where $p < 0.05$.

Principal coordinate analyses (PCoA) were used to summarize the variation in vascular plant community composition (relative abundance) and microbial PLFA composition (mol%), using Bray–Curtis dissimilarity matrices, with permutational analysis of variance (PERMANOVA) used to test for field-treatment effects on plant and microbial structures. To assess if responses in above- and belowground community structures were linked to the administered field treatments, the PCo1 scores for microbial PLFA composition were regressed against the PCo1 scores for vascular plant composition. To further explore the PLFA markers driving the variation in microbial PLFA composition among treatments (see “Results” section), we also conducted a principal component analysis (PCA) of the PLFA composition. Statistical analyses were performed using JMP Pro 15.0 for Mac (SAS Institute) and PAST software version 3.24 (Hammer and others 2001).

RESULTS

Soil Physiochemistry

The field treatments had no distinguishable effect on soil water content, SOM content, soil C/N ratio and the concentration of $\text{NO}_3\text{-N}$ or inorganic P (Table 1). However, the concentration of $\text{NH}_4\text{-N}$ in soils varied more than twofold among the field treatments ($p = 0.004$), being highest in the N addition treatments (ca. $180 \mu\text{g NH}_4\text{-N g SOM}$), and lowest in the litter addition treatments (ca. $80 \mu\text{g NH}_4\text{-N g SOM}$), with the control intermediate (ca. $90 \mu\text{g NH}_4\text{-N g SOM}$; Table 1). Soil pH ($p = 0.001$) and electrical conductivity ($p = 0.03$) were also both affected by the field treatments. Soil pH ranged from 6.3 to 6.7, being lower in the extreme N treatment (+XN) than the other treatments, while electrical conductivity ranged between 65 and $111 \mu\text{S cm}^{-1}$, being almost twice as high in the extreme N addition treatment (+XN) than the control.

Plant Productivity and Composition

Plant productivity (proxied by the NDVI) varied significantly among field treatments ($p < 0.001$). Compared to the control, the NDVI was about 7% higher in the +N plots that received chronic N additions for three years, and about 14% higher in

the extreme +XN plots that received the same amount of N but as a single addition at the start of the growing season (Figure 2). At the time of the NDVI measurements, two months after the treatment applications, litter covered about 16%, 12% and 32% of the surface area in the chronic litter addition (+L), chronic nitrogen and litter (+NL) and high litter addition (+XL) plots, respectively. After accounting for the effect of this litter on the NDVI measurements, there was no significant effect of the litter additions on plant productivity compared to the control (Figure 2).

Twenty-four different vascular plants were identified to species-level within the studied plots (Table 2). All plots were dominated by shrubs and graminoids, comprising ca. 60% and 30% of the vascular plant composition, respectively. Dominant shrubs included the deciduous shrubs *Betula nana* and *Vaccinium uliginosum* and the evergreen shrub *Empetrum hermaphroditum*, while dominant graminoids included *Carex parallella*, *Carex vaginata* and *Carex rupestris*. Generally, there was no significant effect of the field treatments on the relative abundance of different plant functional types. This was

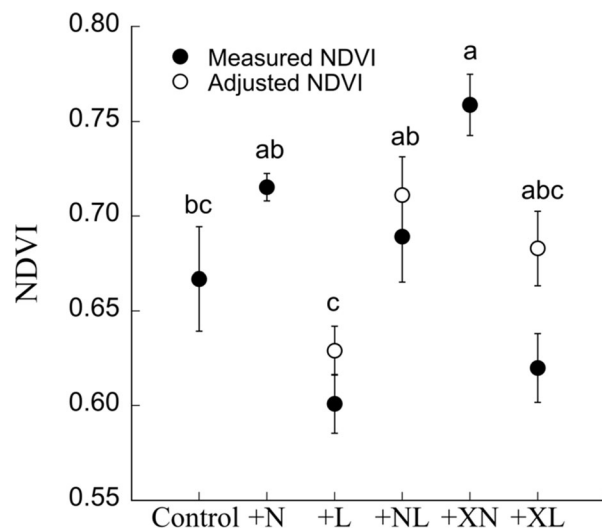


Figure 2. Normalized difference vegetation index (NDVI) as an estimate of plant productivity in response to inorganic N and litter addition field treatments (see Figure 1 for legend description). Closed symbols represent measured NDVI values and open symbols represent NDVI values adjusted to account for the surface covered by dead plant material in the litter addition plots (see Materials and Methods for further information). Data represent mean \pm SE ($n = 6$). Lowercase letters denote significant difference according to Tukey's HSD pairwise comparisons (in the case of litter treatments, the adjusted NDVI values were used for comparisons).

with the exception of graminoids ($p = 0.007$), where there were more graminoids in the combined N and litter treatment (+NL) than in the extreme litter treatment (+XL) plots. The first axis from a PCoA of vascular plant composition explained 31.0% of the variation in the data, while the second axis explained another 16.6% (Figure 3A). However, there was no significant effect of the field treatments on plant community structure.

Microbial Biomass and PLFA Composition

For most microbial biomass parameters that we measured (microbial biomass C, total PLFA concentration, bacterial PLFA concentration and ergosterol concentration), there was no distinguishable difference among the field treatments (Table 3). The concentration of fungal PLFAs did, however, vary significantly among field treatments ($p = 0.03$), with the highest concentration of fungal PLFAs occurring in the extreme N addition treatment (+XN) and the lowest concentration occurring in the chronic litter addition treatment (+L).

The first axis from a PCoA of microbial PLFA composition explained 84.5% of the variation in the data, while the second axis explained another 8.4% (Figure 3B). In this case, the field treatments had a marginal effect on the PLFA composition ($p = 0.08$), with the most pronounced separation occurring between the chronic +N and extreme +XN addition treatments. This separation appeared to be related to higher relative abundances of markers associated with gram-negative bacteria (including 18:1 ω 7 and cy19:0) in the +N soils and a higher relative abundance of the fungal biomarker (18:2 ω 6,9) and markers associated with

gram-positive bacteria (including a15:0 and i15:0) in the +XN soils (Supplementary Figure S1).

Linkage Between Above- and Belowground Community Structures

There was no significant relationship between above- and belowground community structures when data were aggregated by field treatments (not shown). However, there was a significant relationship between the PCo1 scores for microbial and plant composition at the individual subplot scale ($p < 0.001$; Figure 3C), demonstrating linked responses in above- and belowground community structures.

Soil C and N Mineralization

Field treatments affected the rate of gross N mineralization ($p = 0.02$), but had no significant effect on soil C mineralization (Figure 4A). Compared to the control, gross N mineralization was three-times higher in soils which had received additional inorganic N (that is, in the +N, +NL and +XN treatments). Gross N mineralization in the chronic litter addition treatment (+L) was very similar to the control, but was nearly two times higher in the extreme litter addition treatment (+XL). As such, the C/N ratio of SOM mineralization (that is, the ratio of soil C mineralization to gross N mineralization) also varied among soils (Figure 4B; $p = 0.002$), being generally lower in all treated soils, and significantly lower in soils which had received additional inorganic N, compared to the control.

In all soils, rates of gross $\text{NH}_4\text{-N}$ consumption were higher than rates of gross N mineralization (that is, there was net N immobilization). Although

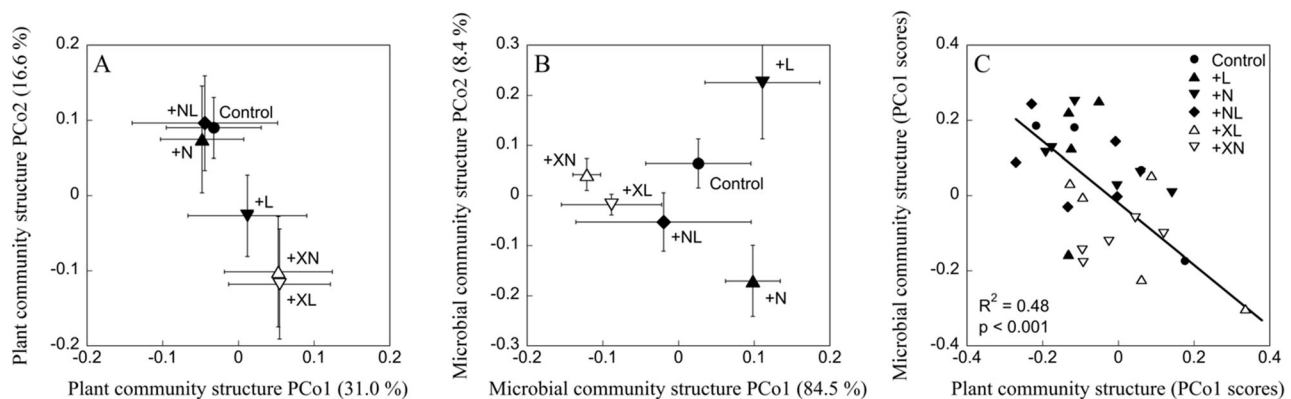


Figure 3. Principal coordinate analysis of **A** vascular plant community structure (relative abundance) and **B** microbial community structure (mol%) and **C** the relationship between PCo1 scores for microbial and plant community structures in response to inorganic N and litter addition field treatments (see Figure 1 for legend description).

Table 3. Microbial Biomass Parameters in Soils Sampled from a Subarctic Tundra Heath in Response to Inorganic N and Litter Addition Field Treatments.

	Control	+N	+L	+NL	+XN	+XL
Microbial biomass C (mg C g ⁻¹ SOM)	6.4 (0.4)	6.7 (0.8)	5.2 (0.3)	5.7 (0.9)	7.1 (0.4)	6.4 (0.7)
Total PLFA (nmol g ⁻¹ SOM)	924 (65)	878 (150)	742 (11)	674 (127)	979 (86)	849 (94)
Bacterial PLFA (nmol g ⁻¹ SOM)	500 (45)	495 (85)	413 (22)	358 (74)	497 (39)	439 (41)
Fungal PLFA (nmol g ⁻¹ SOM)	120 (20) ^{ab}	100 (19) ^a	77 (15) ^b	99 (25) ^{ab}	173 (19) ^a	147 (30) ^{ab}
Ergosterol (µg g ⁻¹ SOM)	150 (7)	131 (3)	160 (16)	140 (14)	176 (15)	145 (8)

Data represent mean (SE; $n = 6$).

Lowercase letters denote significant difference according to Tukey's HSD pairwise comparisons.

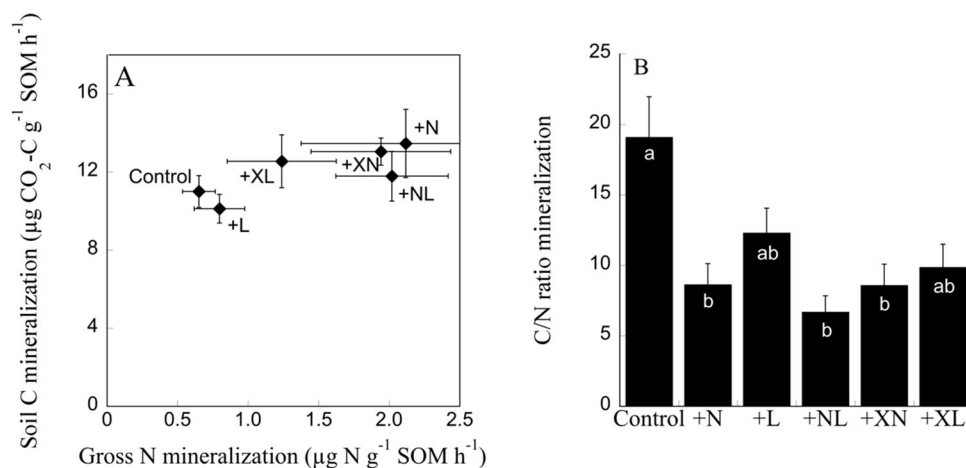


Figure 4. **A** Carbon and gross nitrogen mineralization rates and **B** the resulting C/N ratio of mineralization in soils from inorganic N and litter addition field treatments in a subarctic tundra heath (see Figure 1 for legend description). Data represent mean \pm SE ($n = 6$). Lowercase letters denote significant difference according to Tukey's HSD pairwise comparisons.

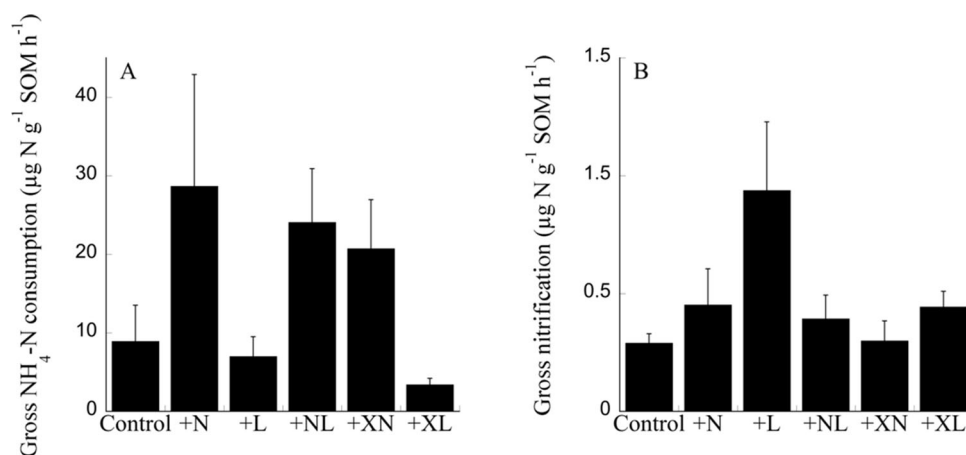


Figure 5. Rate of **A** gross NH₄-N consumption and **B** gross nitrification in soils from inorganic N and litter addition field treatments in a subarctic tundra heath (see Figure 1 for legend description). Data represent mean \pm SE ($n = 6$). No significant differences among field treatments.

and 22% mass loss of the rooibos tea (Supplementary Figure S2A-B). Although there were no significant differences in mass loss depending on the field treatments, for both tea types there was a tendency for greater mass loss in response to the +N and +NL treatments compared to the +L treatment. Consequently, the rate of litter decomposition (k) tended to be higher in the soils which had been exposed to chronic N additions (ca. 0.018 g g^{-1}), compared to the control soils and soils where litter had been added alone (ca. 0.015 g g^{-1}), although the differences were not significant ($p = 0.30$; Supplementary Figure S2C). There was no significant effect of the field treatments on the litter stabilization factor (S-factor) which was ca. 0.35 for all field treatments (Supplementary Figure S2D).

DISCUSSION

Above- and Belowground Community Responses to Inorganic N and Litter Additions

Plant productivity responded strongly to the field treatments, with N additions resulting in higher plant growth (Figure 2). This was consistent with findings from other arctic and subarctic ecosystems, and confirmed that this was a N-limited system (Shaver and Chapin, 1980). Surprisingly, plant productivity was higher in the plots which had received one large N addition (+XN) compared to the plots which had received the same total amount of N, but as chronic N additions over three years (+N), indicating that the plant response to elevated N availability did not saturate at $5 \text{ g N m}^{-2} \text{ year}^{-1}$ and could occur rapidly, manifesting within one growing season. A previous study of high-arctic tundra in Greenland found that the stimulation of plant growth in response to N additions saturated at just $1 \text{ g N m}^{-2} \text{ year}^{-1}$ (Arens and others 2008). However, our findings suggest that in subarctic tundra there is a high potential for a rapid stimulation of plant productivity due to elevated N availability occurring as a result of warming-accelerated N mineralization (Salazar and others 2020) or N deposition (Kühnel and others 2011), which is unlikely to saturate even in response to changes in N availability above $5 \text{ g N m}^{-2} \text{ year}^{-1}$.

Plants supply microorganisms with C, meaning that changes in plant productivity may affect the composition of the belowground microbial community. We observed differences in microbial community structure among field treatments, with the most pronounced difference in PLFA composi-

tion occurring between the chronically N fertilized soils and the soils which had received a single high N addition (Figure 3B). The chronically N fertilized soils were characterized by a high relative abundance of gram-negative bacteria (Supplementary Figure S1), which have been associated with the use of labile root exudates (De Deyn and others 2011; Chaudhary and Dick 2016). This suggests that the stimulation of plant productivity (Figure 2) and thus increased rhizosphere inputs in these soils had driven this response. In contrast, the soils from the extreme N addition plots were characterized by a higher relative abundance of fungi (Table 3, and Supplementary Figure S1). Laboratory-based studies have often shown that the addition of inorganic N suppresses bacterial growth but stimulates fungal growth (Rousk and Bååth 2007; Kristensen and others 2018; Silva-Sanchez and others 2019), which is likely due to the competitive release of fungi with bacterial inhibition (Rousk and others 2008; Hicks and others 2019). In our field experiment, although the high N addition stimulated plant productivity (Figure 2), one growth-season may have been insufficient time for this change to be reflected in the microbial community structure. Instead, the high-level of N addition may have initially had a direct influence on the microbial community by inhibiting bacterial growth and thereby stimulating fungal growth (Rousk and Bååth 2007; Kristensen and others 2018; Silva-Sanchez and others 2019), explaining the higher relative abundance of fungal PLFAs in these soils. At the time of our assessment of soils, two months after the annual treatment application, however, no clear effect of the field treatments on bacterial and fungal growth rates remained (Figure 6). This suggests that the negative effects of the inorganic N additions on soil bacteria were transient and did not persist two months after the treatment had been applied. Indeed, in a controlled microcosm experiment where the effect of inorganic N on microbial growth was resolved over time, although N addition initially inhibited bacterial growth and stimulated fungal growth, after two-month microbial growth had converged with the control (Silva-Sanchez and others 2019).

In addition to the role of overall plant productivity, the composition of aboveground plant communities can also characterize belowground microbial structure (De Deyn and Van der Putten 2005; Delgado-Baquerizo and others 2019). As such, it is expected that the quality and type of plant input, as well as the presence of plants able to form symbiotic mycorrhizal associations, will determine the assembly of the microbial commu-

nity. Consistent with this expectation, we found a close link between the variation in above- and belowground communities (Figure 3C). This suggests that the functional traits of the plant community directly affect the composition of microbial traits. As we did not find significant shifts in the composition of vascular plants in response to the field treatments (Figure 3A), the observed relationship between above- and belowground communities was likely driven by the inherent heterogeneity in plant composition across the tundra heath (Table 2). However, systematic changes in plant composition are expected in the Subarctic, as deciduous and evergreen shrubs expand into the tundra in response to climate warming (Vowles and Björk, 2019; Myers-Smith and others 2019). Our results suggest that these changes in plant communities will correspond to systematic changes in belowground microbial community structure. How specific plant traits map to microbial traits could therefore characterize how subarctic ecosystems sequester C or provide plant fertility (Malik and others 2019).

Microbial Functional Responses to Inorganic N and Litter Additions

We predicted that higher plant productivity as a result of N addition would lead to more bacterially dominated decomposition (Moore and others 2003; Wardle and others 2004), and a shift towards microbial use of more N-rich SOM resulting in a reduction in the ratio of soil C mineralization to gross N mineralization (Hicks and others 2020b). Although the N additions stimulated plant productivity, and induced a shift in microbial community structure, there was no clear effect of the field treatments on microbial growth (Figure 6), soil C mineralization (Figure 4A) or litter decomposition (Supplementary Figure S2). However, gross N mineralization was higher in N fertilized soils, resulting in a lower ratio of soil C mineralization to gross N mineralization (Figure 4). Higher rates of gross $\text{NH}_4\text{-N}$ consumption than gross N mineralization indicates high N immobilization. Moreover, nitrification accounted for a low fraction (< 10%) of gross $\text{NH}_4\text{-N}$ consumption in all the soils (Figure 5), suggesting high microbial immobilization, indicative of a N-poor environment. It is thought that in N-poor soils, depolymerization of N-containing compounds by extracellular enzymes is rate-limiting for the provisioning of bioavailable N (Schimel and Bennet 2004). This would imply that microorganisms can increase the synthesis of N-acquiring enzymes when supplied with inor-

ganic N (Weintraub and Schimel 2003), which would stimulate rates of N depolymerization and gross N mineralization (Wild and others 2015) resulting in a lower ratio of C to gross N mineralization (Kristensen and others 2018). In a controlled laboratory study using subarctic soils, it was also found that inputs of labile C simulating root exudates stimulated N mineralization more than C mineralization, which was interpreted as selective microbial mining of N from N-rich organic matter (Rousk and others 2016; Hicks and others 2020b). As such, by independent mechanisms both (1) the higher N availability and (2) increased belowground C inputs as a consequence of stimulated plant productivity due to fertilization may have contributed to the higher rates of N mineralization, and selective targeting of N-rich organic matter in these soils.

The litter addition treatments had a limited effect on all the microbial parameters that we measured, with no distinguishable effect on the microbial PLFA composition (Figure 3B), microbial growth (Figure 6) or C mineralization rates (Figure 4A). A previous study in the Canadian high-arctic also observed a limited response of microbial community composition and C mineralization to increases in vegetation induced by simulated climate change (Lamb and others 2011). Likewise, a long-term litter manipulation experiment with 23 years of doubling aboveground litter inputs had no significant effect on rates of bacterial and fungal growth, or soil C mineralization (Rousk and Frey 2015). In contrast, litter addition in a laboratory experiment immediately increased microbial growth and respiration, but rates then declined and converged with the control within 20 days (Silva-Sanchez and others 2019). This transient response was likely driven by the initial release of labile soluble compounds from the litter (Ibrahima and others 1995), which after being depleted resulted in the return of rates towards control-levels. The change in SOM quality driven by the short-lived history of higher microbial growth with litter additions may, however, have longer-term implications for C and N cycling. The microbial necromass which accumulated following the period of stimulated growth would be relatively C-poor but N-rich (that is, have a low C/N ratio) compared to plant residues and other components of SOM (Cleveland and Liptzin 2007; Strickland and Rousk 2010). As such, microbial necromass with a low C/N ratio may be preferentially targeted by living microorganisms as a N-rich source of organic matter. Consistent with this, the ratio of soil C mineralization to gross N mineralization in the litter amended soils tended to

be lower than in the control (Figure 4B). The high C/N ratio of the added litter may have also contributed to this response, by exacerbating microbial demand for N. The added litter had a C/N ratio of 37.5, which is higher than that of microbial biomass, which is typically in the range of 5–15 (Cleveland and Liptzin 2007; Strickland and Rousk 2010). To use the added litter, microorganisms would therefore have needed to immobilize N from the soil, leading to high N demand. This may have resulted in selective microbial mining for N from SOM (Murphy and others 2015; Rousk and others 2016; Hicks and others 2020b), driving the shift towards use of more N-rich organic matter via a similar mechanism to that occurring in the N fertilized soils as a result of higher plant productivity and belowground C input (see above).

Microbial CUE was also affected by the litter addition treatments, being lower in the treatment where soils received one large litter addition compared to the control and soils which received the same amount of litter addition but gradually over three years (Figure 6D). It is thought that microbial CUE decreases under nutrient limited conditions (Sterner and Elser 2002; Manzoni and others 2012). In the extreme treatment, it is likely that the large addition of litter with a high C/N ratio will have led to high N immobilization (see above) and hence transiently induced N limitation, potentially explaining the reduced CUE. In contrast, the more gradual addition of litter would not have induced such strong nutrient limitation, resulting in no change to the microbial CUE in these soils. These results suggest that the rate of increase in litter inputs (that is, either gradual or punctuated) will be important in determining the fate of C in soils (Liang and others 2017). However, as we only studied the effect of increased deciduous leaf-litter inputs, the effect of increased litter inputs from other plant functional types (Hobbie 1996; Parker and others 2018), as well litter mixtures reflecting the diversity of litter inputs in the subarctic tundra (McLaren and others 2017), merits further investigation.

Implications for Future C and N Cycling in Subarctic Ecosystems

In all cases, plant and microbial responses to the combined N and litter addition treatment (+NL) were statistically indistinguishable from the responses to the chronic N addition treatment (+N). This suggests that increased N availability will have the most pronounced effect on the functioning of subarctic tundra ecosystems, by supporting increased plant productivity, driving coupled changes in above and belowground community structures and accelerating rates of gross N mineralization due to microbial N-mining. Large increases in N availability ($> 5 \text{ g N m}^{-2}$), in particular, have a high potential to drive rapid changes in subarctic tundra systems, possibly more so than in arctic systems where the plant response to increased N availability is likely to saturate at lower levels (Arens and others 2008). In contrast, increased litter inputs, expected due to shrub expansion (Myers-Smith and others 2019; Nielsen and others 2019), will have more subtle effects on C- and N-cycling in subarctic soils, by primarily altering the ratio between C and N derived from SOM and influencing microbial CUE. The effect on microbial CUE differed between the chronic and extreme litter addition treatments, with a lower CUE induced by the high litter addition. As such, large inputs of leaf litter associated with litterfall after a period of high plant productivity, for example, following summer heatwaves (Marchland and others 2005) have the potential to reduce soil C sequestration, by reducing the fraction of microbially processed C which is used for growth and subsequently can be stored relative to that respired to the atmosphere (Liang and others 2017).

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