



Simulated rhizosphere deposits induce microbial N-mining that may accelerate shrubification in the subarctic

Hicks, Lettice; Leizeaga, Ainara; Rousk, Kathrin; Michelsen, Anders; Rousk, Johannes

Published in:
Ecology

DOI:
[10.1002/ecy.3094](https://doi.org/10.1002/ecy.3094)

Publication date:
2020

Document version
Publisher's PDF, also known as Version of record

Document license:
[CC BY](https://creativecommons.org/licenses/by/4.0/)

Citation for published version (APA):
Hicks, L., Leizeaga, A., Rousk, K., Michelsen, A., & Rousk, J. (2020). Simulated rhizosphere deposits induce microbial N-mining that may accelerate shrubification in the subarctic. *Ecology*, *101*(9), [e03094].
<https://doi.org/10.1002/ecy.3094>



Simulated rhizosphere deposits induce microbial N-mining that may accelerate shrubification in the subarctic

LETTICE C. HICKS,^{1,4} AINARA LEIZEAGA,¹ KATHRIN ROUSK ,^{2,3} ANDERS MICHELSEN,^{2,3} AND JOHANNES ROUSK ¹

¹Section of Microbial Ecology, Department of Biology, Lund University, Ecology Building, Lund 223 62 Sweden

²Terrestrial Ecology Section, Department of Biology, University of Copenhagen, Universitetsparken 15, Copenhagen DK-2100 Denmark

³Centre for Permafrost (CENPERM), University of Copenhagen, Øster Voldgade 10, Copenhagen DK-1350 Denmark

Citation: Hicks, L. C., A. Leizeaga, K. Rousk, A. Michelsen, and J. Rousk. 2020. Simulated rhizosphere deposits induce microbial N-mining that may accelerate shrubification in the subarctic. Ecology 101(9): e03094. 10.1002/ecy.3094

Abstract. Climate change is exposing high-latitude systems to warming and a shift towards more shrub-dominated plant communities, resulting in increased leaf-litter inputs at the soil surface, and more labile root-derived organic matter (OM) input in the soil profile. Labile OM can stimulate the mineralization of soil organic matter (SOM); a phenomenon termed “priming.” In N-poor subarctic soils, it is hypothesized that microorganisms may “prime” SOM in order to acquire N (microbial N-mining). Increased leaf-litter inputs with a high C/N ratio might further exacerbate microbial N demand, and increase the susceptibility of N-poor soils to N-mining. We investigated the N-control of SOM mineralization by amending soils from climate change–simulation treatments in the subarctic (+1.1°C warming, birch litter addition, willow litter addition, and fungal sporocarp addition) with labile OM either in the form of glucose (labile C; equivalent to 400 µg C/g fresh [fwt] soil) or alanine (labile C + N; equivalent to 400 µg C and 157 µg N/g fwt soil), to simulate rhizosphere inputs. Surprisingly, we found that despite 5 yr of simulated climate change treatments, there were no significant effects of the field-treatments on microbial process rates, community structure or responses to labile OM. Glucose primed the mineralization of both C and N from SOM, but gross mineralization of N was stimulated more than that of C, suggesting that microbial SOM use increased in magnitude and shifted to components richer in N (i.e., selective microbial N-mining). The addition of alanine also resulted in priming of both C and N mineralization, but the N mineralization stimulated by alanine was greater than that stimulated by glucose, indicating strong N-mining even when a source of labile OM including N was supplied. Microbial carbon use efficiency was reduced in response to both labile OM inputs. Overall, these findings suggest that shrub expansion could fundamentally alter biogeochemical cycling in the subarctic, yielding more N available for plant uptake in these N-limited soils, thus driving positive plant–soil feedbacks.

Key words: carbon and nitrogen mineralization; climate change; microbial carbon use efficiency; nitrogen limitation; nitrogen-mining; rhizosphere biogeochemistry; soil priming effect; subarctic tundra.

INTRODUCTION

Arctic and subarctic soils are a globally important store of terrestrial carbon (C), which is vulnerable to loss because of climate change (Schuur et al. 2009, Crowther et al. 2016, van Gestel et al. 2018). In addition to the direct effect of climate warming on soil C turnover (Davidson and Janssens 2006), climate change is also driving rapid shifts in vegetation across high-latitude systems (Martin et al. 2017, Myers-Smith et al. 2019). Shrub expansion in the Arctic (“shrubbyfication”) will affect the type, quantity, and chemistry of plant-derived

organic matter (OM) inputs to soil (Parker et al. 2015, McLaren et al. 2017), resulting in increased leaf-litter inputs at the soil surface and increased root production and associated rhizosphere input in the soil profile (Chapin et al. 1996; Sistla et al. 2013, Pausch and Kuz'yakov 2018). Roots exude a mixture of soluble organic substances, including sugars, amino acids, organic acids and enzymes, creating hotspots of microbial activity (Kuz'yakov and Blagodatskaya 2015, Sokol and Bradford 2019). These inputs of labile plant-derived OM therefore have the potential to stimulate the decomposition of soil organic matter (SOM), with consequences for future soil C storage (Mack et al. 2004, Hartley et al. 2012, Wild et al. 2014, Guenet et al. 2018). However, the mechanisms determining the responses to labile OM inputs in soils are currently not well understood. We also lack an

Manuscript received 23 January 2020; revised 6 March 2020; accepted 3 April 2020. Corresponding Editor: Joseph B. Yavitt.

⁴E-mail: lettice.hicks@biol.lu.se

understanding of how factors such as warming and increased litter inputs—as a result of climate change—will affect the susceptibility of soils to C loss.

Microbial mineralization of SOM can be accelerated by the input of labile plant-derived OM by a phenomenon known as “priming.” Although several different mechanisms for priming have been proposed (see Blagodatskaya and Kuzyakov 2008 for a review), responses are often strongly influenced by soil nutrient availability and microbial nutrient demand (Dijkstra et al. 2013, Carrillo et al. 2014, Chen et al. 2014, Meier et al. 2017). The nutrient mining interpretation for priming suggests that labile OM is used as a source of energy to support microbial activity, with microorganisms catabolizing SOM to liberate and acquire nitrogen (N) from soil (Craine et al. 2007, Meier et al. 2017, Macdonald et al. 2018). In N-poor arctic and subarctic soils (Shaver and Chapin 1980, Weintraub and Schimel 2003, Mack et al. 2004, Sistla et al. 2012, Hicks et al. 2019), microbial responses to inputs of labile OM may therefore be driven by microbial demand for N. In one study of an acidic tundra heath, labile OM input did not affect the decomposition of SOM (Lynch et al. 2018). However, in another study of subarctic mountain birch forest and tundra soils, labile OM did prime the decomposition of soil C, and the priming response was reduced when labile OM was added in combination with inorganic N, suggesting that priming was induced by microbial demand for N (Hartley et al. 2010). Increased litter inputs with a high C/N ratio as a result of shrub expansion in the Arctic could exacerbate N limitation to microorganisms, thereby increasing the susceptibility of N-poor soils to priming. A stimulation of SOM decomposition associated with microbial N-mining may explain why less soil C is stored in subarctic forest soils compared to the subarctic tundra, despite higher plant productivity in the forest (Hartley et al. 2012, Parker et al. 2015). Conversely, an acceleration of N mineralization due to warmer temperatures (Salazar et al. 2020) could reduce microbial demand for N, leading to reduced microbial N-mining in response to increased labile OM inputs in the rhizosphere.

During decomposition, microorganisms break down OM into its constituent inorganic parts. Although rates of C and N mineralization in soils are often assumed to be coupled, recent studies have shown a strong decoupling of C and N mineralization after labile C addition, with microorganisms specifically targeting N-rich components of SOM (Murphy et al. 2015; Rousk et al. 2016; Ehtesham and Bengtson 2017). In one study of boreal forest soils, however, although the addition of labile C increased microbial N demand, this did not lead to microbial N-mining from SOM, with microorganisms instead more efficiently immobilizing available N (Wild et al. 2017). Other recent studies have also challenged the N-mining hypothesis (Mason-Jones et al. 2018, Wild et al. 2019). Hence, further investigation is required in order to assess the N-control of

SOM mineralization in response to labile OM inputs in high-latitude soils.

To investigate the N-control of SOM mineralization, soils from climate change–simulation treatments in the subarctic (+1.1°C warming, birch litter addition, willow litter addition, and fungal sporocarp addition, generating a wide-range of C/N treatments) were amended with labile OM either in the form of glucose (labile C) or alanine (labile C+N), simulating rhizosphere inputs. We hypothesized that (1) glucose addition would induce greater mineralization of N than C sourced from SOM (N-mining). Moreover, we expected that (2) the N-mining effect would be more pronounced in climate change–simulation treatments of higher C/N (plant litter) than treatments with lower C/N (fungal sporocarps and warming), with the control treatments intermediate. We also hypothesized that (3) alanine (labile C+N) addition would not result in selective N-mining, but that it would instead reduce the mineralization of both soil C and N, due to preferential utilisation of C and N from the added labile OM.

MATERIALS AND METHODS

Field experiments

Experimental field treatments to simulate climate change in the subarctic were established in a tundra heath, close to the Abisko Scientific Research Station in northern Sweden (68°21' N, 18°49' E), where the mean annual temperature is 0.2°C and mean annual precipitation is 340 mm (30 yr average 1986–2015; Abisko Scientific Research Station 2016). The vegetation is dominated by dwarf shrubs and mosses, with scattered forbs and graminoids. Common ericaceous dwarf shrubs include *Vaccinium uliginosum* L., *Empetrum hermaphroditum* L., *Andromeda polifolia* L., and *Rhododendron lapponicum* (L.) Wahlenb. along with the nonericaceous dwarf shrubs *Dryas octopetala* L. and *Betula nana* L., as well as the dominant moss *Tomentypnum nitens* (Hedw.) Loeske, together with *Hylocomium splendens* (Hedw.) Schimp and other mosses. Soils have formed on a base-rich schist and are classed as Histosols (International Union of Soil Sciences World Reference Base for soils, 2006). The organic soil horizon at the site is ~5–15 cm deep, and pH is close to neutral (Table 1).

Soil was sampled from five field treatments; (1) control, (2) warming, (3) mountain birch litter addition (*Betula pubescens* (Ehrh.); hereafter referred to as *Betula*), (4) dark-leaved willow litter addition (*Salix myrsinifolia* (Salisb.); hereafter referred to as *Salix*) and (5) fungal sporocarp (organic N) addition with mixed field-collected *Russula* spp., *Cortinarius* spp., and *Lecaninum* spp. sporocarps. The litter addition treatments were intended to simulate shrub expansion in the subarctic, and the fungal sporocarp addition was used as a high organic N addition, in order to generate an extreme endpoint to examine the effect of altered litter inputs on

TABLE 1. Soil physiochemical and microbial characteristics.

	Control		Warming		Betula litter		Salix litter		Fungal sporocarp	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Water content (g H ₂ O/g)	2.6	0.4	2.5	0.3	2.8	0.1	2.9	0.2	2.6	0.2
Soil pH _{H₂O}	6.8	0.03	6.8	0.06	6.8	0.04	6.8	0.05	6.8	0.03
Conductivity (μS/cm)	51	8	50	4	57	4	47	4	67	12
Soil organic matter (mg/g)	645	70	668	58	760	32	765	26	702	35
Total soil C (mg C/g)	322	40	339	34	381	14	379	12	364	12
Total soil N (mg N/g)	17.7	2.1	19.1	2.2	20.4	1.0	21.7	0.8	20.4	1.0
Soil C/N	18.8	0.3	17.7	0.7	17.6	0.4	18.2	0.4	18.0	0.7
Soil extractable NH ₄ (μg N/g)	13.2	2.0	14.1	2.4	14.8	3.6	16.3	3.5	11.9	3.4
Total PLFA (nmol/g SOM)	835	23	856	39	780	69	897	64	688	41
Bacterial PLFA (nmol/g SOM)	454	35	449	51	411	22	467	37	367	37
Fungal PLFA (nmol/g SOM)	29	1	48	10	32	7	41	8	24	3

Notes: Mean and SE ($n = 6$ except for total PLFA, fungal PLFA, and bacterial PLFA where $n = 3$). No significant differences among field treatments.

responses to labile OM additions. The field treatments were established in 1 × 1 m experimental squares, replicated in a randomized block design with six replicates. The litter and fungal sporocarp addition treatments were initiated in September 2011 and the warming treatment was initiated in May 2012. The warming treatment was achieved using open-top chambers (OTCs), designed to increase air temperature, which were installed according to the setup of the International Tundra Experiment (Marion et al. 1997). The OTCs increased the mean annual surface temperature by 0.7°C from 2.0°C to 2.7°C, and soil temperature by 1.1°C from 1.0°C to 2.1°C (1-yr average), with no distinguishable effects on soil moisture between treatments (Rousk and Michelsen 2017). Betula litter, Salix litter and fungal sporocarps, with C/N ratios of 45 ± 4.5 , 22 ± 0.8 , and 11 ± 0.2 , respectively (mean ± SE, $n = 3$), were added at a rate of $90 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$, corresponding to the annual litter fall in the nearby open birch forest (Bylund and Nordell 2001). Fungal sporocarps were first cut into ~1-cm³ pieces before being distributed evenly on the surface of the plot. These additions were made in the autumn every year, with the exception of the fungal sporocarp treatment, which could not be administered in 2012 and 2013 because of a scarcity of fruit bodies.

At the time of sampling in late July 2016, the climate change treatments had been active for 5 yr. Soil samples were collected from 6–8 randomized cores (2-cm diameter) from each plot, including only the O-horizon up to ~10-cm depth. Visible stones and coarse roots were removed and soils were homogenised by sieving, resulting in 30 independent samples (5 field treatments × 6 replicates). After sieving, soils were stored at 5°C until use (see Labile ¹³C addition experiment).

Soil physiochemistry

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 C and N content was determined using a CN analyzer (Dumas

combustion). Soil pH was measured in a 1:5 (w:v) water extraction using an electrode. Electrical conductivity (EC) was measured using the same solution, with a EC meter. The concentration of extractable NH₄⁺ was determined in diffusion traps in a 1-mol/L KCl soil extract (see Gross N mineralization).

Labile ¹³C addition experiment

Forty-five grams of fresh (fwt) soil was weighed into 150-mL microcosms with lids, which were stored at 5°C for 3 d until the start of the experiment. The soil microcosms were then moved to a climate-controlled room (16°C) shortly before soils were amended with either ¹³C-glucose (labile C), ¹³C-alanine (labile C+N), or distilled water (as a control). Alanine was chosen as the source of labile C + N used in this study, as it is one of the most abundant free amino acids found in tundra soils nearby (Andresen et al. 2008). ¹³C-labeled substrates were created by mixing ¹³C-labeled glucose or alanine with equivalent unlabeled substrates. This resulted in glucose with 3.75 atom% enrichment and alanine with 3.67 atom% enrichment (determined by isotope-ratio mass spectrometry; IRMS; see Stable isotope calculations). Soils were amended with 1,000 μg glucose or alanine/g fwt soil. For both the glucose and alanine treatments, this resulted in a C addition of ~400 μg C/g fwt soil (equivalent to 4 mg C/g soil C), and for the alanine treatment this also resulted in a N addition of ~157 μg N/g fwt soil (equivalent to 28 mg N/g soil N). Substrates were dissolved in distilled water, administered as 10 μL solution/g fwt soil, before being thoroughly mixed into the soil using a metal spatula. For controls, deionized water was added instead of glucose or alanine solutions. This resulted in 90 microcosms (5 field treatments × 3 substrate treatments × 6 replicates).

Microcosms were incubated in a climate controlled room at $16^\circ \pm 1^\circ\text{C}$ for 7 d, which matched the mean daily soil temperature during the peak growing season at the site

(Rousk and Michelsen 2017). These systems were sampled repeatedly over 1 week to measure fungal growth, bacterial growth, respiration (along with its ^{13}C enrichment), gross N mineralization, and microbial phospholipid fatty acid (PLFA) composition (along with its ^{13}C enrichment).

Respiration and ^{13}C - CO_2 enrichment

Respiration (i.e., C mineralization) 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 5–18 h at 16°C in the dark, adapting the incubation time dependent on the desired temporal resolution of measurements (i.e., shorter incubation times to capture the dynamics immediately after the substrate additions). The headspace CO_2 concentration was analyzed using a gas chromatograph equipped with a methanizer and flame ionization detector. Headspace subsamples (1.5 mL) were also transferred to He-purged 12-mL glass exetainers (Labco, Lampeter, UK) using a gastight syringe, for subsequent isotope ratio measurements of the produced CO_2 (see Stable isotope calculations).

Gross N mineralization

Gross N mineralization rates were determined using the ^{15}N pool-dilution method, as described by Rousk et al. (2016). Briefly, at each time point, two subsamples of soil (each 3.0 g fwt) were weighed into 50-mL gastight polypropylene tubes, to which 67 μL NH_4Cl (45 μg N/mL, enriched to 5 atom% ^{15}N) was administered using a pipette. The ^{15}N addition to these soils increased the inorganic N concentration by approximately 1 μg N/g, corresponding to limited changes in mineral N concentrations, at <10% increases in NH_4^+ concentrations above initial levels (Table 1). Soils were immediately vigorously mixed with a spatula to ensure even distribution of the added NH_4Cl before tubes were lidded. One set of subsamples were extracted using a 1-mol/L KCl solution approximately 1 h after ^{15}N addition, and the second set was treated identically after 6–18-h incubation at 16°C without light. The amount of $^{15}\text{NH}_4^+\text{-N}$ and $^{14}\text{NH}_4^+\text{-N}$ were determined by IRMS after diffusion to acidified glass fiber traps, according to standard procedures (International Atomic Energy Agency; IAEA 2001). The $^{15}\text{N}/^{14}\text{N}$ content of the glass fiber traps were measured with a Flash 2000 elemental analyzer coupled to a Delta V plus via the ConFlow interface (Thermo Fisher Scientific, Bremen, Germany), at the Stable Isotope Facility at the Department of Biology, Lund, Sweden. Gross N mineralization rates were estimated according to the equations described by Bengtson et al. (2005).

Microbial growth and carbon use efficiency

Microbial growth rates were measured using soils subsampled from the microcosms over time. Bacterial

growth was determined by measuring the rate of ^3H -thymidine (TdR) incorporation into extracted bacteria (Bååth 1992), which estimates the rate of DNA synthesis as a measure of bacterial growth. Briefly, 0.30 g soil was mixed with 20 mL demineralized water, vortexed for 3 min, and centrifuged (10 min at 1,000 g). The resulting bacterial suspension was incubated at 16°C for 2 h, with 100 nmol/L methyl- ^3H thymidine (37 MBq/mL). Bacterial growth was terminated after 2 h by adding 75 μL of 100% trichloroacetic acid. Centrifugation and washing was performed as described by Bååth et al. (2001). Scintillation cocktail (Ultima Gold; Perkin Elmer, USA) was added and the radioactivity was measured using a liquid scintillation counter. The amount of thymidine incorporated into extracted bacteria (pmol TdR incorporated $\cdot\text{g}^{-1}$ SOM $\cdot\text{h}^{-1}$) was used as a measure of bacterial growth. The rate of thymidine incorporation was converted into bacterial-C production (μg C $\cdot\text{g}^{-1}$ SOM $\cdot\text{h}^{-1}$) based on the empirical relationship determined by Soares and Rousk (2019).

Fungal growth and biomass (ergosterol concentration) were measured using the acetate-in-ergosterol incorporation method (Newell and Fallon 1991) adapted for soil (Bååth 2001, Rousk et al. 2009), which estimates the rate of ergosterol synthesis as a measure of fungal growth. To do so, 0.30 g soil was mixed with 20 μL of ^{14}C -acetate solution ([^{14}C] acetic acid, sodium salt, 2.07 GBq/mmol; Perkin Elmer, Waltham, MA, USA) and unlabeled sodium acetate, resulting in a final acetate concentration of 220 $\mu\text{mol/L}$ in the soil slurry. Samples were incubated at 16°C for 2 h before growth was terminated by addition of formalin. Ergosterol and incorporated acetate were measured according to Rousk and Bååth (2007). Ergosterol concentration was estimated from the UV absorbance at 282 nm compared with external standards. The amount of acetate incorporated into ergosterol (pmol $\cdot\text{g}^{-1}$ SOM $\cdot\text{h}^{-1}$) was used as a measure of fungal growth. The rate of acetate incorporation was converted into fungal-C production (μg C $\cdot\text{g}^{-1}$ SOM $\cdot\text{h}^{-1}$) based on the empirical relationship determined by Soares and Rousk (2019). Microbial carbon use efficiency (CUE) was estimated as the ratio between total microbial growth (bacterial + fungal C production) to the total microbial C use (total microbial growth + respiration).

PLFA composition and ^{13}C -PLFA enrichment

A subset of the time points (days 2, 4, and 7; see Appendix S1: Fig. S3) were extracted for microbial PLFAs for three of the replicates. The microbial PLFA composition was determined from 0.30-g frozen subsamples, according to Frostegård et al. (1993), with modifications (Nilsson et al. 2007). An internal standard (methyl nonadecanoate fatty acid 19:0) was added before the methylation step for quantification. The derived fatty acid methyl esters (FAMES) were quantified on a gas chromatograph with flame ionization detector, and then analyzed for their $\delta^{13}\text{C}$ content (see Stable isotope

calculations). Bacterial- (i14:0, i15:0, a15:0, i16:0, 16:1 ω 9, 16:1 ω 7, 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, and 18:0 in addition to those listed as bacterial and fungal biomarkers), was used as a measure of total microbial abundance.

Stable isotope calculations

Enrichment of ^{13}C in respired CO_2 and microbial PLFAs were expressed as $\delta^{13}\text{C}$ (‰ Vienna Pee Dee Belemnite; PDB). All IRMS measurements were performed using various appliances coupled to a Delta V Plus via the ConFlow IV interface (Thermo Fisher Scientific), at the Stable Isotope Facility at the Department of Biology, Lund University, Sweden. For measurements of $\delta^{13}\text{C}$ in respired CO_2 , a GasBench II was used, and for the $\delta^{13}\text{C}$ in FAMES derived from extracted PLFAs, a Trace GC Ultra gas chromatograph was used. The $\delta^{13}\text{C}$ in respired CO_2 was corrected for the background signal of $\delta^{13}\text{C}$ in the sampled headspace using standard procedures. The $\delta^{13}\text{C}$ values for PLFAs were corrected for the C atom added during the methylation step, as in Williams et al. (2006).

The total CO_2 efflux and microbial PLFAs from each soil was partitioned into labile substrate C- and soil-derived components, using a mass balance approach, where f_{sub} was the fraction of the total C that was substrate derived, δT was the isotopic signature of the treated sample obtained at each time point, δC was the isotopic signature of the control treatment without labile OM addition and δS was the isotopic signature of the added substrate:

$$f_{\text{sub}} = \frac{\delta\text{T} - \delta\text{C}}{\delta\text{S} - \delta\text{C}}.$$

In order to distinguish the effect of labile OM additions on microbial processes, all rates were relativized to the control (no C substrate addition), by subtracting the basal rate of the no-C substrate addition from all values, before normalizing to that value. With this transformation, any deviation from zero would indicate how the labile OM addition modulated the rate, either by stimulation (positive values) or inhibition (negative values).

In the case of gross N mineralization, for soils amended with alanine it was first necessary to partition the N mineralized from the added substrate and N mineralized from SOM. To do so, the amount of N mineralized from the added alanine was estimated based on the measured ^{13}C -substrate derived C mineralization for individual samples at each time point (Appendix S1: Figs. S1B and S2B). This value was then subtracted from the total gross N mineralization to estimate the N

mineralization from SOM (Appendix S1: Fig. S2), before the priming of soil N mineralization was calculated as previously described. Although it is possible that some alanine-N may have been released by extracellular deamination of the amino group, very low concentrations of deamination products (<2%) and a rapid uptake of amino acids (96% of alanine lost from soil solution within 1 min) have been measured in soils (Geisseler and Horwath 2014, Wilkinson et al. 2014). Hence, our assumption that the alanine was taken up directly (Geisseler et al. 2009, 2010), and that the substrate-derived C mineralization reflects the substrate-derived N mineralization, is supported.

Data analysis

The effect of field treatments on initial soil properties (including soil physiochemical properties, basal microbial process rates, and microbial biomass parameters) were assessed by one-way analysis of variance (ANOVA). A principal-component analysis (PCA) was used to screen for differences in the PLFA composition of the soil microbial community, using the relative abundances (mol%) of PLFAs in unamended soils, after standardizing to unit variance. The scores of the principal components were also subjected to ANOVA.

As there was no evidence for significant field-treatment effects on soil properties, microbial process rates or community composition (see Results), and no clear effect of field treatment on priming responses, data from all field treatments were combined to test the main and interactive effects of substrate addition (control, glucose, or alanine) and time by repeated-measures ANOVA. In order to avoid pseudoreplication, we first averaged the data from the different field treatments across replicates, thus preserving the replication of $n = 6$. The tested response variables included priming of soil C and N mineralization as well as priming of bacterial and fungal growth and microbial CUE over the 7 d study. Prior to analysis, dependent data were log transformed in order to meet the assumptions (homogeneity of variance) of ANOVA. Pairwise comparisons of significant effects were conducted using Tukey's honestly significant difference (HSD) test, with significant differences identified where $P < 0.05$. Soil C and gross N mineralization rates were also regressed against the rate of substrate mineralization, in order to investigate the relationship between the use of substrates and the observed priming responses. All statistical analyses were performed using JMP Pro 15 for Mac (SAS Institute, North Carolina, USA).

RESULTS

Field treatment effects

Soil physiochemistry was unaffected by climate change-simulation treatments (Table 1). There was also no significant effect of field treatments on microbial

process rates (Fig. 1) or microbial biomass parameters (Table 1). Microbial C use in all soils was dominated by fungi (as suggested by the ratio >1 for fungal-to-bacterial growth; Fig. 1D), with all soils exhibiting a microbial CUE of ~ 0.16 (Fig. 1E).

Although microbial community structure did not vary significantly among field treatments, there was a tendency for separation along PC1 ($P = 0.12$), which was strongest between the fungal sporocarp addition treatment and warming treatment, towards positive and negative variable loadings, respectively (Fig. 2A). Differences along PC1 appeared to be related to higher relative abundances of the fungal marker 18:2 ω 6,9 towards negative variable loadings and a higher relative abundance of some PLFA markers associated with gram-positive bacteria (a15:0 and i15:0) towards positive variable loadings (Fig. 2B). In the case of PLFA markers associated with gram-negative bacteria, there was a higher relative abundance of 18:1 ω 7, cy19:0 and 18:1 ω 9 towards negative variable loadings, and a higher relative abundance of the cy17:0 marker towards positive variable loadings.

Effect of labile OM addition on SOM mineralization

Amendment of soils with labile substrates quickly stimulated total respiration, within 3 h (Appendix S1: Fig. S1A). In order to assess the change in soil C

mineralization induced by glucose and alanine additions, total respired C was partitioned into substrate- and soil-derived components (Appendix S1: Fig. S1B, C). There was no effect of field treatment on the cumulative priming of soil C mineralization stimulated over the 7-d study, and also no difference between glucose and alanine, with both substrates stimulating C mineralization overall by $\sim 10\%$ (Fig. 3A). There were, however, differences in the C mineralization response to glucose and alanine over time, as indicated by the significant Substrate \times Time interaction ($P < 0.001$). Immediately after the addition of glucose, soil C mineralization was increased by $\sim 50\%$, whereas the addition of alanine stimulated soil C mineralization by only $\sim 25\%$ (Fig. 3C). The priming of soil C induced by glucose remained higher than that induced by alanine for up to 15 h. After this, the priming of soil C induced by glucose declined, and the priming of soil C induced by alanine remained relatively constant until the end of the 7-d study.

Over the course of the 7-d study, the priming of soil N mineralization induced by alanine was approximately four times greater than induced by the glucose addition (Fig. 3B), with no clear effect of field treatment. The responses to glucose and alanine also varied over time, as demonstrated by the significant Substrate \times Time interaction ($P < 0.001$). Immediately after the substrate additions, soil N mineralization was increased by $\sim 150\%$, with no significant difference between glucose

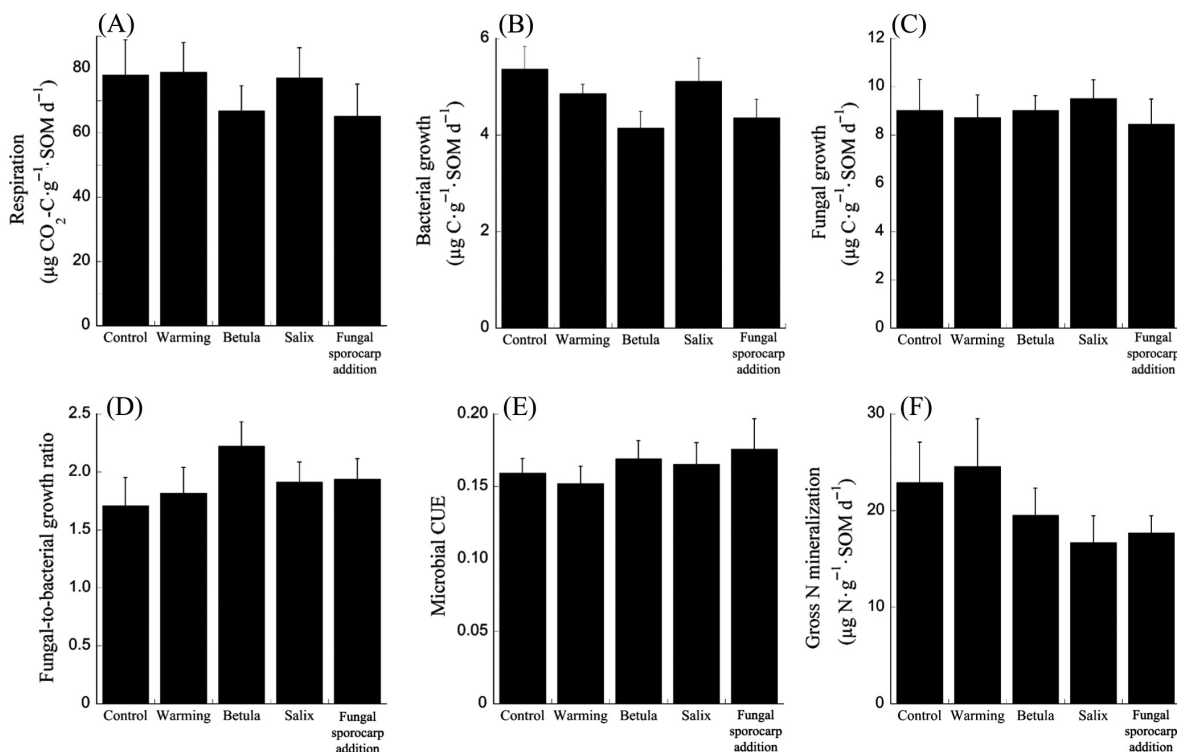


FIG. 1. (A) Respiration, (B) bacterial growth, (C) fungal growth, (D) fungal-to-bacterial growth ratio, (E) microbial carbon use efficiency (CUE), and (F) gross N mineralization in soils resulting from climate change-simulation field treatments in a subarctic heath (see x-axis). Bars represent mean ± 1 SE ($n = 6$). SOM, soil organic matter.

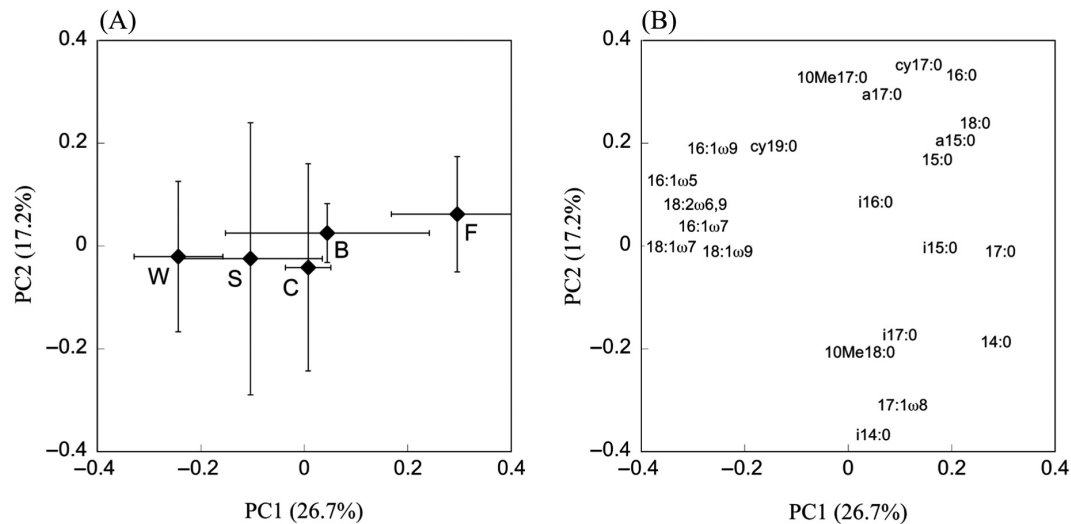


FIG. 2. Microbial community composition in soils dependent on field treatment (C, control; W, warming; B, Betula litter; S, Salix litter; F, fungal sporocarp addition), according to a principal-component analysis (PCA) of the PLFA composition (expressed as mol%). (A) Scores of the first two principal components, together explaining 43.9% variation (data represent mean \pm 1 SE, $n = 3$). (B) Loadings of the first two components from the PCA of PLFA composition, showing the PLFA markers driving the separation among samples in (A).

and alanine (Fig. 3D). However, 15 h after the substrate additions, the priming of N mineralization induced by alanine increased dramatically to reach \sim 800%, and although the priming response generally decreased after this peak, it remained substantially higher than that induced by glucose for the duration of the study (Fig. 3D).

In soils amended with glucose, there was a strong relationship between the mineralization of the added substrate and priming of both soil C and N mineralization (Fig. 4). In contrast, for alanine, although there was a trend for higher priming of soil N mineralization with greater mineralization of the added substrate ($R^2 = 0.43$, $P = 0.23$), there was no significant relationship between substrate mineralization and the priming of soil C or N (Fig. 4).

Effect of labile OM addition on microbial growth

The addition of glucose stimulated priming of both bacterial and fungal growth, whereas the addition of alanine only induced priming of bacterial growth (Fig. 5A, B). There was no effect of field treatment on bacterial or fungal growth responses to either substrate addition; hence we combined the data from all field treatments to examine the responses to glucose and alanine additions over time. Initially, immediately after the addition of glucose and alanine, bacterial and fungal growth was statistically indistinguishable from the control (Fig. 5C, D). However, over the following 7 d, different microbial growth dynamics were induced by the added substrates, as shown by the significant Substrate \times Time interaction ($P < 0.001$ for bacterial growth and $P = 0.01$ for fungal growth). After

day 1, bacterial growth was \sim 40% higher in soils amended with glucose and \sim 60% higher in soils amended with alanine, compared to the control. In the soils amended with glucose, bacterial growth remained high until day 2 and then gradually declined towards the control. In contrast, for the soils amended with alanine, the priming of bacterial growth increased further by day 2 and remained elevated until the end of the 7-d study. In the case of fungi, after day 1, fungal growth was \sim 20% higher in soils amended with glucose than the control, and remained elevated for the duration of the study (Fig. 5D). In contrast, there was no effect of alanine on the priming of fungal growth.

The responses of bacterial and fungal growth to labile OM inputs were broadly reflected by the incorporation of ^{13}C -glucose and ^{13}C -alanine into microbial PLFAs (Appendix S1: Fig. S3). Tracing the incorporation of ^{13}C into microbial PLFAs revealed a particularly high incorporation of ^{13}C -labeled substrates into gram-negative bacteria, compared to the incorporation into gram-positive bacterial and fungal PLFAs (Appendix S1: Fig. S3).

Effect of labile OM addition on microbial carbon use efficiency

Microbial CUE was affected by the added substrates ($P < 0.001$) and varied over time ($P < 0.001$), with a significant Substrate \times Time interaction ($P < 0.001$). The CUE in the unamended control soils was initially \sim 0.10, but quickly increased and stabilized at \sim 0.15 after 1 d. Compared to the control, the addition of glucose and alanine immediately reduced the CUE followed by a gradual recovery. In the soils amended with glucose, microbial CUE converged with the control by day 4,

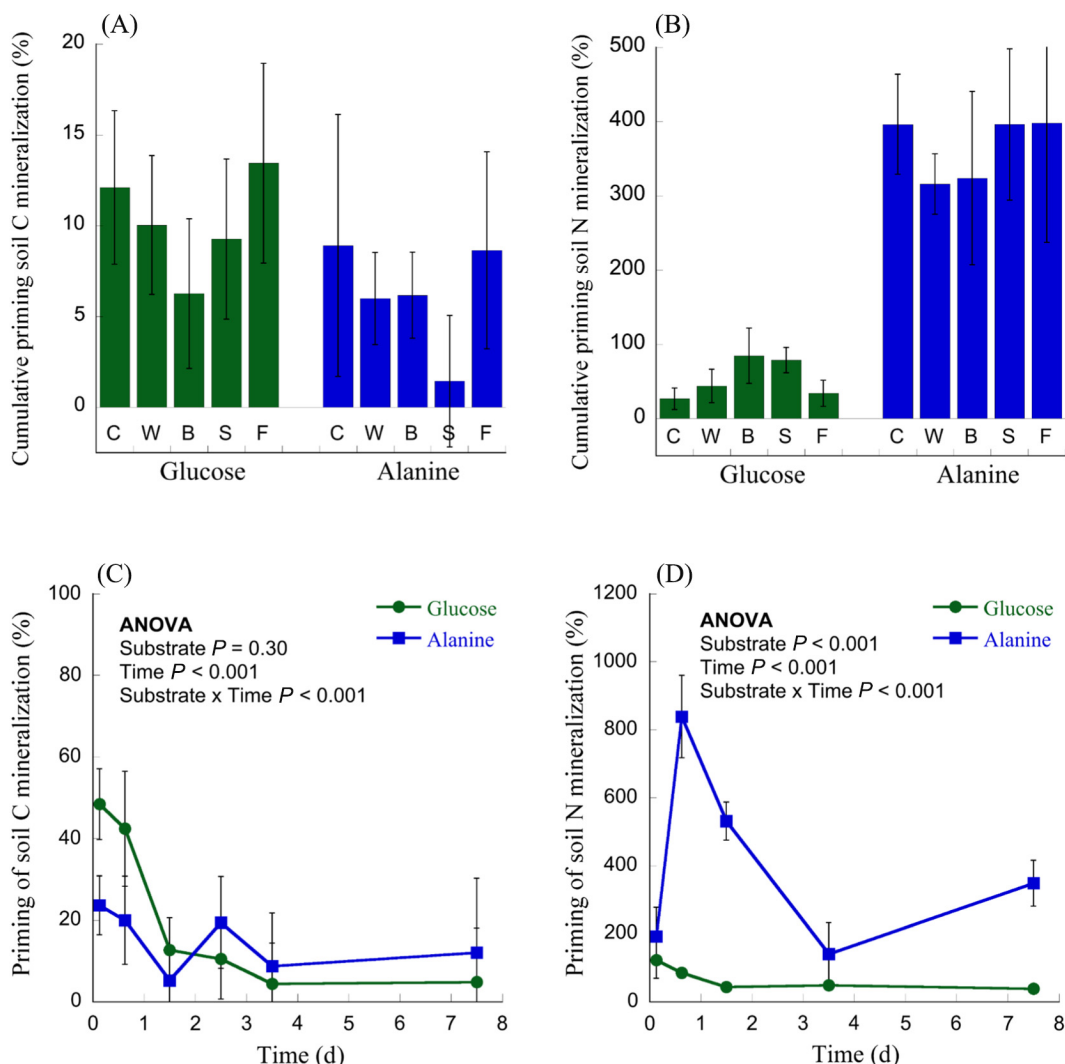


FIG. 3. The effect of glucose and alanine additions on the cumulative priming of (A) C mineralization and (B) gross N mineralization in soils from climate change simulation treatments in the subarctic (see Fig. 2 for legend description), and the priming of (C) soil C mineralization and (D) gross N mineralization over time. Deviation from zero indicates a change compared with unamended controls, whereby positive values indicate stimulated rates and negative values indicate reduced rates. Data represent mean \pm SE ($n = 6$). Where error bars cannot be seen, the bar is smaller than the symbol. Note difference in y-axis scales.

whereas in soils amended with alanine the microbial CUE did not recover to the control level until day 7.

DISCUSSION

Simulating climate change in the subarctic

Climate change will have a profound impact on arctic and subarctic ecosystems, both directly because of increased temperatures (Intergovernmental Panel on Climate Change; IPCC 2013), and indirectly because of changes in the quantity and quality of litter inputs to soil as a result of shrub expansion into the tundra (Martin et al. 2017, Myers-Smith et al. 2019). Here we used soils from a climate change–simulation field experiment—

including warming and litter-addition treatments—to investigate how these changes would influence the susceptibility of SOM to priming. Using open-top chambers, soil temperature was increased by 1.1°C above ambient, which is in line with the level of warming achieved by this approach in other subarctic studies (Rinnan et al. 2007, Weedon et al. 2012), and also reflects the magnitude of warming that is expected to occur in this ecosystem over the coming years (IPCC 2013). The annual litter addition rate (90 g/m²) represented a high resource input compared to the annual aboveground net primary productivity of 100–200 g/m² reported for arctic tundra systems (Mack et al. 2004), but corresponded to the annual litterfall of a nearby open birch forest (Bylund and Nordell 2001), thereby

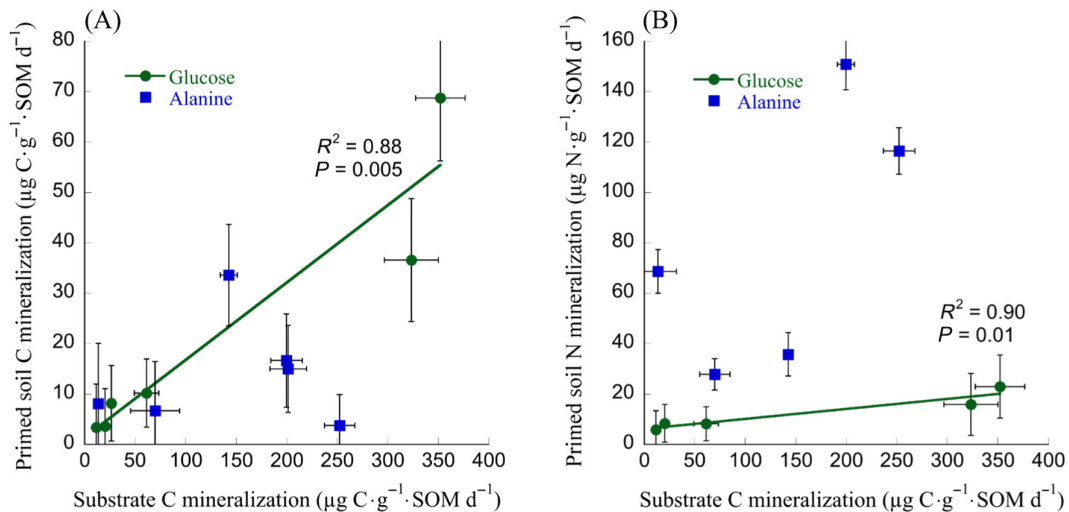


FIG. 4. The relationship between substrate mineralization and (A) priming of C mineralization and (B) priming of gross N mineralization in subarctic soils following the addition of glucose and alanine. Data represent mean \pm SE ($n = 6$). Note differences in y-axis scales.

reflecting potential inputs as a result of deciduous shrub expansion.

Although the field treatments had been active for 5 yr at the time of soil sampling, there was no significant effect on soil physiochemical parameters (Table 1) or microbial process rates (Fig. 1), and only subtle effects on microbial community structure (Fig. 2). It is possible that the physical disturbance during soil sampling could have impacted measured microbial parameters, reducing our sensitivity to detect differences among the treatments. However, previous studies found no effects of sieving field-moist soils on microbial respiration (Meyer et al. 2019), and only minor effects on PLFA composition, with sieving primarily reducing the concentration of the fungal biomarker (18:2 ω 6,9), likely due to the filamentous nature of fungi making them more susceptible to the disturbance (Petersen and Klug 1994). Despite this, we found that the fungal biomarker was one of the PLFAs that drove separation in the microbial community structure among the field treatments, indicating that the signal driven by the field treatments remained stronger than that potentially introduced by the sampling disturbance. We also found that fungi were responsive to the labile OM additions (Fig. 5), suggesting that they were not negatively impacted by the sampling procedure.

Several studies have also shown a limited responsiveness of microbial community structure and process rates to simulated climate change. For example, after 7 yr of litter additions, although some differences in microbial community structure were identified in the topsoil, there was no effect of the treatment evident below 5-cm depth (Rinnan et al. 2008). Warming has also been shown to have no clear effect on microbial composition (Rinnan et al. 2007, Weedon et al. 2012), even in response to long-term warming of up to 20 yr (Rinnan et al. 2013).

Considered together, these studies along with ours, suggest that microorganisms in subarctic soils will be resistant to increases in temperature and litter inputs as a result of future climate change. As there was no significant change in the belowground microbial community in response to the field treatments, it was not surprising that there was no clear effect of the field treatments on priming responses to labile OM additions (Fig. 3A, B). However, by combining the responses from all field treatments, our power to test for substrate effects on priming responses was enhanced.

Labile OM induces a decoupling of C and N mineralization in subarctic soils

In our study, we used ^{13}C -glucose and ^{13}C -alanine to simulate rhizosphere inputs. Tracing the incorporation of these ^{13}C -labeled substrates into microbial PLFAs revealed that the added glucose and alanine was predominantly used by gram-negative bacteria. Gram-negative bacteria are often associated with the use of root exudates derived from recently photosynthesized C (De Deyn et al. 2011, Chaudhary and Dick 2016), indicating that our additions successfully simulated sources of labile OM found in the rhizosphere. Few studies have quantified root exudation, partly because of technical difficulties in assessing gross exudation rates (Pausch and Kuzyakov 2018). However, it has been estimated that root exudation may account for up to 10% of net primary productivity (Jones et al. 2004). One study that did measure the exudation of organic C from ryegrass roots (Paterson and Sim 1999) found exudation rates corresponding to $1,500 \mu\text{g C}\cdot\text{g}^{-1} \text{soil}\cdot\text{d}^{-1}$ (Paterson et al. 2007), thus being of the same order of magnitude as the OM additions used to simulate rhizosphere inputs

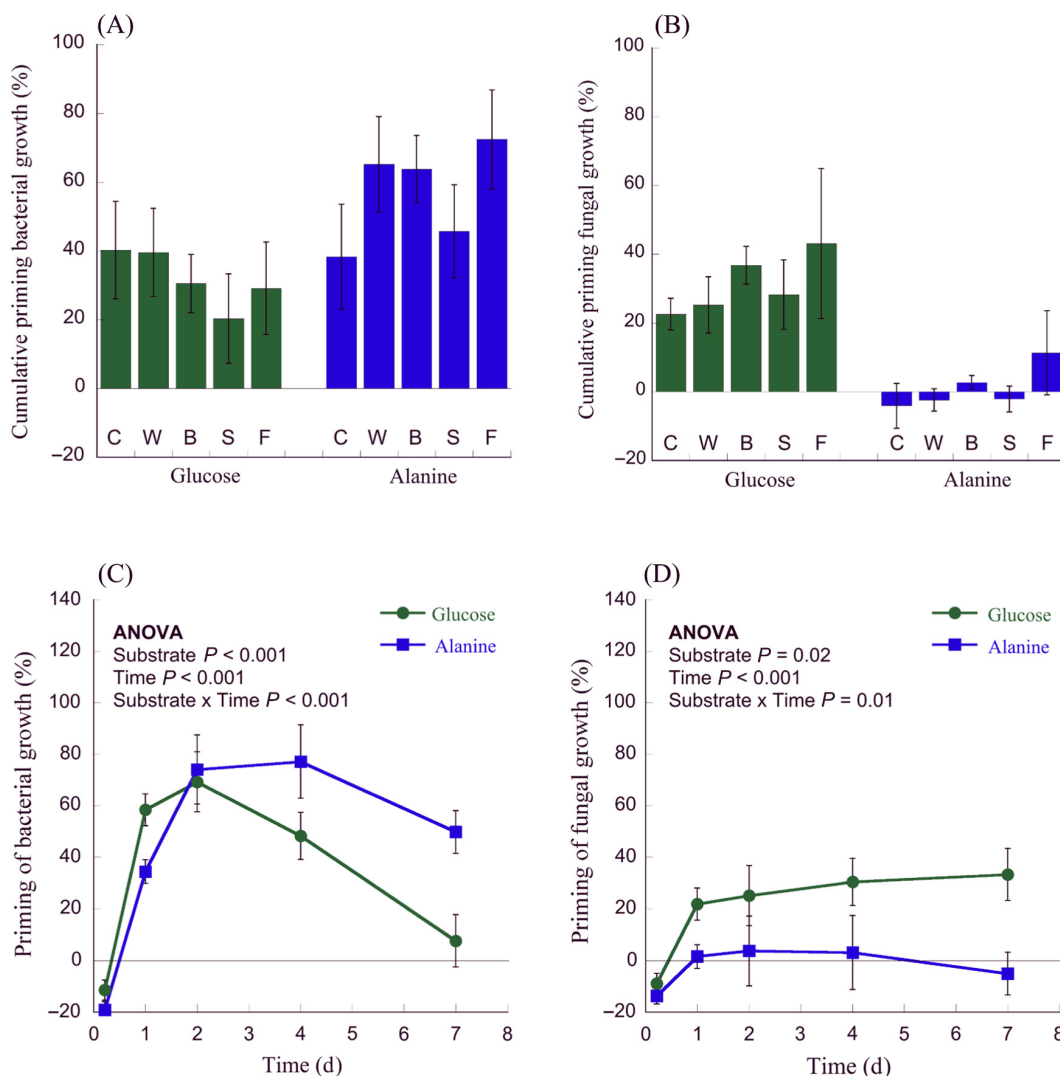


FIG. 5. The effect of glucose and alanine additions on the cumulative priming of (A) bacterial and (B) fungal growth in soils from climate change-simulation treatments in the subarctic (see Fig. 2 for legend description), and the priming of (C) bacterial growth and (D) fungal growth over time. Deviation from zero indicates a change compared with unamended controls, whereby positive values indicate stimulated rates and negative values indicate reduced rates. Data represent mean \pm SE ($n = 6$). Where error bars cannot be seen, the bar is smaller than the symbol.

in our study. The quantification of rhizodeposition rates for arctic plants, and the role of symbiotic fungi in the decomposition of SOM (Hewitt et al. 2020), which we do not include here, clearly remains an important avenue for future research. However, a meta-analysis of 31 studies found that woody plant species induced stronger priming effects than grasses (Huo et al. 2017). Moreover, given that root exudation comprises $\sim 10\%$ of net primary productivity (Jones et al. 2004), it is likely that inputs of root-derived OM and thus the potential for priming will increase in concert with increases in plant growth and shrubification associated with climate change in the Arctic (Myers-Smith et al. 2019).

We hypothesized that glucose addition to these N-poor subarctic soils would induce a specific targeting of

N-rich SOM by microbes. Consistent with this prediction, following the addition of glucose, soil C mineralization was increased by $\sim 50\%$, and soil N mineralization was increased by $\sim 120\%$ (Fig. 3C, D), indicating selective microbial N-mining. These responses are consistent with those previously found for these same subarctic tundra soils, whereby glucose addition led to a decoupling of C and N mineralization, resulting in a lower C/N ratio of OM that was mineralized (Rousk et al. 2016).

The N-mining interpretation of priming suggests that in N-poor soils, soil microorganisms mineralize SOM in order to acquire N. As such, we hypothesized that the addition of alanine would reduce microbial mineralization of soil N, because microorganisms could use the

stoichiometrically balanced supply of C and N from the added labile substrate. Contrary to expectation, we found that the addition of alanine stimulated the microbial mineralization of soil N to an even greater degree than that stimulated by the glucose addition. A recent study of agricultural soils also found that the addition of alanine induced priming of SOM (Mason-Jones et al. 2018). In the agricultural soils, there was a positive relationship between the mineralization of the added substrate and the priming of soil C mineralization, suggesting that the priming effect may be explained by the C-induced synthesis of SOM-degrading extracellular enzymes. In contrast, in our study, although there was a positive relationship between the mineralization of glucose and the priming of soil C and N mineralization, there was no significant relationship between the mineralization of alanine and priming responses (Fig. 4). This points towards a different mechanism for priming in our soils, with the response differing depending on the input of labile substrates containing C alone or nitrogenous substrates containing both C and N.

Although the stimulated gross N mineralization (i.e., N-mining) response to the addition of a nitrogenous substrate is seemingly counterintuitive, our finding may be explained in light of the theory forwarded by Schimel and Bennett (2004). It was suggested that in N-poor ecosystems, depolymerization of N-containing compounds by extracellular enzymes is rate limiting for the production of bioavailable N. If N availability increases, however, microorganisms may be able to increase the synthesis of N-acquisitioning enzymes (Weintraub and Schimel 2003), resulting in enhanced rates of organic N depolymerization and mineralization (Wild et al. 2015). In a chronically N-depleted environment—such as subarctic tundra ecosystems—this strategy of using and investing the source of labile C and N in order to acquire more N by N-mining should “future proof” the microbial community against N limitation. As such, this could be a trait that has been selected for within the studied N-poor subarctic soils, explaining the dramatic N mineralization response following the addition of alanine. Analogous trait–environment relationships have been shown for plants (Keddy 1992), whereby resource-poor environments promote plants with traits that are resource conservative and retentive in order to sustain the population under low levels of resource supply (Reich 2014, Bruelheide et al. 2018). For instance, at a nearby subarctic tundra site, the nutrient-conservative dwarf shrub *V. uliginosum* did not respond to long-term nutrient or labile C addition, whereas the more opportunistic grass *Festuca ovina* was promoted by nutrient enrichment and retarded by microbial N immobilization caused by labile-C–stimulated microbial growth (Michelsen et al. 1999). The release of inorganic N as a result of microbial N-mining would also benefit plants in N-limited subarctic soils (Michelsen et al. 1999, Hicks et al. 2019). This could generate positive plant–soil feedbacks, whereby the

elevated N availability supports increased plant productivity, potentially accelerating the rate of subarctic shrubification.

Microbial growth responses to labile OM additions

The addition of glucose and alanine quickly stimulated priming of SOM, with priming of soil C mineralization increasing by 25–50% and gross soil N mineralization increasing by 150% within 3 h of the substrate additions (Fig. 3C, D). This observed priming of SOM did not, however, coincide with the priming of microbial growth, as a stimulation of bacterial and fungal growth did not occur until 24 h after the substrate additions (Fig. 5C, D). There was also a mismatch in the subsequent dynamics of microbial growth and SOM mineralization, with the priming of bacterial growth peaking 2 d after the addition of both glucose and alanine, at a time when the priming of C and N mineralization in the soils was decreasing. Taken together, these results suggest that the priming of SOM mineralization was driven by catabolic, rather than anabolic, responses of the resident microbial community. In a previous study of the same subarctic soils, the priming of SOM induced by glucose was also associated with a catabolic microbial response (Rousk et al. 2016). As the response occurred rapidly (i.e., within 3 h) after the addition of the glucose and alanine, the most likely explanation is that the labile OM triggered a catabolic response of the resident microbial community and its associated extracellular enzymes (Drake et al. 2011, Bengtson et al. 2012).

Immediately following the addition of glucose and alanine to soils, microbial CUE was reduced compared to the control (Fig. 6). It has been suggested that there is an inverse relationship between microbial CUE and N-use

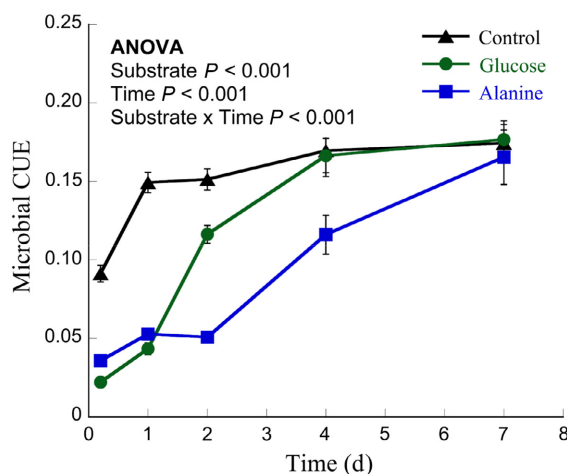


FIG. 6. Microbial carbon use efficiency (CUE) in soils following the addition of glucose (labile C) and alanine (labile C + N) compared to control (unamended) soils. Data represent mean \pm SE ($n = 6$). Where error bar cannot be seen, the bar is smaller than the symbol.

efficiency (NUE) driven by microbial stoichiometry, whereby under N limitation microbes use N with high efficiency (i.e., a low fraction of inorganic N is released as ammonium) and that this is accompanied by a low CUE (Mooshammer et al. 2014). There is, however, no evidence that this occurred in our study, as microbial CUE was reduced by the addition of labile OM, and this coincided with increased gross N mineralization, indicating that NUE was also low. This suggests that the stimulated N-mineralization response to added glucose and alanine occurred extracellularly, which is consistent with our interpretation that the N-mining was driven by a triggering of extracellular enzymes (Drake et al. 2011, Bengtson et al. 2012, Rousk et al. 2016). The sustained reduction in microbial CUE in response to alanine compared to glucose (Fig. 6) may also reflect that more energy was used for the synthesis of N-acquisitioning enzymes, which sustained the increased N-mineralization response over the duration of the 7-d study (Fig. 3D).

CONCLUSIONS AND OUTLOOK

Shrub expansion and increased primary productivity associated with climate change in the Arctic and subarctic will increase inputs of labile OM in the rhizosphere (Jones et al. 2004, Huo et al. 2017, Myers-Smith et al. 2019). A stimulation of soil C mineralization in response to increased plant productivity has been observed *in situ* at a nearby experimental site in Abisko (Ravn et al. 2017) as well as in other high-latitude ecosystems (Mack et al. 2004, Hartley et al. 2012), demonstrating the potential for soil C loss in response to future climate change. Our results, rather, suggest that labile OM inputs will stimulate the mineralization of both C and N, and that N mineralization would be stimulated more than C mineralization. This would make more N available for plant uptake in these N-limited soils, resulting in positive plant–soil feedbacks whereby increased N availability driven by microbial N-mining would support increased plant productivity (Michelsen et al. 1999, Hicks et al. 2019). The importance of soil microorganisms in decomposing OM to maintain plant productivity is widely recognized (Van Der Heijden et al. 2008). However, our findings suggest that rather than just maintaining plant productivity, plant–soil feedbacks initiated by climate change–driven shrub expansion could act as a positive feedback accelerating plant productivity in subarctic tundra ecosystems. Eventually, these plant–soil feedbacks may lead to the loss of old SOM, causing tundra soil properties to converge towards those of subarctic forest soils (Hartley et al. 2012, Parker et al. 2015).

ACKNOWLEDGMENTS

We thank the Abisko Scientific Research Station (ANS) for providing logistical support and Eva Berglund for laboratory assistance. This study was supported by grants from the Crafoord Foundation (Grant 20150561), the Carl Tryggers Foundation (Grant CTS16: 385), the Knut and Alice Wallenberg

Foundation (Grant KAW 2017.0171), and the Swedish Research Council Formas (Grant 2018-01315). The research is a contribution to the strategic research area Biodiversity and Ecosystems in a Changing Climate (BECC) at Lund University.

LITERATURE CITED

- Abisko Scientific Research Station. 2016. Temperature and precipitation data 1913–2015. Abisko Scientific Research Station, Polarforskningssektretariatet. <http://polar.se/abisko>
- Andresen, L. C., S. Jonasson, L. Ström, and A. Michelsen. 2008. Uptake of pulse injected nitrogen by soil microbes and mycorrhizal and non-mycorrhizal plants in a species-diverse subarctic heath ecosystem. *Plant and Soil* 313:283–295.
- Bååth, E. 1992. Thymidine incorporation into macromolecules of bacteria extracted from soil by homogenization-centrifugation. *Soil Biology and Biochemistry* 24:1157–1165.
- Bååth, E. 2001. Estimation of fungal growth rates in soil using ¹⁴C-acetate incorporation into ergosterol. *Soil Biology and Biochemistry* 33:2011–2018.
- Bååth, E., M. Pettersson, and K. H. Söderberg. 2001. Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. *Soil Biology and Biochemistry* 33:1571–1574.
- Bengtson, P., J. Barker, and S. J. Grayston. 2012. Evidence of a strong coupling between root exudation, C and N availability, and stimulated SOM decomposition caused by rhizosphere priming effects. *Ecology and Evolution* 2:1843–1852.
- Bengtson, P., U. Falkengren-Grerup, and G. Bengtsson. 2005. Relieving substrate limitation soil moisture and temperature determine gross N transformation rates. *Oikos* 111:81–90.
- Blagodatskaya, E., and Y. Kuzyakov. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils* 45:115–131.
- Bruelheide, H., et al. 2018. Global trait–environment relationships of plant communities. *Nature Ecology & Evolution* 2:1906–1917.
- Bylund, H., and O. Nordell. 2001. Biomass proportion, production and leaf nitrogen distribution in a polycoformic mountain birch stand (*Betula pubescens* ssp. *czerepanovii*) in northern Sweden. Pages 115–126 in F. E. Wielgolaski, editors. *Nordic mountain birch ecosystems*, New York, London: UNESCO.
- Carrillo, Y., F. A. Dijkstra, E. Pendall, D. LeCain, and C. Tucker. 2014. Plant rhizosphere influence on microbial C metabolism: the role of elevated CO₂, N availability and root stoichiometry. *Biogeochemistry* 117:229–240.
- Chapin, F. S., M. S. Bret-Harte, S. E. Hobbie, and H. Zhong. 1996. Plant functional types as predictors of transient responses of arctic vegetation to global change. *Journal of Vegetation Science* 7:347–358.
- Chaudhary, D. R., and R. P. Dick. 2016. Identification of metabolically active rhizosphere microorganisms by stable isotopic probing of PLFA in switchgrass. *Communications in Soil Science and Plant Analysis* 47:2433–2444.
- Chen, R., M. Senbayram, S. Blagodatsky, O. Myachina, K. Dittert, X. Lin, E. Blagodatskaya, and Y. Kuzyakov. 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. *Global Change Biology* 20:2356–2367.
- Craine, J. M., C. Morrow, and N. Fierer. 2007. Microbial nitrogen limitation increases decomposition. *Ecology* 88:2105–2113.
- Crowther, T. W., et al. 2016. Quantifying global soil carbon losses in response to warming. *Nature* 540:104–108.
- Davidson, E. A., and I. A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440:165–173.

- De Deyn, G., H. Quirk, S. Oakley, N. Ostle, and R. Bardgett. 2011. Rapid transfer of photosynthetic carbon through the plant–soil system in differently managed species-rich grasslands. *Biogeosciences* 8:1131–1139.
- Dijkstra, F. A., Y. Carrillo, E. Pendall, and J. A. Morgan. 2013. Rhizosphere priming: a nutrient perspective. *Frontiers in Microbiology* 4:216.
- Drake, J. E., et al. 2011. Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO₂. *Ecology Letters* 14:349–357.
- Ehteshami, E., and P. Bengtson. 2017. Decoupling of soil carbon and nitrogen turnover partly explains increased net ecosystem production in response to nitrogen fertilization. *Scientific Reports* 7:46286.
- Frostegård, Å., and E. Bååth. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils* 22:59–65.
- Frostegård, Å., A. Tunlid, and E. Bååth. 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Applied and Environmental Microbiology* 59:3605–3617.
- Geisseler, D., and W. R. Horwath. 2014. Investigating amino acid utilization by soil microorganisms using compound specific stable isotope analysis. *Soil Biology and Biochemistry* 74:100–105.
- Geisseler, D., W. R. Horwath, and T. A. Doane. 2009. Significance of organic nitrogen uptake from plant residues by soil microorganisms as affected by carbon and nitrogen availability. *Soil Biology and Biochemistry* 41:1281–1288.
- Geisseler, D., W. R. Horwath, R. G. Joergensen, and B. Ludwig. 2010. Pathways of nitrogen utilization by soil microorganisms — a review. *Soil Biology and Biochemistry* 42:2058–2067.
- Guenet, B., M. Camino-Serrano, P. Ciais, M. Tifafi, F. Maignan, J. L. Soong, and I. A. Janssens. 2018. Impact of priming on global soil carbon stocks. *Global Change Biology* 24:1873–1883.
- Hartley, I. P., M. H. Garnett, M. Sommerkorn, D. W. Hopkins, B. J. Fletcher, V. L. Sloan, G. K. Phoenix, and P. A. Wookey. 2012. A potential loss of carbon associated with greater plant growth in the European Arctic. *Nature Climate Change* 2:875–879.
- Hartley, I. P., D. W. Hopkins, M. Sommerkorn, and P. A. Wookey. 2010. The response of organic matter mineralisation to nutrient and substrate additions in sub-arctic soils. *Soil Biology and Biochemistry* 42:92–100.
- Hewitt, R. E., M. R. DeVan, I. V. Lagutina, H. Genet, A. D. McGuire, D. L. Taylor, and M. C. Mack. 2020. Mycobiont contribution to tundra plant acquisition of permafrost-derived nitrogen. *New Phytologist* 226:126–141.
- Hicks, L. C., K. Rousk, R. Rinnan, and J. Rousk. 2019. Soil microbial responses to 28 years of nutrient fertilization in a subarctic heath. *Ecosystems*. <http://dx.doi.org/https://doi.org/10.1007/s10021-019-00458-7>
- Huo, C., Y. Luo, and W. Cheng. 2017. Rhizosphere priming effect: a meta-analysis. *Soil Biology and Biochemistry* 111:78–84.
- IAEA. 2001. IAEA Training Courses Series, No 14. Use of isotope and radiation methods in soil and water management and crop nutrition. IAEA, Vienna, Austria.
- IPCC. 2013. Summary for policymakers. *In* T. F. Stocker, D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. M. Midgley, editors. *Climate change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK.
- IUSS Working Group WRB. 2006. World reference base for soil resources 2006. *World Soil Resources Reports* 103.
- Jones, D. L., A. Hodge, and Y. Kuzyakov. 2004. Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist* 163:459–480.
- Keddy, P. A. 1992. A pragmatic approach to functional ecology. *Functional Ecology* 6:621–626.
- Kuzyakov, Y., and E. Blagodatskaya. 2015. Microbial hotspots and hot moments in soil: concept & review. *Soil Biology and Biochemistry* 83:184–199.
- Lynch, L. M., M. B. Machmuller, M. F. Cotrufo, E. A. Paul, and M. D. Wallenstein. 2018. Tracking the fate of fresh carbon in the Arctic tundra: Will shrub expansion alter responses of soil organic matter to warming? *Soil Biology and Biochemistry* 120:134–144.
- Macdonald, C. A., M. Delgado-Baquerizo, D. S. Reay, L. C. Hicks, and B. K. Singh. 2018. Soil nutrients and soil carbon storage: modulators and mechanisms. Pages 167–205 *in* B. Singh, editor. *Soil carbon storage: modulators, mechanisms and modelling*. Cambridge, MA: Academic Press.
- Mack, M. C., E. A. Schuur, M. S. Bret-Harte, G. R. Shaver, and F. S. Chapin III. 2004. Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature* 431:440–443.
- Marion, G. M., et al. 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. *Global Change Biology* 3:20–32.
- Martin, A. C., E. S. Jeffers, G. Petrokofsky, I. Myers-Smith, and M. Macias-Fauria. 2017. Shrub growth and expansion in the Arctic tundra: an assessment of controlling factors using an evidence-based approach. *Environmental Research Letters* 12:085007.
- Mason-Jones, K., N. Schmücker, and Y. Kuzyakov. 2018. Contrasting effects of organic and mineral nitrogen challenge the N-mining hypothesis for soil organic matter priming. *Soil Biology and Biochemistry* 124:38–46.
- McLaren, J. R., K. M. Buckeridge, M. J. van de Weg, G. R. Shaver, J. P. Schimel, and L. Gough. 2017. Shrub encroachment in Arctic tundra: *Betula nana* effects on above- and belowground litter decomposition. *Ecology* 98:1361–1376.
- Meier, I. C., A. C. Finzi, and R. P. Phillips. 2017. Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. *Soil Biology and Biochemistry* 106:119–128.
- Meyer, N., G. Welp, and W. Amelung. 2019. Effect of sieving and sample storage on soil respiration and its temperature sensitivity (Q₁₀) in mineral soils from Germany. *Biology and Fertility of Soils* 55:825–832.
- Michelsen, A., E. Graglia, I. K. Schmidt, S. Jonasson, D. Sleep, and C. Quarmby. 1999. Differential responses of grass and a dwarf shrub to long-term changes in soil microbial biomass C, N and P following factorial addition of NPK fertilizer, fungicide and labile carbon to a heath. *New Phytologist* 143:523–538.
- Mooshammer, M., et al. 2014. Adjustment of microbial nitrogen use efficiency to carbon: nitrogen imbalances regulates soil nitrogen cycling. *Nature Communications* 5:3694.
- Murphy, C. J., E. M. Baggs, N. Morley, D. P. Wall, and E. Paterson. 2015. Rhizosphere priming can promote mobilisation of N-rich compounds from soil organic matter. *Soil Biology and Biochemistry* 81:236–243.
- Myers-Smith, I. H., et al. 2019. Eighteen years of ecological monitoring reveals multiple lines of evidence for tundra vegetation change. *Ecological Monographs* 89:e01351.
- Newell, S. Y., and R. D. Fallon. 1991. Toward a method for measuring instantaneous fungal growth rates in field samples. *Ecology* 72:1547–1559.
- Nilsson, L. O., E. Bååth, U. Falkengren-Grerup, and H. Wallander. 2007. Growth of ectomycorrhizal mycelia and

- composition of soil microbial communities in oak forest soils along a nitrogen deposition gradient. *Oecologia* 153:375–384.
- Parker, T. C., J. A. Subke, and P. A. Wookey. 2015. Rapid carbon turnover beneath shrub and tree vegetation is associated with low soil carbon stocks at a subarctic treeline. *Global Change Biology* 21:2070–2081.
- Paterson, E., T. Gebbing, C. Abel, A. Sim, and G. Telfer. 2007. Rhizodeposition shapes rhizosphere microbial community structure in organic soil. *New Phytologist* 173:600–610.
- Paterson, E., and A. Sim. 1999. Rhizodeposition and C-partitioning of *Lolium perenne* in axenic culture affected by nitrogen supply and defoliation. *Plant and Soil* 216:155–164.
- Pausch, J., and Y. Kozuyakov. 2018. Carbon input by roots into the soil: quantification of rhizodeposition from root to ecosystem scale. *Global Change Biology* 24:1–12.
- Petersen, S. O., and M. J. Klug. 1994. Effects of sieving, storage, and incubation temperature on the phospholipid fatty acid profile of a soil microbial community. *Applied Environmental Microbiology* 60:2421–2430.
- Ravn, N. R., P. Ambus, and A. Michelsen. 2017. Impact of decade-long warming, nutrient addition and shading on emission and carbon isotopic composition of CO₂ from two subarctic dwarf shrub heaths. *Soil Biology and Biochemistry* 111:15–24.
- Reich, P. B. 2014. The world-wide ‘fast–slow’ plant economics spectrum: a traits manifesto. *Journal of Ecology* 102:275–301.
- Rinnan, R., A. Michelsen, and E. Bååth. 2013. Fungi benefit from two decades of increased nutrient availability in tundra heath soil. *PLoS ONE* 8:e56532.
- Rinnan, R., A. Michelsen, E. Bååth, and S. Jonasson. 2007. Fifteen years of climate change manipulations alter soil microbial communities in a subarctic heath ecosystem. *Global Change Biology* 13:28–39.
- Rinnan, R., A. Michelsen, and S. Jonasson. 2008. Effects of litter addition and warming on soil carbon, nutrient pools and microbial communities in a subarctic heath ecosystem. *Applied Soil Ecology* 39:271–281.
- Rousk, J., and E. Bååth. 2007. Fungal biomass production and turnover in soil estimated using the acetate-in-ergosterol technique. *Soil Biology and Biochemistry* 39:2173–2177.
- Rousk, J., P. C. Brookes, and E. Bååth. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied Environmental Microbiology* 75:1589–1596.
- Rousk, K., and A. Michelsen. 2017. Ecosystem nitrogen fixation throughout the snow-free period in subarctic tundra: effects of willow and birch litter addition and warming. *Global Change Biology* 23:1552–1563.
- Rousk, K., A. Michelsen, and J. Rousk. 2016. Microbial control of soil organic matter mineralization responses to labile carbon in subarctic climate change treatments. *Global Change Biology* 22:4150–4161.
- Ruess, L., and P. M. Chamberlain. 2010. The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. *Soil Biology and Biochemistry* 42:1898–1910.
- Salazar, A., K. Rousk, I. S. Jónsdóttir, J. P. Bellenger, and Ó. S. Andrésón. 2020. Faster nitrogen cycling and more fungal and root biomass in cold ecosystems under experimental warming: a meta-analysis. *Ecology* 101:e02938.
- Schimel, J. P., and J. Bennett. 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85:591–602.
- Schuur, E. A., J. G. Vogel, K. G. Crummer, H. Lee, J. O. Sickman, and T. E. Osterkamp. 2009. The effect of permafrost thaw on old carbon release and net carbon exchange from tundra. *Nature* 459:556–559.
- Shaver, G. R., and F. S. Chapin III. 1980. Response to fertilization by various plant growth forms in an Alaskan tundra: nutrient accumulation and growth. *Ecology* 61:662–675.
- Sistla, S. A., S. Asao, and J. P. Schimel. 2012. Detecting microbial N-limitation in tussock tundra soil: implications for Arctic soil organic carbon cycling. *Soil Biology and Biochemistry* 55:78–84.
- Sistla, S. A., J. C. Moore, R. T. Simpson, L. Gough, G. R. Shaver, and J. P. Schimel. 2013. Long-term warming restructures Arctic tundra without changing net soil carbon storage. *Nature* 497:615–618.
- Soares, M., and J. Rousk. 2019. Microbial growth and carbon use efficiency in soil: links to fungal–bacterial dominance, SOC-quality and stoichiometry. *Soil Biology and Biochemistry* 131:195–205.
- Sokol, N. W., and M. A. Bradford. 2019. Microbial formation of stable soil carbon is more efficient from belowground than aboveground input. *Nature Geoscience* 12:46–53.
- Van Der Heijden, M. G., R. D. Bardgett, and N. M. Van Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11:296–310.
- Van Gestel, N., et al. 2018. Predicting soil carbon loss with warming. *Nature* 554:E4–E5.
- Weedon, J. T., G. A. Kowalchuk, R. Aerts, J. van Hal, R. van Logtestijn, N. Taş, W. F. M. Röling, and P. M. van Bodegom. 2012. Summer warming accelerates sub-arctic peatland nitrogen cycling without changing enzyme pools or microbial community structure. *Global Change Biology* 18:138–150.
- Weintraub, M. N., and J. P. Schimel. 2003. Interactions between carbon and nitrogen mineralization and soil organic matter chemistry in arctic tundra soils. *Ecosystems* 6:129–143.
- Wild, B., S. Alaei, P. Bengtson, S. Bodé, P. Boeckx, J. Schneckler, W. Mayerhofer, and T. Rütting. 2017. Short-term carbon input increases microbial nitrogen demand, but not microbial nitrogen mining, in a set of boreal forest soils. *Biogeochemistry* 136:261–278.
- Wild, B., et al. 2014. Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil. *Soil Biology and Biochemistry* 75:143–151.
- Wild, B., et al. 2015. Microbial nitrogen dynamics in organic and mineral soil horizons along a latitudinal transect in western Siberia. *Global Biogeochemical Cycles* 29:567–582.
- Wild, B., J. Li, J. Pihlblad, P. Bengtson, and T. Rütting. 2019. Decoupling of priming and microbial N mining during a short-term soil incubation. *Soil Biology and Biochemistry* 129:71–79.
- Wilkinson, A., P. W. Hill, J. F. Farrar, D. L. Jones, and R. D. Bardgett. 2014. Rapid microbial uptake and mineralization of amino acids and peptides along a grassland productivity gradient. *Soil Biology and Biochemistry* 72:75–83.
- Williams, M. A., D. D. Myrold, and P. J. Bottomley. 2006. Carbon flow from ¹³C-labeled straw and root residues into the phospholipid fatty acids of a soil microbial community under field conditions. *Soil Biology and Biochemistry* 38:759–768.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.3094/supinfo>