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Microbial carbon use and associated changes in microbial community structure in high-Arctic tundra soils under elevated temperature

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ABSTRACT

In the high-Arctic, increased temperature results in permafrost thawing and increased primary production. This fresh plant-derived material is predicted to prime microbial consortia for degradation of the organic matter stored in tundra soils. However, the effects of warming and plant input on the microbial community structure is hardly known. We assessed the use of glycine, a readily available C and N source, and cellulose, a long C-biopolymer, by prokaryotic and fungal communities using DNA-SIP in tundra soils incubated at 8 °C or 16 °C. Glycine addition contributed mainly to instantaneous microbial carbon use and priming of soil organic matter decomposition, particularly under elevated temperature. By contrast, cellulose was linked to the dominant and active microbial communities, with potential carbon stabilization in soils. Our findings stress the importance of the type of plant-derived material in relation to microbial metabolism in high-Arctic soils and their consequences for the carbon cycle in response to global warming.

1. Introduction

Microbial mediated mineralization of soil organic matter (SOM) stored in Arctic soils is expected to be one of the most important feedback effects on the global climate system in response to altered precipitation regimes and increased temperature (Schuur et al., 2015). This is because elevated temperature accelerates microbial processes and increases the availability of easily decomposable organic matter (Chapin III et al., 1995; Donhauser et al., 2020), resulting in higher heterotrophic respiration and C release from soils (Dorrepaal et al., 2009; Lulakova et al., 2019; Schädel et al., 2016). Elevated temperature, however, leads to increased primary production (Bintanja and Andry, 2017; Elmendorf et al., 2012; Hartley et al., 2012; Lynch et al., 2018; Sistla et al., 2013), but how both climate change and increased plant biomass will influence the carbon stock in tundra soils is poorly known.

Recent studies revealed that an increase in plant-derived material may have a potential positive priming effect (i.e. acceleration of C mineralization following a substrate addition), whereby the excess of C and N from fresh plant biomass (via litter and root exudates) boosts microbial activities (Adamczyk et al., 2020, 2021) and the breakdown of organic matter stored in tundra soils (Wild et al., 2016). Additional C may provide prokaryotes and fungi with energy that facilitates the decomposition of SOM (Blagodatskaya and Kuzyakov, 2008; Fontaine et al., 2007), while additional N may fuel the synthesis of extracellular enzymes breaking down polymeric compounds of SOM (Sinsabaugh et al., 2014). However, C and N sources from plant-derived material might alternatively reduce the microbial decomposition of native and relatively recalcitrant SOM. This, termed “negative priming” or “entombing effect”, involves a decrease in the microbial dependence on more complex substrates of the native SOM (Kuzyakov et al., 2000) or the synthesis of compounds stabilizing the soil by the formation of mineral-organic aggregates (Liang et al., 2017). It follows that enhanced plant-derived input to Arctic tundra soils might either increase loss of native soil C, or alternatively promote the formation of newly stabilized soil C (Street et al., 2020). This implies that the response of vegetation to warming and its feedback effects on soil microbial communities is a critical, yet poorly understood, regulator of global C cycling (Blok et al., 2018; Wild et al., 2016).

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Moreover, the response of soils to warming is also largely governed by the soil hydrologic regime (Christiansen et al., 2017; Schädel et al., 2016), with higher rates of C mineralization effects in drier soils (aerobic decomposition) than in moist soils (anaerobic decomposition). Alternating surface hydrology due to glacier thawing, permafrost thawing and regional changes in precipitation in the Arctic has expanded the portion of the tundra that is a fragmented landscape of drier and wetter soil ecosystems with varying vegetation and C stocks in soils (Jorgenson et al., 2013; Kern et al., 2019; Schädel et al., 2016). Following a typical Arctic vegetation toposequence (Kern et al., 2019; Walker et al., 2011), soil moisture varies from upslope zones (drier) to downslope zones (wetter). This variation in the Arctic microtopography is expected to add complexity for predicting the responses of soils to climate change.

Carbohydrates, carboxylic acids, peptides and amino acids are common components of the organic carbon pool found in Arctic tundra soils. Polymers such as cellulose, hemicellulose and pectin are the major structural components of plant litter input in soils (Foster et al., 2016; Pushkareva et al., 2020; Tveit et al., 2013). Cellulose is a structural C pool derived from the most abundant plant component in high-latitude soils (Ivanova et al., 2016; Segura et al., 2017; Tveit et al., 2013). Glycine, a common component of plant root exudate, is one of the most abundant amino acids available in tundra soils (Ravn et al., 2017; Weintraub and Schimel, 2005) and microorganisms as well as most plants can easily metabolize glycine-derived C and N (Adamczyk et al., 2021; Andresen et al, 2009, 2014; Sorensen et al., 2008). Decomposition of plant-derived organic matter in the Arctic tundra requires a cooperation of functional microbial groups (Rime et al., 2016; Tao et al., 2020; Tveit et al., 2015). However, we do not have a complete picture of which specific microorganisms carry out critical C transformations in high-Arctic tundra soils under elevated temperature and varying soil moisture, and which groups drive the priming effect.

Our aim was to assess the main microbial utilizers of glycine and cellulose in both upslope and downslope high-Arctic tundra soils under current mean and elevated summer soil temperatures. We hypothesized that (1) readily available substrates, such as glycine, cause a more important priming effect on C utilization than structural plant components such as cellulose, leading to (2) a stronger impact on the microbial community structure, promoting fast growing taxa with putative copiotrophic lifestyle. We also expect that (3) environmental factors such as temperature and soil types with different moisture levels influence the microbial community structure and its response to C substrate utilization.

2. Materials and methods

2.1. Site description and soil collection

The soils used for the incubation experiment were collected in summer 2017 at Knudsenheia (N78°56.544 E11°49.055), situated in the vicinity of the research settlement of Ny-Ålesund (Svalbard). Surface soils (0–10 cm depth) were collected from three upslope and three downslope plots along a natural topographic gradient (Kern et al., 2019; Pushkareva et al., 2020). The upslope plots (N 78°56′22.1'', E 11° 48′28.1'', elevation 36.2 m ASL) were situated on a small hilltop or ridge. Downslope plots (N 78°56′30.7, E 11° 49′41.0, elevation 26.8 m ASL) were situated close to a pond. Soil samples were collected from three replicated plots of 1 m^2 (at least 10 m away from each other). At each plot, a composite sample was collected and pooled together. The soil samples were kept at 0.5 °C in air-tight bags in order to minimize microbial activities and changes within the microbial communities until being used for the experiment (4 months after collection). Soil physico-chemical parameters (Table 1) were measured as described in Kern et al. (2019). Soil organic C and N were quantified after HCl-fumigation (Walthert et al., 2010).

Upslope soils were drier (15.4 ± 0.7% of soil moisture for 0–10 cm depth, mean ± SD) than in the downslope ones (21.3 ± 2.8%; Table 1).

Table 1
Physico-chemical characteristics of the upslope and downslope tundra soils (topsoil 0–10 cm) at the Knudsenheia site near Ny Ålesund. Differences between the soil types were tested using one-way ANOVA. SOM = soil organic matter, C = carbon, N = nitrogen, BSCs = biological soil crusts. Parameters with a "*" are data reported from Kern et al. (2019), where upslope soil correspond to "dry" and downslope soils to "wet" in Kern et al. (2019). N = 3.

Parameters	Upslope		Downslope		Upslope vs. Downslope	
	mean	± std dev.	mean	± std dev.	F	P
Elevation (m ASL)	36.2		26.8			
Soil moisture 0–10 cm depth (%)	15.4	±0.7	21.3	±2.8	14.2	**0.020**
pH	6.3	±0.9	7.2	±0.3	41.3	**0.003**
SOM (%)	16.9	±14.5	20.7	±20.9	1.8	0.253
Total C (%)	5.0	±1.1	8.0	±8.6	0.0	0.937
Organic C (%)	4.9	±1.0	7.7	±7.6	0.4	0.846
Total N (%)	0.3	±0.1	0.5	±0.6	0.3	0.618
Organic N	0.3	±0.1	0.5	±0.5	0.4	0.575
Sand (%)	71.1	±9.1	84.7	±5.4	7.8	**0.049**
Silt (%)	20.6	±8.0	7.3	±2.6	17.4	**0.014**
Clay (%)	8.3	±1.6	8.0	±5.1	0.07	0.804
*Total vegetation cover (%)	86.7	±2.9	87.0	±8.2	0.0	0.982
*Coverage higher plants (%)	21.7	±5.8	5.0	±1.7	34.9	**0.004**
*Coverage mosses (%)	0.7	±0.6	6.0	±3.5	10.0	**0.034**
*Coverage lichens (%)	59.3	±8.3	8.7	±6.4	27.5	**0.006**
*Coverage BSCs (%)	5.0	±4.4	67.3	±2.5	43.3	**0.003**

Upslope soils had a significantly lower pH and sand content but contained more silt than the downslope soils. Soil organic matter and organic C content in both upslope and downslope soils were in similar range as other high-Arctic soils (Kern et al., 2019). Moreover, the low nitrogen content in both soils was comparable with high-Arctic tundra soils from other regions (Kern et al., 2019). The total vegetation cover did not differ between upslope and downslope plots, but the type of vegetation varied (Table 1, data from Kern et al., 2019). Upslope zone was dominated by lichens, corresponding to the snow bed zone along a typical Arctic landscape toposequence (Kern et al., 2019). In contrast, biological soil crusts prevailed in the downslope zone which reflected the topographic entity of an Arctic wetland (Kern et al., 2019).

2.2. Experiment set-up

Stable isotope probing (SIP) incubation experiments were carried out in microcosms prepared with 15 g (dry mass) of either upslope or downslope Arctic tundra soil. The soils were previously sieved at 2 mm and homogenized before being transferred into 100 ml polypropylene containers (Sarstedt AG, Sevelen, Switzerland) which served as microcosms. The soils were primed with 30 mg of either glycine or cellulose substrate or without substrate (control). Glycine represented a readily available substrate serving as a source of both C and N. Glycine substrates contributed up to 0.8% of the total C content in the microcosms which is about 10% of the total soil C content and in the range of plant input in Arctic soils (Sorensen et al., 2008). Cellulose represented a C source requiring cellulase enzymes prior to microbial C assimilation from that substrate. Cellulose substrates contributed up to 1% of the total C content in the microcosms. For both glycine and cellulose, microcosms contained either ^{12}C substrate or ^{13}C-labelled substrate in order to control GC-content in microbial cell during the SIP procedure (see "*Data Analyses*"). The ^{12}C- and ^{13}C- (>99% of ^{13}C atoms) glycine molecules used in the incubation were artificially synthesized (Sigma-Aldrich, Buchs, Switzerland). The ^{12}C (<1.2% of ^{13}C atoms) and the ^{13}C-enriched (>97% of 13C atoms) cellulose substrate were extracted from maize leaves (Isolife, Wageningen, The Netherlands).

Soils were gently air dried (at 25 °C) for an optimized mix with the

substrates, accomplished with an end-over-end shaker mixing the soils and the substrates for 1 h. Subsequently, the soil moisture in the microcosms was adjusted to their original soil moisture levels (15% for upslope and 21% for downslope) with sterile milliQ water. Microcosms were then incubated in climate chambers either at 8 °C or at or 16 °C corresponding to soil mid-summer (July) mean temperature and elevated temperature, respectively. The microcosms were incubated for 21 days with continuous monitoring and adjustment of water content. In total 120 microcosms were prepared accounting for: 2 soil types (upslope and downslope) × 5 labelled/non-labelled substrates (^{12}C-glycine, ^{13}C-glycine, ^{12}C-cellulose, ^{13}C-cellulose and control without substrate) × 2 incubation temperatures (8 and 16 °C) × 2 sampling times (7 days and 21 days) × 3 replicates.

2.3. CO_2 and $\delta^{13}CO_2$ fluxes

To measure CO_2 and $\delta^{13}CO_2$ gas fluxes from soils, the microcosms were placed in air-tight glass jars (volume = 1L) with a lid equipped with a septum, enabling gas sampling with a syringe. CO_2 production was measured over a 24 h period at days 1, 3, 5, 7, 10, 14 and 20 of incubation. On these days, jars were hermetically closed for 24h and 1 ml of accumulated gas was collected and released into an exetainer tube (Labco limited, Lampeter, United Kingdom). The CO_2 and $^{\delta 13}CO_2$ were measured with a gas chromatograph (Trace GC Ultra, Thermo Fisher Scientific, Waltham, MA, USA) coupled with an isotope-ratio mass spectrometer (IRMS; Delta V Advantage, Thermo Fisher Scientific). Because the IRMS used to detect the $\delta^{13}C$–CO_2 signature was not equipped for measuring a high percentage of ^{13}C atoms, gas sampled from glycine-amended soils was diluted 100 times with atmospheric gas. The $\delta^{13}C$–CO_2 values were expressed per mill (‰) in relation to the Vienna-Pee Dee Belemnite gauged reference material. The percentage of the derived C from the substrate (*%Csubstrate*) was derived from $\delta^{13}C$ values of CO_2 fluxes from amended and non-amended soils (Tao et al., 2020):

$$\%Csubstrate = \frac{\delta control - \delta resp}{\delta control - \delta substrate} \times 100$$

where *δcontrol* is the $\delta^{13}C$–CO_2 of the control non-amended soils, *δtreatment* is the $\delta^{13}C$–CO_2 of the treated soil with glycine or cellulose amendment and *δsubstrate* is the $\delta^{13}C$ value of ^{13}C-glycine or ^{13}C-cellulose. From there, the percentage of CO_2 derived for the original soil organic carbon was calculated as 100 - %Csubstrate. CO_2 emission rates from the soil organics carbon were integrated over the whole incubation period and priming was calculated as the difference in respired soil organic carbon between amended samples and controls, as described in Mau et al. (2015).

2.4. DNA extraction, DNA fractionation and amplicon sequencing

Total DNA was extracted from 0.6 g of soil from each microcosm after 7 and 21 days of incubation with the DNeasy Powersoil Kit (Qiagen, Hilden, Germany). DNA concentration was quantified with the Picogreen dsDNA quantification Kit (Thermo Fisher). DNA from ^{13}C-enriched samples (glycine or cellulose ^{13}C-enriched substrate) and unlabelled samples (^{12}C-enriched substrate and control) were retrieved by ultracentrifugation (Neufeld et al., 2007; Rime et al., 2016; Zumsteg et al., 2013b). Five μg DNA was suspended in 5 ml of CSCl buffer and adjusted to an optical density of 1.4029 ± 0.0002 with a Refracto 30PX (Mettler-Toledo, Greifensee, Switzerland), corresponding to a volumetric density of 1.720 g ml^{-1}. The samples were subsequently sealed in polyallomer tubes (Beckman Coulter, Indianapolis, CA, USA) and ultracentrifuged at 177,000×*g* for 40 h at 20 °C (Vti-65.1 vertical rotor and optimaTM L-80 XP ultracentrifuge, Beckman Coulter). The samples were then separated into 21 fractions of 250 μl each, collected drop-wise from the bottom of the tubes. The optical density of each fraction was measured (Refracto 30XP). Each fraction was then precipitated with 0.7 ml PolyEthylGlycerol buffer (30% PEG and 1.5 M NaCl; incubation at 37 °C for 1h, centrifugation at 4 °C for 30 min), washed with 150 μl of 70% ethanol and eluted in 30 μl of 10 mM Tris buffer. The DNA content of each fraction was quantified with Nanodrop (Thermo Fisher Scientific) and with gel visualization subsequent to amplification of the 16S rRNA gene. The light fraction was identified as the three fractions directly below a density of 1.720 g ml^{-1} and the heavy as the three fractions directly above a density of 1.730 g ml^{-1}. At least four fractions had a density falling between the light and heavy fractions. The three light and three heavy fractions were each pooled to form the light (^{12}C) and heavy (^{13}C) fractions for each sample.

PCR amplification of the 16S rRNA gene (region V3–V4, 341F and 806R primers, Frey et al., 2016) and fungal ribosomal internal transcribed spacers (region ITS2, Its and ITS4 primers, Tedersoo et al., 2014) was performed with both light and heavy fractions, as described previously (Frey et al., 2016; Frossard et al., 2018). The light and heavy samples were amplified in triplicate and pooled before purification with Agencourt AMPure XP beads (Beckman Coulter). Amplicons were sent to the Génome Québec Innovation Centre (Montreal, Canada) for barcoding using the Fluidigm Access Array technology and paired-end sequencing on the Illumina MiSeq v3 platform (Illumina Inc., San Diego, CA, USA).

Prokaryotic and fungal raw sequences were quality filtered, chimeras and singletons were removed, and high-quality reads were clustered into operational taxonomic units (OTUs) based on 97% identity as described previously (Frossard et al., 2018), using a customized pipeline based on UPARSE (Edgar, 2013; Edgar and Flyvbjerg, 2015) and implemented in USEARCH v.9.2 (Edgar et al., 2011). Taxonomic assignment of the OTUs was achieved by querying centroid sequences against the SILVA v.138 (for 16S; Quast et al., 2013) and UNITE v.8.0 (for ITS; Abarenkov et al., 2010) reference databases using the Bayesian classifier (Wang et al., 2007) with a minimum bootstrap support of 60% implemented in MOTHUR (Schloss et al., 2009). Rarefication curves reached saturation, indicating that sequencing effort was sufficient. Raw sequences were deposited in the NCBI database under the accession number PRJNA640992.

2.5. Data analyses

Statistical analyses were performed in the R software v 3.6.2 (R Development Core Team, 2020). Analyses of variances (ANOVAs) were conducted on the repeated CO_2 fluxes measurements with a linear mixed effects model ("lme" function in *lme4* package) to test for significance between substrates (cellulose, glycine and control), soils (upslope and downslope) and temperatures (8 and 16 °C), with times (1, 3, 5, 7, 10, 14 and 21 days of incubation) considered as a random factor (repeated measures design). Tukey's post-hoc tests were used to determine significances among Glycine, cellulose and control treatments. Variables were log-transformed to achieve normality of the residual distribution and variance homoscedasticity to ensure validity of the test.

OTUs responding to substrates (i.e. responsive community) were defined as OTUs whose relative abundance increased by at least a $\log_2$-fold change ratio between the heavy and light fractions of labelled samples and between amended and control samples. To correct for DNA GC-content which can impact on the density gradient (i.e. light and heavy fractions), we selected OTUs which were increased by a $\log_2$-fold change factor between the amended (cellulose or glycine) ^{13}C-labelled and the amended non-labelled (^{12}C only). Richness, Shannon diversity and Pielou's evenness indices were based on randomly rarefied OTU abundance matrices. A linear model (lm function) was used to test significance between substrates, soils, temperature and incubation time (7 or 21 days) for the relative abundance of the responsive OTUs and alpha-diversity metrics.

Bray-Curtis dissimilarity matrices were generated based on the relative abundance of the total and responsive prokaryotic and fungal

communities, and the overall variability was examined by non-metric multidimensional scaling (NMDS) ordination plots. Permutational multivariate analysis of variance (PERMANOVA) was performed to assess significant between substrates, soil types, temperatures and times with the function 'Adonis' in the *vegan* package v2.5-6 (Oksanen et al., 2011). Calculations were based on Bray-Curtis dissimilarities and 10, 000 permutations. Taxonomic networks of the abundant (≥10 reads) prokaryotic and fungal responsive OTUs were generated with Cytoscape (Cytoscape.org, version 3.7.2) with an edge-weighted algorithm.

3. Results

3.1. CO_2 and $\delta^{13}CO_2$ fluxes

Cumulative CO_2 fluxes over the 21 days of incubation from the soils amended with glycine reached 37.5 ± 10.2 and 51.5 ± 9.8 mg C–CO_2 gDM^{-1} (g Dry Mass, mean ± standard eror) for soils incubated at 8 and 16 °C, respectively (Fig. 1A). These values were about 10 times higher than those for soils amended with cellulose, which reached 4.7 ± 0.9 and 5.4 ± 1.1 mg C–CO_2 gDM^{-1} at 8 and 16 °C, respectively (Fig. 1B, Table 2). Cumulative CO_2 fluxes in the control soils were minimal, reaching 1.8 ± 0.8 and 2.1 ± 0.8 mg C–CO_2 gDM^{-1} for soils incubated at 8 and 16 °C, respectively. While the higher incubation temperature led to significantly higher cumulative CO_2 fluxes, soil type (i.e. upslope or downslope) had no effect on these fluxes (Table 2).

Likewise, both amended substrate type and temperature had

Table 2
Statistical differences (ANOVA) in cumulative CO_2 fluxes over the 21 days of incubation and in $\delta^{13}C$–CO_2 values between substrate (glycine, cellulose and control), soil type (upslope and downslope) and incubation temperatures (8 and 16 °C), as well as all possible interactions among factors. Cumulative CO_2 fluxes were extrapolated for the length of the incubation experiment by adjusting the rates at each new measurement. Significant difference at $P < 0.05$ are shown in bold. N = 21.

Factors *Pairwise comparisons*		Cumulative CO_2		$\delta^{13}C$–CO_2	
	DF	F	P	F	P
Substrate	2, 234	686.4	**<0.001**	1454.9	**<0.001**
Control vs. cellulose			***<0.001***		***<0.001***
Control vs. glycine			***<0.001***		***<0.001***
Cellulose vs. glycine			***<0.001***		***0.001***
Soil type	1, 234	1.8	0.176	0.1	0.718
Temperature	1, 234	14.6	**<0.001**	9.8	**0.002**
Substrate × Soil type	2, 234	0.8	0.458	0.3	0.072
Substrate × Temperature	2, 234	9.5	**<0.001**	2.5	0.087
Soil type × Temperature	1, 234	0.7	0.408	0.6	0.432
Substrate × Soil type × Temperature	2, 234	2.9	0.057	1.9	1.145

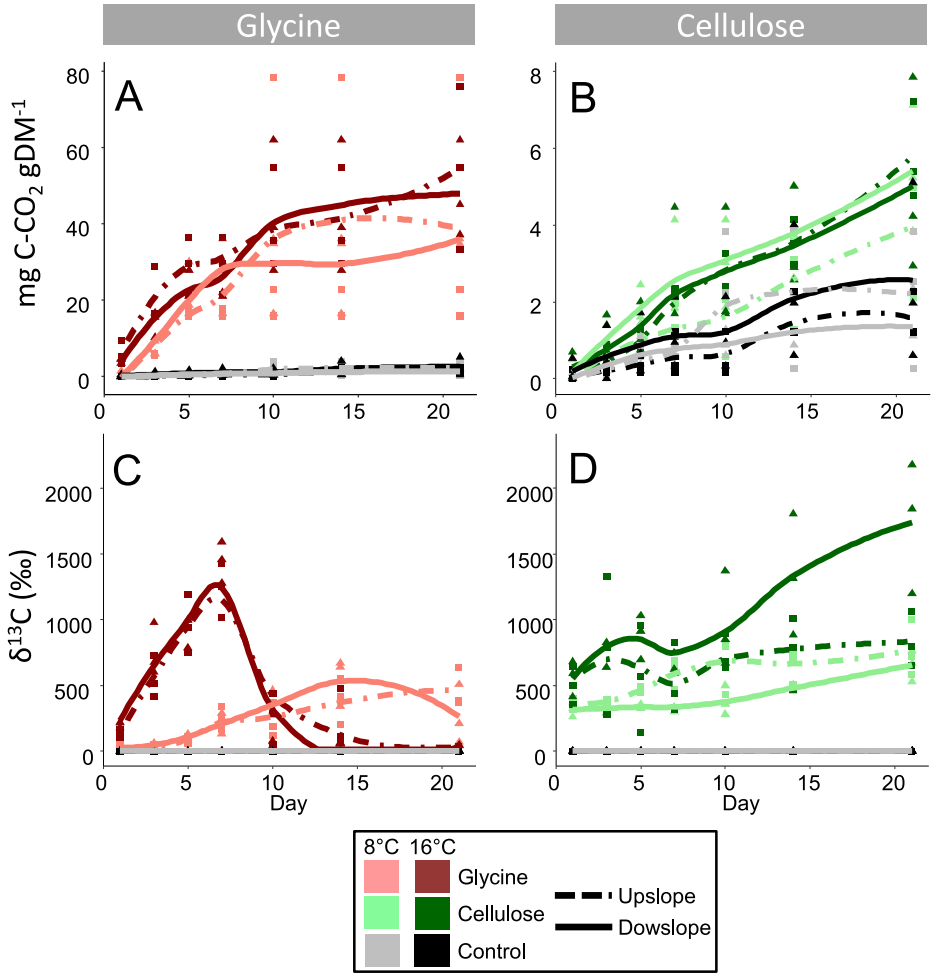

Fig. 1. Cumulative fluxes of C–CO_2 (A and B) and $\delta^{13}C$–CO_2 signatures (C and D) from the upslope and downslope soils amended with either glycine, cellulose or no substrate (control) during the 21-day incubation experiment. Cumulative CO_2 fluxes were extrapolated for the length of the incubation experiment by adjusting the rates at each new measurement. Lines represent mean rates and individual measurements are shown as data points (N = 3).

significant effects on $\delta^{13}C–CO_2$ values emitted from the soils (Table 2). The $\delta^{13}C–CO_2$ values from soil amended with glycine incubated at 16 °C rapidly increased and peaked on day 7, while the values of the glycine-amended soil incubated at 8 °C peaked only on day 15 (Fig. 1C). The $\delta^{13}C–CO_2$ values of the soil amended with cellulose exhibited a slower increase and was highest at the end of the 21-day incubation period (Fig. 1D). At the end of the experiment, most of the C-glycine was respired in the soils incubated at 16 °C (65.4 ± 16.3% for upslope soils and 95.6 ± 25.5% for downslope soils), while only a small percentage was respired at 8 °C (14.0 ± 12.9% for upslope and 20.7 ± 2.2% for downslope soils). Similarly, a small percentage of the C-cellulose was respired at 8 °C (18.0 ± 8.1% for upslope and 29.5 ± 11.1% for downslope), while the percentage was higher at 16 °C (30.1 ± 11.7% for upslope and 68.4 ± 46.9% for downslope).

Priming was positive for both substrates but was highly enhanced in the soils amended with glycine, with rates 14 times higher than in cellulose-amended soils (Fig. 2, Table 3). While priming in glycine-amended soils was up to 97.3 ± 35.8 and 134.1 ± 26.7 mgC-CO_2 gDM^{-1} for soil incubated at 8 and 16 °C, respectively, it only reached 7.7 ± 2.2 and 8.7 ± 2.6 mgC-CO_2 gDM^{-1} soil for 8 and 16 °C, respectively, in soils amended with cellulose.

3.2. Prokaryotic and fungal alpha-diversity

Subsequent to quality filtering, a total of 5,903,964 prokaryotic and 3,509,049 fungal sequences mapped into 12,053 prokaryotic and 1992 fungal OTUs were recovered from the samples. The ratio of responsive to total prokaryotic taxa, defined as the percentage of prokaryotic OTUs responding significantly to substrate addition, was higher in soils amended with cellulose (average 34.1 ± 4.1%) than in those soil amended with glycine (average 14.5 ± 3.6%; Fig. 3, Supp. Table S1). However, the percentage of reads of responsive prokaryotic OTUs was higher in glycine-amended soils (averaging 86.4 ± 10.0%) than in cellulose-amended soils (averaging 55.9 ± 3.8%; Supp. Table S1). Thus,

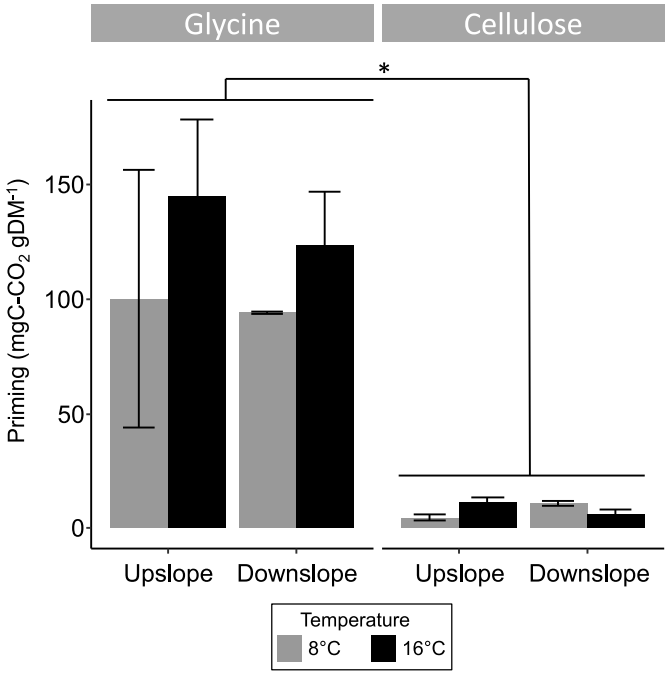

Fig. 2. Cumulative priming rates at the end of the experiment (after 21 days of incubation) for glycine and cellulose amended to upslope and downslope tundra soils, calculated as the difference in respired soil organic matter between amended samples and controls. Bars represent mean values and error bars represent standard errors (N = 3). Significance between substrates is shown with a *.

Table 3
Statistical differences (ANOVA) for cumulative priming (mgC-CO_2 gDM^{-1}) at the end of the experiment (after 21 days) between amended substrate (glycine and cellulose), soil type (upslope and downslope) and incubation temperature (8 and 16 °C), as well as all possible interactions among factors. Significant difference at $P < 0.05$ are shown in bold. N = 3.

Factors		Cumulative Priming	
	DF	F	P
Substrate	1, 16	38.46	**<0.001**
Soil	1, 16	0.03	0.725
Temperature	1, 16	0.40	0.196
Substrate × Soil type	1, 16	0.00	0.938
Substrate × Temperature	1, 16	0.16	0.416
Soil type × Temperature	1, 16	1.18	**0.035**
Substrate × Soil type × Temperature	1, 16	0.39	0.203

the prokaryotic community that assimilated glycine only comprised a small number of abundant taxa. The ratio of prokaryotic OTUs responsive to cellulose or glycine did not vary between soil types, temperatures, or incubation times. Prokaryotic richness, Shannon diversity and evenness indices of the total and responsive communities were lower in the glycine-amended soils than in the cellulose ones (Supp. Fig. S1, Supp. Table S2).

The percentage of fungal OTUs responding to the substrates, although small, was higher in glycine-amended (average 20.3 ± 4.1%) than in cellulose-amended soils (average 10.4 ± 4.2%; Fig. 3, Supp. Table S1). Moreover, the ratio of the abundance of reads of responsive to total fungal OTUs was very low for both cellulose (average 9.2 ± 7.4%) and glycine (average 10.7 ± 5.2%) substrates. In contrast, the Shannon diversity of the total fungal community was lower in glycine-amended soils than in cellulose-amended ones (Supp. Fig. S1). This difference was smaller than for the fungal responsive community and was not significant (Supp. Table S3). In general, alpha-diversity did not differ between soil types, incubation temperatures and incubation times for the prokaryotic or fungal community.

3.3. Prokaryotic and fungal beta-diversity

The structure of the responsive prokaryotic community differed significantly from that of the total prokaryotic community ($F_{1,143}$ = 26.3, $P < 0.001$; Fig. 4). However, the structure of both responsive and total prokaryotic communities shifted in response to substrate, soil type, incubation temperature and incubation time (Table 4). In the total prokaryotic community, glycine-amended samples differed more from the control than the cellulose-amended samples. Similar to prokaryotes, the structure of the responsive fungal community was significantly different from that of the total fungal community ($F_{1,143}$ = 10.4, $P <$ 0.001). The structure of both responsive and total fungal communities varied with substrate, soil type and time but not with temperature (Fig. 4, Supp. Figs. S2–S5, Table 4). In the total fungal community, the structure of the glycine-amended samples differed significantly from the control, whereas the cellulose-amended samples exhibited a community structure similar to the control samples.

3.4. Prokaryotic and fungal responsive taxa

The majority of responsive prokaryotic taxa utilized cellulose as a carbon source, whereas only a minority used glycine or both substrates (Fig. 5, Supp. Table S4). Prokaryotic taxa that assimilated cellulose predominantly belonged to the classes Planctomycetaia (10.0% for upslope soils and 10.7% for downslope soils; phylum Planctomycetes), Thermoleophilia (6.4% for upslope and 5.0% for downslope; Actinobacteria), Alphaproteobacteria (6.0% for upslope and 6.5% for downslope), candidate class C6-1 (4.8% for upslope and 4.3% for downslope; Acidobacteria), Betaproteobacteria (4.5% for upslope and 4.6% for downslope) and Deltaproteobacteria (4.2% for upslope and 4.6% for

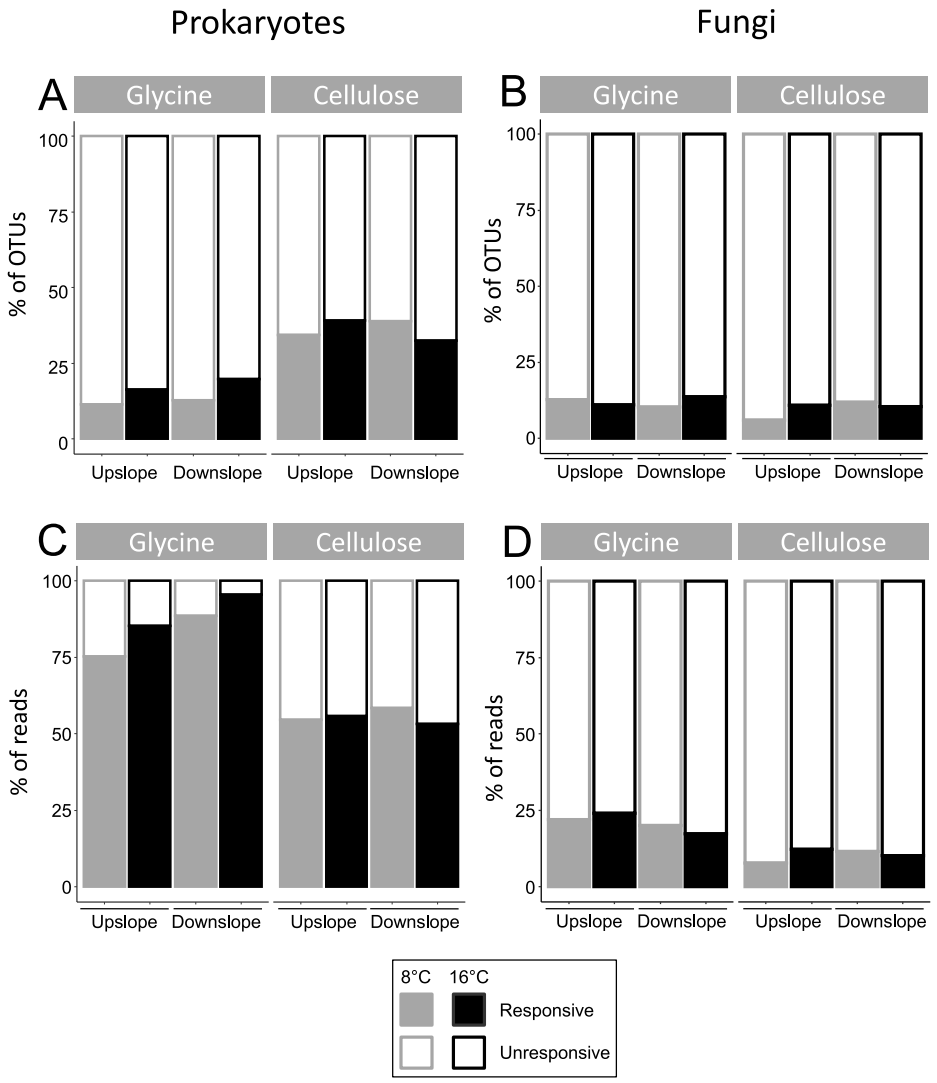

Fig. 3. Number of total and responsive prokaryotic and fungal OTUs (A and B) and read abundances (C and D) for the two substrates (glycine and cellulose), two soil types (upslope and downslope) and two incubation temperature (8 and 16 °C).

downslope). Prokaryotic taxa incorporating ^{13}C-glycine were almost exclusively OTUs from the Actinobacteria class (Actinobacteria), and accounted for 19.6% and 18.1% of the responsive prokaryotic reads in the upslope and downslope, respectively. Most of the prokaryotic responsive taxa were able to use both or one of the substrates at 8 °C, whereas a lower percentage of taxa assimilated the substrates at 16 °C (Table S4).

The most abundant responsive prokaryotic taxa were *Pseudomonas migulae* (Proteobacteria), using both substrates, and *Pseudarthrobacter* sp. (Actinobacteria), utilizing glycine (Fig. 5, Supp. Table S5). Other abundant responsive taxa included *Cellvibrio gandavensis* (Proteobacteria), *Pelomonas* sp. (Proteobacteria), *Luteobacter* sp. (Verrucomicrobia) and Gemmatimonadaceae (Gemmatimonadetes), all utilizing cellulose.

The responsive fungal taxa mainly used only glycine or only cellulose (Fig. 5, Supp. Table S4). A minority of taxa were able to utilize both substrates. Fungal taxa assimilating glycine belonged to the classes Eurotiomycetes (13.5% and 21.2% of fungal responsive taxa for upslope and downslope soils, respectively; Ascomycota) and Leotiomycetes (16.2% and 24.2%; Ascomycota).

The most abundant responsive fungal taxon, *Pseudogymnoascus* sp. (Leotiomycetes, Ascomycota) used cellulose as a C source (Fig. 5, Supp. Table S6). Within the Leotiomycetes order, *Tetracladium* sp. was able to assimilate glycine, whereas *Cistella albidolutea* responded to cellulose. Moreover, two abundant OTUs, both identified as *Trichocladium opacum* (Sordariales, Ascomycetes) assimilated cellulose. Similar to prokaryotes, most of the fungal responsive taxa were able to use the substrates at 8 °C and to a lesser extent at 16 °C (Table S4).

4. Discussion

The considerably higher respiration and greater positive priming in soils amended with glycine compared with those amended with cellulose (Figs. 1 and 2) suggests a rapid metabolization of readily available C and N, potentially enhancing microbial metabolism (Blagodatskaya and Kuzyakov, 2008), which confirms our first hypothesis. Although comparisons among studies are difficult due to differing experimental conditions, the positive priming effects observed in our study are in line with reports of priming effects from various soil types across five continents (Perveen et al., 2019). Despite being an order of magnitude smaller than in soils amended with glycine, positive priming in our high-Arctic tundra soils amended with cellulose was higher than that measured in grassland, cropland, forest and Savannah soils (Hopkins et al., 2014; Perveen et al., 2019). Moreover, cumulative CO_2 emissions from glycine-amended soils were about ten-fold higher than from soils amended with cellulose, which were in the range of respirations rates reported for Siberian Arctic soils amended with cellulose (Wild et al.,

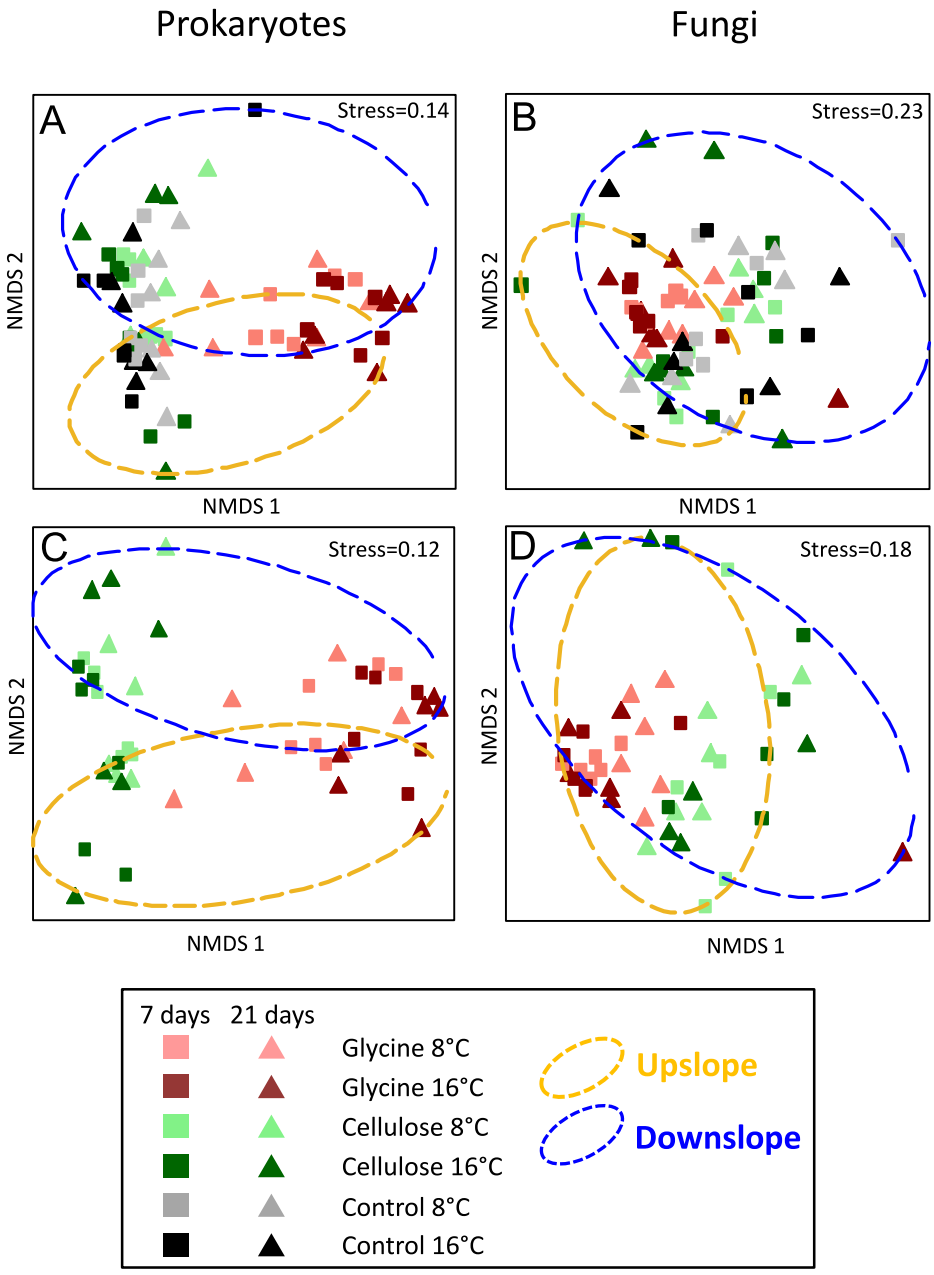

Fig. 4. Non-metric multidimensional scaling (NMDS) of prokaryotic and fungal total (A and B) and responsive (C and D) communities. Samples from upslope (orange) and downslope (blue) tundra soils are defined by dashed ellipses (95% confidence interval).

2016).

The glycine content in proteins can be up to 60% (Andreesen, 1994), and microorganisms as well as plants have been observed to take up glycine directly (Andresen et al, 2009, 2014; Sorensen et al., 2008). Moreover, glycine dehydrogenase genes have been found frequently in metagenomes and metatranscriptomes of microbial communities from soils close to our Knudsenheia sampling site (Tveit et al., 2015). Furthermore, the availability of glycine as an N source might be of special importance in these high-Arctic soils in which N has been observed to be a limiting factor (Kern et al., 2019) and long-term warming has had no detectable effect on the soil N pool despite an increase in plant biomass (Sistla et al., 2013). As shown in several studies on priming effects, the amount of primed C often depends on N availability (Blagodatskaya and Kuzyakov, 2008). Specifically, the addition of N is known to stimulate the activity of cellulases (Carreiro et al., 2000). Our findings indicate a fast microbial assimilation of a readily available substrate as an N and C source, resulting in a positive priming of the SOM by boosting the synthesis of extracellular enzymes.

The relatively low CO_2 emission from soils amended with cellulose (Fig. 1) can be explained by the specific enzymes being required to acquire C molecules from the cellulose biopolymer. Nonetheless, the majority of responsive prokaryotic OTUs incorporated ^{13}C from cellulose into their DNA, suggesting that cellulose amendment resulted in more microbial growth and production than with the glycine as substrate. It follows that the type of substrate might affect the stabilization of C in soil, the readily available C and N source (glycine) contributing mainly to instantaneous microbial metabolism (and ultimately release of CO_2 into the atmosphere) and the long biopolymer C source (cellulose) serving essentially for microbial growth. Furthermore, the type of substrate can control the balance between positive priming and an entombing effect (negative priming), via the build-up of microbial biomass and necromass, further regulating C stabilization in the soil.

Table 4
Effects of substrate (cellulose, glycine and control), soil type (upslope and downslope), incubation temperature (8 and 16 °C) and incubation time (7 and 21 days) on the beta-diversity of the responsive (i.e. taxa enriched in ^{13}C from amended labelled substrate) and the total bacterial and fungal communities, as assessed by PERMANOVA.

			Responsive		Total	
	Factors *Pairwise comparisons*	DF	F	P	F	P
Prokaryotes	Substrate	2, 32/48[a]	36.6	**<0.001**	14.6	**<0.001**
	Cellulose vs. control			NA		***0.003***
	Glycine vs. control			NA		***0.003***
	Cellulose vs. glycine			***<0.001***		***0.003***
	Soil type	1, 32/48[a]	9.4	**<0.001**	11.0	**<0.001**
	Temperature	1, 32/48[a]	3.7	**0.007**	2.6	**0.007**
	Time	1, 32/48[a]	2.9	**0.020**	2.4	**0.011**
Fungi	Substrate	2, 32/48[a]	13.5	**<0.001**	4.0	**<0.001**
	Cellulose vs. control			NA		***1.000***
	Glycine vs. control			NA		***0.003***
	Cellulose vs. glycine			***<0.001***		***0.003***
	Soil type	1, 32/48[a]	7.7	**<0.001**	6.6	**<0.001**
	Temperature	1, 32/48[a]	1.4	0.1452	1.4	0.60
	Time	1, 32/48[a]	2.5	**0.010**	2.2	**0.001**

Bold: significant difference at $P < 0.05$, NA: not available.
[a] denominator for Responsive/Total communities.

The accumulated biomass and necromass can act as a potential C sink or source of plant-derived C, according to the microbial C pump concept (Liang et al., 2017). The microbial C pump, first developed in marine systems, focuses on the role of long-term microbial assimilation and stabilization of organic compounds in the soil via SOM decomposition and formation. Hence, microbial necromass could contributes directly to soil C storage via the stabilization of microbial C molecules, which would further incorporated into organo-mineral complexes of soil. Although we can only hypothetically suggest these mechanisms from the output of our study, it would be important to further assess them, considering the increased amount of C available to soil microorganisms with the acceleration of permafrost thaw (Schuur et al., 2015) and the densification of vegetation cover (Pearson et al., 2013) in the Arctic.

In accordance with our second hypothesis (stating that readily available substrate would impact the microbial community structure and promote fast growing taxa), the elective effect of substrate amendment was especially strong for glycine, where both prokaryotic and fungal richness and the Shannon index decreased drastically (Sup. Fig. S1, Sup. Table 3). Moreover, and in particular for the prokaryotic community, only a minority of highly abundant taxa responded to glycine (Fig. 5, Sup. Table S4), reducing the community evenness index. Our results support the common understanding of microbial community dynamics associated with positive priming (Fontaine et al., 2003), in which microbial r-strategists (Blagodatskaya and Kuzyakov, 2008; De Graaff et al., 2010) or fast-growing copiotrophic taxa (Koch, 2001; Perez-Mon et al., 2020) are postulated to exhibit rapid growth and assimilation of available labile C substrates, leading to changes in the microbial community structure (Mau et al., 2015). Hence, glycine significantly modified the microbial community structures, as illustrated by the considerable shift in the prokaryotic community in glycine-amended soils relative to the control and cellulose-amended soils. Nonetheless, we cannot rule out that cross-feeding (i.e. the incorporation of ^{13}C into DNA of secondary consumers or microbial commensals) occurred in our experiment, making it impossible to distinguish between populations involved in primary and secondary C utilization (Bell et al., 2011).

Prokaryotic taxa using glycine or cellulose were predominantly members of phyla that have been suggested to harbour a putative copiotrophic lifestyle (Koch, 2001; Ho et al., 2017), such as Proteobacteria, Actinobacteria and Planctomycetes (Fig. 5, Sup. Table S5). *Pseudomonas migulaea* (Proteobacteria), containing by far the most abundant OTUs responding to both glycine and cellulose amendment, is a known cold-adapted and a potential plant-growth-promoting bacteria (Suyal et al., 2014). Furthermore, the abundant *Pseudoarthrobacter* sp. taxa (Actinobacteria), which responded to glycine amendment, are psychrotrophic bacteria which have previously been observed in a rock habitat of Svalbard (Choe et al., 2018). Although *Pseudoarthrobacter* sp. taxa were formerly grouped with the genus *Arthrobacter*, which has been reported to have a copiotrophic lifestyle, several members of this group sustain an oligotrophic lifestyle strategy (Choe et al., 2018), suggesting that this taxonomical group encompasses taxa with different growth strategies. Moreover, the relative abundance of Actinobacteria were found to be increased in sub-Arctic tundra soils amended with N (Männistö et al., 2016).

Only a few responsive fungal taxa were identified in comparison with the many prokaryotic ones, particularly when comparing the number of reads of responsive OTUs (Fig. 5). This is not surprising, however, considering the relatively short duration of the incubation; the initial phase of priming experiments is usually dominated by the response of bacteria (Blagodatskaya and Kuzyakov, 2008), while the fungal to bacterial biomass ratio is expected to gradually increase following successional changes in the microbial community structure (Lundquist et al., 1999). Nonetheless, the few responsive fungal taxa were almost all from the soil amended with glycine collected after 7 days, indicating that they are r-strategists.

The majority of the fungal taxa that responded to the added substrates belonged to the order Helotiales (Sup. Table S6), which is in line with the relatively high diversity and dominance of this fungal order in tundra soils in the Ny Ålesund region (Zhang et al., 2016b). Members of Helotiales can have mycorrhizal, parasitic, saprobic or root symbiotic lifestyles (Vrålstad et al., 2002), and they have been observed to be the principal ectomycorrhizal fungi associated with Ericaceae in the Arctic tundra (Walker et al., 2011). Moreover, *Tetracladium* sp., an abundant taxon within the Helotiales that utilized glycine in our study, was the dominant fungal genus in a study assessing the fungal diversity in the Ny-Ålesund region (Zhang et al., 2016a). Interestingly, the relative abundance of Helotiales was observed to increase following a long-term N and P fertilization in Arctic tundra soil, suggesting their dominance when conditions become less oligotrophic (Koyama et al., 2014).

Pseudogymnoascus roseus, another fungal taxon from the Leotiomycete class and the most abundant fungal taxon utilizing cellulose in both upslope and downslope soils, is frequently observed in polar regions, including Svalbard (Santiago et al., 2015; Yogabaanu et al., 2017; Zhang et al., 2016b). Members of the genus *Pseudogymnoascus* are broadly distributed and thrive in various environments (Yogabaanu et al., 2017). Moreover, their ability to produce a wide range of extracellular enzymes including cellulase (Krishnan et al., 2016), as well as their capacity to exhibit antimicrobial activity, give them a clear competitive advantage. Therefore, our findings suggest that the addition of fresh C (and N in the case of glycine) mostly promoted fungal taxa that were already abundant in the indigenous soil microbial community and able to readily utilize the substrate, without profoundly changing the composition of the entire microbial community.

The increase in microbial C-metabolism in response to elevated

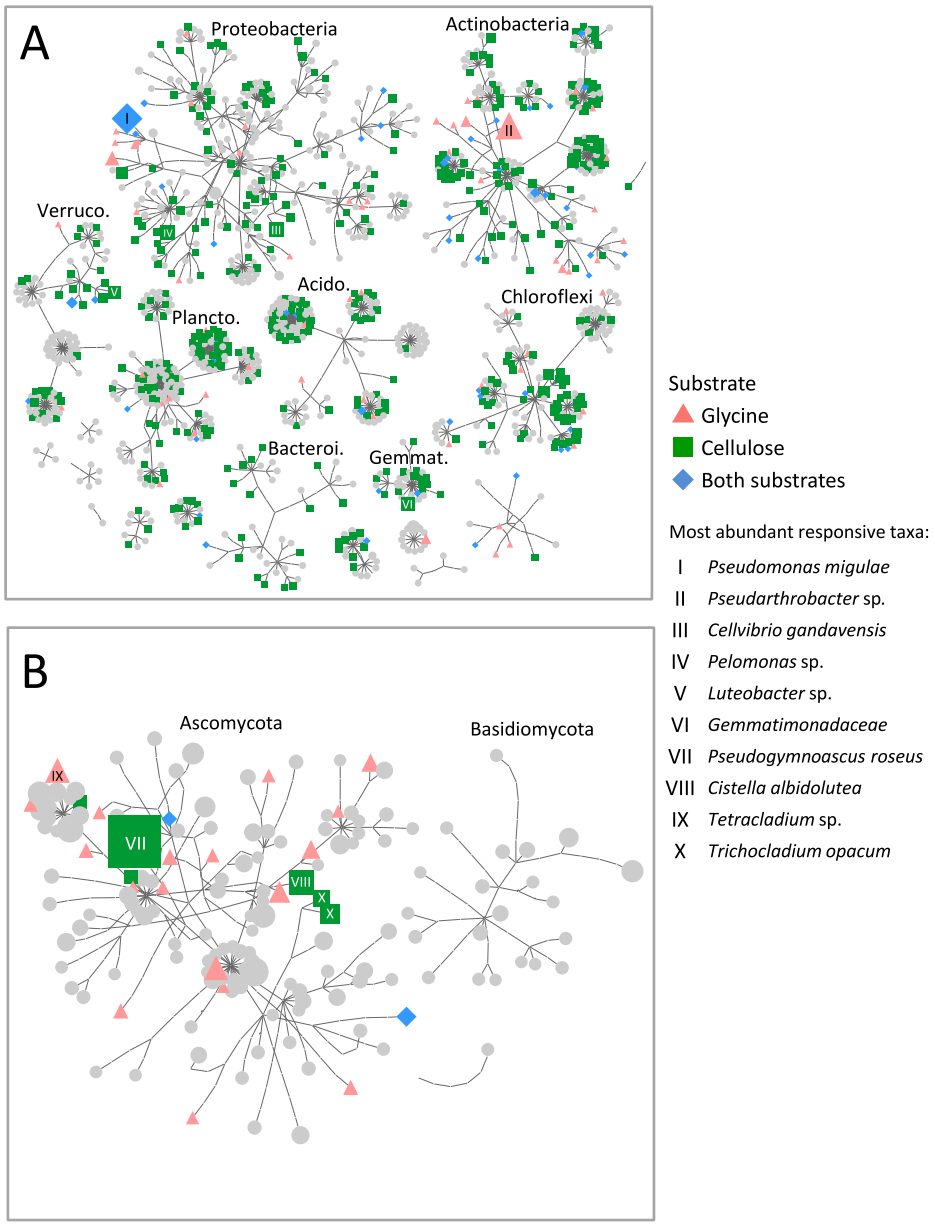

Fig. 5. Taxonomic network of the 10% most abundant prokaryotic (A) and fungal (B) OTUs for both upslope and downslope soils. Each node is a single OTU and its size is in proportion to its relative abundance (based on the number of reads). OTUs responsive to glycine are depicted as pink triangles, to cellulose as green squares and to both substrates as blue diamonds. Highly abundant OTUs are highlighted with roman numbers: I = *Pseudomonas migulae*, II = *Pseudarthorbacter* sp., III = *Cellvibrio gandavensis*, IV = *Pelomonas* sp., V = *Luteobacter* sp., VI = Gemmatimonadaceae, VII = *Pseudogymnoascus roseus*, VIII = *Cistella albidolutea*, IX = *Tetracladium* sp. and X = *Trichocladium opacum*. Verruco. = Verrucomicrobia, Plancto. = Planctomycetes, Acido. = Acidobacteria, Bacteroi. = Bacteroidetes, Gemmat. = Gemmatimonadetes.

temperature is confirmed by the rapid increase in CO_2 emission from soils amended with glycine and incubated at 16 °C (Fig. 1), endorsing our third hypothesis (stating that environmental factors would influence the microbial community structure and its response to C substrate utilization). This pronounced peak in respiration was brief and observed only during the first 7 days of incubation, however decreased over time, due to the depletion of the energy-rich glycine source (Rime et al., 2016). Rapidly increasing respiration rates after the amendment of glycine have been similarly observed in sub-Arctic soils (Sorensen et al., 2008), in nutrient-poor soils of an alpine glacier forefield (Zumsteg et al., 2013a), as well as in temperate soils (Andresen et al., 2014). Thus, our results are in line with the well-known increase in soil microbial activity in response to elevated temperatures observed repeatedly in Arctic soils (e.g. Dorrepaal et al., 2009; Schädel et al., 2016).

The soil type, i.e. upslope or downslope tundra soils, did not affect the microbial functional response (i.e. heterotrophic respiration) to substrate amendment, in contrast to our expectation. This outcome is however to take with caution due to the limited number of sites included in our study. This is however somewhat surprising considering that the structure of both prokaryotic and fungal communities was different between upslope and downslope tundra soils (Table 4), and that – along with temperature – soil moisture is a major factor driving microbial activity in soils (Christiansen et al., 2017; Schädel et al., 2016). Therefore, prokaryotic and fungal taxa in these communities must include a high level of functional redundancy.

5. Conclusions

By testing the microbial utilization of glycine, a readily available substrate, and cellulose, a polymeric C substrate, this experiment demonstrates that the input of organic material with distinct chemical structures into high-Arctic soils has important implications for microbial community diversity and its functional feedback on soil C turnover and stability. Further, elevated temperature promoted CO_2 release from the soils amended with both substrates, but a greater positive priming of SOM in warmer soils was observed subsequent to the addition of glycine. Soil moisture did not affect the fluxes of CO_2, although distinct microbial communities characterized the upslope and the downslope tundra soils.

Only few prokaryotic taxa responded to glycine amendment, whereas a high proportion of responsive taxa utilized only cellulose. Prokaryotic and fungal taxa responding to the substrates were mainly abundant taxa known to be fast growers, r-strategists or taxonomically acknowledged to have a putative copiotrophic lifestyle. The output of this experiment helps tackle the knowledge gap concerning the identification of microbial taxa actively participating in C cycling in high-Arctic tundra soils, a topic of high importance considering the increasing amount of C made available through the thawing of permafrost and increases in vegetation biomass.

Author contributions

AF, BF and MS designed the experiment, AF, BF and LDM performed the experiment, AF analysed the data, and AF and BF wrote the manuscript, which was revised by all authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2021.108419.

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