Fast and persistent responses of alpine permafrost microbial communities to in situ warming

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HIGHLIGHTS
- The impact of global warming on the alpine permafrost microbiome is poorly known.
- 1 m deep alpine permafrost was transplanted to topsoils to simulate in situ warming.
- In situ permafrost warming benefited copiotrophic prokaryotes and ascomycete fungi.
- Warming loosened microbial association networks in the permafrost.
- Permafrost warming enhanced microbial biomass and substrate use but not respiration.

GRAPHICAL ABSTRACT

Abstract

Global warming in mid-latitude alpine regions results in permafrost thawing, together with greater availability of carbon and nutrients in soils and frequent freeze-thaw cycles. Yet it is unclear how these multifactorial changes will shape the 1 m-deep permafrost microbiome in the future, and how this will in turn modulate microbially-mediated feedbacks between mountain soils and climate (e.g. soil CO2 emissions). To unravel the responses of the alpine permafrost microbiome to in situ warming, we established a three-year experiment in a permafrost monitoring summit in the Alps. Specifically, we simulated conditions of warming by transplanting permafrost soils from a depth of 160 cm either to the active-layer topsoils in the north-facing slope or in the warmer south-facing slope, near the summit. qPCR-based and amplicon sequencing analyses indicated an augmented microbial abundance in the transplanted permafrost, driven by the increase in copiotrophic prokaryotic taxa (e.g. Noviherbaspirillum and Massilia) and metabolically versatile psychrotrophs (e.g. Tundrisphaera and Granulicella); which acclimatized to the changing environment and potentially benefited from substrates released upon thawing. Metabolically restricted Patescibacteria lineages vastly decreased with warming, as reflected in the loss of α-diversity in the transplanted soils. Ascomycetous sapro-pathophots (e.g. Tetractadium) and a few lichenized fungi (e.g. Aspicilia) expanded in the transplanted permafrost, particularly in soils transplanted to the warmer south-facing slope, replacing basidiomycetous yeasts (e.g. Glacioclasm). The transplantation-induced loosening of microbial association networks in the permafrost could potentially indicate lesser cooperative interactions between neighboring microorganisms. Broader substrate-use microbial activities measured in the transplanted permafrost could relate to altered soil C dynamics. The three-year simulated warming did not, however, enhance heterotrophic respiration, which was limited by the carbon-depleted permafrost conditions. Collectively, our quantitative findings suggest the vulnerability of the alpine permafrost...
1. Introduction

Global warming is amplified in polar and mid-latitude alpine regions, with predicted increases in air temperature between 2 and 8 °C by 2100 (IPCC, 2014; Hock et al., 2019). Permafrost, i.e. soil at subzero temperatures located under a seasonally frozen active layer, is a major component of polar and alpine cryoenvironments (Margesin, 2009; Donhauser and Frey, 2018). Elevated temperatures are causing extensive permafrost thawing, which results in increased soil water availability and the release of organic materials previously occluded in the frozen layers (Abbott et al., 2014; Gobiet et al., 2014; Chen et al., 2016). Reduced snowfall results in poorly insulated active layers, which therefore experience frequent freeze–thaw cycles (FTCs; Henry, 2008). The warming-associated expansion of plants towards higher latitudes and altitudes (Elmendorf et al., 2012; Steinbauer et al., 2018) might translate into permafrost soils with augmented inputs of carbon (C) and nutrients (Hagedorn et al., 2019; Keuper et al., 2020). The warming-induced changes in soil temperature, moisture, C and nutrients impact the polar and alpine permafrost microbiome (i.e. prokaryotes and fungi), with direct consequences on microbiologically mediated soil processes (e.g. C mineralization) that might influence soil fertility (Fahad et al., 2021; Sönmez et al., 2021) and projected soil–climate feedbacks (e.g. soil CO2 release) at latitudinal and global scales (Mackelprang et al., 2016; Nikrad et al., 2016; Donhauser and Frey, 2018; Hock et al., 2019; Jansson and Hofmockel, 2020).

The microbial responses of permafrost to warming have mainly been investigated in the polar regions, especially in the Arctic, whereas the alpine permafrost at lower latitudes is still poorly studied. Mid-latitude permafrost often occurs on poorly vegetated or barren steep slopes of mountains, above 2500 m a.s.l. (Margesin, 2009; Donhauser and Frey, 2018). In comparison with the permafrost soils at the polar regions, alpine permafrost is "warm" (0 to −2 °C) and located at soil depths below 1 m. Similar to Antarctic soils and unlike Arctic permafrost, alpine permafrost soils at lower latitudes are rocky, well-drained and depleted in organic carbon (i.e. global SOC stock of 66 Pg; Bockheim and Munroe, 2014).

Despite being C-poor, alpine permafrost soils harbor taxonomically and metabolically diverse prokaryotic (i.e. Bacteria and Archaea) and fungal communities (Margesin, 2012; Hu et al., 2015; Frey et al., 2016; Margesin and Collins, 2019; Perez-Mon et al., 2021) that are sensitive to the changing climate (Donhauser and Frey, 2018). Short-term (< 1 year) laboratory-controlled experiments conducted on Asian and European alpine permafrost soils have shown that conditions of elevated soil temperatures (Luláková et al., 2019; Donhauser et al., 2020; Chen et al., 2021), greater availability of soil C and nutrients (Adamczyk et al., 2021) or frequent FTCs (Perez-Mon et al., 2020) shift the structure of the soil prokaryotic communities. Fast-growing and metabolically versatile (i.e. copiotrophic) taxa respond quickly to the increased temperatures and substrates, outcompeting stress-tolerant and slow-growing (i.e. oligotrophic) taxa (Fierer et al., 2007; Ho et al., 2017), which abound in the energy-limited and freezing permafrost environments (De Maeyer et al., 2014; Frey et al., 2016; Mackelprang et al., 2017). Furthermore, C and nutrient amendments in alpine permafrost and mineral Arctic soils have been shown to boost fungal growth, promoting the expansion of fungi over bacteria and compositional variations within the fungal communities (Deslippe et al., 2012; Adamczyk et al., 2020; Adamczyk et al., 2021). The enrichment in copiotrophs and fungi could translate into soils with broader substrate-use microbial activities (Llado and Baldrian, 2017), as indicated by Biolog assays applied to thawed alpine permafrost amended with labile C (Adamczyk et al., 2021) and Siberian soils incubated at increased temperatures (Ernakovich and Wallenstein, 2015). Greater soil microbial abundances and heterotrophic respiration rates have also been observed in alpine permafrost exposed to laboratory-induced warming (Bao et al., 2016; Adamczyk et al., 2021).

Soil transplantation field experiments conducted in the Tibetan mountains have made it possible to extrapolate the results from laboratory studies to natural scenarios, where the soil microbiota respond to multifactorial conditions of warming over the time span of years. Congruent with laboratory observations, downward transplantations of Tibetan grassland topsoils from >3600 m a.s.l. to the warmer thermal regimes of vegetated land at <3200 m a.s.l. led to an augmented microbial biomass in mineral layers (Gou et al., 2015) and long-lasting increases in copiotrophic bacterial groups (e.g. Actinomycetales; Rui et al., 2015; Ho et al., 2017), as well as altered soil C and nitrogen (N) cycling functions and elevated rates of C mineralization (Zhang et al., 2014; Yue et al., 2015). In the alpine permafrost layers, where habitat conditions have remained stable for millennia, abrupt in situ temperature changes could affect the microbial communities more strongly than in the topsoils. Thawing might hamper long-term trophic or cooperative interactions between permafrost microorganisms, i.e. between neighbors failing to adapt to the changing environmental conditions. This could loosen microbial association networks in the permafrost, as observed for heavily disturbed agricultural soils (Banerjee et al., 2019), and it could potentially enhance the vulnerability of the alpine permafrost microbiome to warming. Existing quantitative data on the impacts of warming on the alpine soil microbiome under field conditions is, however, limited to shallow active layers (< 20 cm of depth). To the best of our knowledge, field–manipulation warming experiments outside the Tibetan regions and including both active and the 1 m-deep permafrost layers are lacking.

We conducted a soil transplantation field experiment at the long-term permafrost monitoring site on Muot da Barba Peider (MBP) in eastern Switzerland (Frey et al., 2016; Haberkorn et al., 2019) for three years (2016 to 2019) to evaluate the in situ effects of climate warming on the diversity, community structure, association networks, abundances and activities of the alpine permafrost and active-layer microbiota. Similar to other mountain systems, permafrost in MBP is found near the summit, below a depth of 150 cm on the barren north-facing (N) slope. The south-facing (S) slope of MBP experiences a warmer climate than the N slope, featuring topsoils with 2–3 °C higher soil temperatures, less snow and more vegetation, which results in greater soil C and nutrient contents. We simulated conditions of warming on MBP permafrost and overlaying active-layer topsoils by transplanting soils near the summit from the N to the S slope, where they were placed in the topsoil at a depth of 18 cm (Fig. 1). Permafrost was also transplanted to the N topsoils. Reciprocally, we simulated conditions of cooling (colder climate) in the active layers by transplanting topsoils from the S to the N slope. Opposing microbial responses between the simulated warming and cooling helped us to identify climate-microbial links in the soils. We hypothesized that three years of in situ simulated warming would: (1) lead to augmented soil microbial abundance, associated with abundance increases of fungi and copiotrophic oligotrophic prokaryotes and shifts in soil microbial diversity; (2) loosen microbial association networks in the soils; and (3) result in enhanced substrate-use microbial activities and soil CO2 effluxes.
2. Materials and methods

2.1. Site description

Muot da Barba Peider (2979 m a.s.l., N 46°29.78040′ E 009°55.88700′) is located in the upper Engadine valley in eastern Switzerland. This site is part of the Swiss Permafrost Monitoring Network (PERMOS; http://www.permos.ch/). Hourly soil temperatures measured at a depth of 5 cm (M-Log5W-SIMPLE sensors; GeoPrecision GmbH, Ettlingen, Germany) during three consecutive years (2016 to 2019) had mean annual values of $-2 ^\circ$C (ranging from $-14 ^\circ$C to $21 ^\circ$C) in the north-facing slope and $1 ^\circ$C (ranging from $-8 ^\circ$C to $24 ^\circ$C) in the south-facing slope. The N slope has permafrost below a depth of 150 cm (temperatures of 0 to $-4 ^\circ$C measured at a depth of 1–2 m from August 2016 to August 2019, PERMOS borehole MBP_0196, 2946 m a.s.l., N 46°29.784′ E 009°55.8645; http://www.permos.ch/data.html) for the entire period of August 2016 to August 2019. Mean annual precipitation in the region was 941 mm for the period of 2016–2019 (automatic meteorological station of Bernina, N 46°26.4656, E 009°59.2132, 2090 m a.s.l.; www.meteoswiss.admin.ch). Soils in the S slope experience more FTCs than in the N slope (Table S1). The bedrock in MBP is gneiss from the upper Austroalpine Languard nappe. Quartz and feldspars are the dominant minerals. Vegetation mostly occurs in the S slope, with scattered occurrences of the taxa Poa, Cerastium and Jacobea spp. (Frey et al., 2016).

2.2. Experiment set up

The soil transplantation experiment in MBP was established during the summer of 2016 (Fig. 1). In the N (N 46.49634, E 9.93145) and the S (N 46.49587, E 9.93226; 2979 m a.s.l.) slope, 10 independent active-layer topsoil samples (approx. 200 g of fresh soil), separated by distances of 2–3 m, were collected using a soil corer (10 cm diameter × 10 cm height). A total of 20 topsoil samples were produced (10 samples × 2 slopes). Constrained by time and logistics, a maximum of three soil profiles of approx. 2 m² and separated by approx. 5 m were excavated with shovels on the N slope, down to a depth of 180 cm. Soils were collected at a depth of 160 cm from each profile using the corer, producing a total of 3 independent permafrost (P) samples. Two additional permafrost samples were produced by pooling soils from the three profiles. All collected samples were homogenized and transferred to 2 l stainless-steel containers (12 cm diameter × 18 cm height, catalogue no. 301.317.16, IKEA, Älmhult, Sweden). Materials were sterilized with 70% ethanol between all steps of sample collection and processing.

![Visualization of Muot da Barba Peider (MBP) and scheme of the experimental set-up. A. The upper panel shows an aerial view of the MBP summit (yellow star), with overlapping images of the north-facing and south-facing sampling locations near the summit, and close-up photographs of the active layers. The lower panel shows the properties of the permafrost and north- and south-facing active-layer topsoils, accompanied by a representation of the transplantations using arrows. Permafrost (at 160 cm depth) and active-layer topsoils (at a depth of 18 cm) were collected in the north- and south-facing slopes near the summit of MBP. The collected soils were transplanted (solid-line arrows) to the north- and south-facing active-layer topsoils (at a depth of 18 cm). Permafrost soils were also transplanted within the permafrost table (dashed-line arrow). B. Tabular representation of the transplantation experiment. Capital letters represent the original soils: permafrost (P), north-facing active layer (N) and south-facing active layer (S), whereas lower-case letters represent the destination to which the soils were transplanted. Ns, Pn and Ps are warming treatments, whereas Sn represents cooling. Ns, Pp and Ps (to be used in future studies) are soil disturbance controls. *Average and range (in brackets) of topsoil temperatures measured during the three years of the experiment. Permafrost temperatures correspond to ground temperature measurements at a depth of 1–2 m (PERMOS borehole MBP_0196, 2946 m a.s.l., N 46°29.784′ E 009°55.8645; http://www.permos.ch/data.html) for the entire period of August 2016 to August 2019. OM: organic matter. The aerial photograph of MBP was provided by Dr. Marcia Phillips.](#)
The containers were divided in seven groups, each with five replicates: three groups of permafrost soils (P) and two groups each of N and S active-layer topsoils (N and S). For the transplantation, the containers filled with the soils were placed in the N slope (Nn, Pn and Sn containers) or the S slope (Ns, Ps and Ss containers). Climatic conditions of warming (i.e. warming treatments) were applied to the N and P soils. One group of N topsoils was transplanted to the S slope (Ns, active-layer warming) and the other to the N slope (Nn, disturbance control) (Fig. 1). Comparably, one group of P soils was transplanted to the topsoil in the N slope (Pn, permafrost warming 1) and a second group was transplanted to the S slope (Ps, permafrost warming 2; warmest climate). The third group of P samples were kept within the permafrost table (Pp). These containers will serve as long-term disturbance controls in future studies (e.g. resampling after ≥5 years) and were not sampled in the present study. Here, intact permafrost soils collected at the start of the transplantation experiment (t0, time zero) were used as controls. Climatic conditions of cooling (i.e. cooling treatments) were applied to the S soils: one group of S soils was transplanted to the N slope (Sn, active-layer cooling) and the other to the S slope (Ss, disturbance control).

In each slope the containers were buried (transplanted) at a depth of 18 cm, in a randomized arrangement. The containers were perforated at the sides and the bottom to prevent waterlogging and to allow the exchange of solutions between the soil and the surroundings (Zumsteg et al., 2013). The Pp long-term controls were produced by burying P soils at a depth of 180 cm, in one of the excavated profiles in the N slope. Profiles were sealed using the excavated soil. The transplantation experiment started on 17 August 2016. The transplanted soils (i.e. transplants) were incubated in the field for three years. During the incubation, soil temperatures were measured continuously (via Ibuttons, Maxim Integrated, San Jose, California, USA) at a depth of 3 cm in five of the transplants incubated in each slope. Soil aliquots of all transplants were collected one (t1), two (t2) and three (t3) years after transplantation (summers of 2017, 2018 and 2019). In addition, N and S topsoils were collected every year around the experimental site to monitor temporal variations in the soil microbiota. Aliquots of intact P, N and S soils were also collected at t0. The collected soils were transported on ice from the field to the WSL laboratory facilities, where they were sieved with a 2 mm mesh and stored at −20 °C (for genetic analyses) or 4 °C (for microbial activity and soil chemical analyses). Intact permafrost samples at t0 were also stored at the in situ temperature of −1.5 °C in a growth chamber at the Swiss Federal Research Institute WSL.

2.3. Amplicon sequencing, OTU clustering and taxonomic assignments

DNA was extracted from each individual soil sample (1–2.5 g aliquots) using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany), and the DNA extracts were quantified with PicoGreen (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. To remove foreign DNA and prevent microbial contaminations, working bench surfaces and non-autoclavable materials were cleaned with 5% sodium hypochlorite and 70% ethanol solutions prior to the DNA extractions. Triplicate PCR amplifications of the V3–V4 region of the 16S rRNA gene (prokaryotes) and of the ITS2 genomic region (fungi) were performed on 10 ng of the extracted DNA samples, using the primer pairs 341F/806R and ITS3/ITS4, and the conditions described in Frey et al. (2016). Negative controls for the DNA extractions (extraction buffer without soil) and PCR amplifications (high-purity water without DNA template) were included. The amplicon triplicates were pooled, purified (AMPure XP beads, Beckman Coulter, Beverly, MA, USA) and sent to the Génome Québec Innovation Centre at McGill University (Montreal, Canada), where the pools were paired-end sequenced using the Illumina MiSeq v3 platform (Illumina Inc., San Diego, CA, USA). Raw sequences were deposited in the NCBI Sequence Read Archive under the BioProject accession identifier PRJNA726951.

Quality filtering of the prokaryotic and eukaryotic sequences, clustering (at 97% identity) into operational taxonomic units (OTUs), and assignment of OTUs to taxa were accomplished as described previously (Frey et al., 2016), using a customized pipeline based on UPARSE (Edgar, 2013; Edgar and Flyvbjerg, 2015) and implemented in USEARCH v9.2 (Edgar, 2010). OTUs were classified to taxa by querying centroid sequences against reference databases, using the naive Bayes classifier (Wang et al., 2007) implemented in MOTHUR (Schloss et al., 2009), with a minimum bootstrap support of 60%. Prokaryotic sequences were queried against the SILVA database v138 (Quast et al., 2013). Eukaryotic OTUs were first curated using a custom-made ITS2 database generated from NCBI GenBank. Centroid sequences assigned to fungi were classified to finer taxonomic levels with the UNITE database v8.2 (Nilsson et al., 2019). Singleton and chimeras were removed on-the-fly during OTU clustering. Prokaryotic or fungal taxa represented by less than 1% of all sequences were considered low-abundant (Shade et al., 2014; Zhao et al., 2019).

2.4. Microbial abundance

Bacterial and fungal abundance were estimated by quantitative PCR (qPCR) on a 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). qPCR reactions were prepared using 6.6 μl of the DNA extracts and the primer pairs 27F/519R and ITS3/ITS4, which amplify the V1–V3 region of the 16S rRNA gene in bacteria and the ITS2 genomic region in fungi, respectively. qPCR programs were performed as described by Hartmann et al. (2014) and Frey et al. (2021). Three standard curves (correlations ≥0.99) per target region (16S or ITS2) were obtained using 10-fold serial dilutions (10⁻¹ to 10⁷ copies per μl) of plasmids cloned with the targets.

2.5. Substrate-use microbial activities

The microbial utilization of 31 substrates (i.e. substrate-use activities) was investigated using 96-well EcoPlates™ (Biolog Inc., Hayward, CA) for the P, N and S soil transplants (five replicates) collected at t3, and for the P samples at t0, stored at −15 °C (three replicates). Soils were pre-incubated for one week at 4 °C, followed by one day of pre-incubation at 15 °C. Soil suspensions were prepared by adding a 0.9% NaCl solution to the soils (10:1 v/w) and shaking the mix for 2 h at 15 °C. To reduce the interference of soil materials in the colorimetric assays, soils were left to settle for 10 min and the clear supernatant was diluted 1:10 fold. The EcoPlate wells were inoculated with 125 μl of the dilutions and incubated at 15 °C in dark growth chambers. Flasks containing water were added to the chambers to minimize evaporation. A soil temperature (at a depth of 5 cm) of 15 °C was frequently measured at the MBP summit during summer (Fig. S1), and short-term incubations of Alpine soils at this temperature have only shown minor effects on the microbial communities (Donhauser et al., 2020; Ruthi et al., 2020; Adamczyk et al., 2021).

Absorbance (A) of the inocula was measured at 595 nm with an INFINITE F200 reader (TECAN, Männedorf, Switzerland) every 2–3 days. Measurements were collected until colour saturation (A ≥ 2), exhaustion of the tetrazolium dye), which occurred between 1 (S soils) and 4 weeks (P soils). The absorbance values of the control wells (water) were subtracted from the values of the wells containing the substrates. Maximum rates (r, h⁻¹) of substrate-use microbial activities were calculated by fitting the time courses (hours, h) of absorbance (A) measurements to logistic growth models (A(t)=…), using the package growthcurve (Sprouffske and Wagner, 2016), implemented in R (R Core Team, 2020). Rates were set to 0 when A did not change over time or when the change in absorbance could not be fitted to the logistic growth model. The fitted curves were checked manually and outliers were removed where necessary to improve the fit, as recommended by Sprouffske (2016). We assumed that the soil microbial communities were not able to utilize a substrate when the substrate-use rates were
0 in >50% of the soil replicates from the same soil group (two out of three replicates for P, three out of five replicates for the soil plants). Activity rates were normalized to g$^{-1}$ DW. Substrates were grouped into classes according to Ernakovich and Wallenstein (2015).

2.6. Heterotrophic respiration

Cumulative CO$_2$ respiration and δ$^{13}$C were measured for the intact P soils (three replicates) and the Pn and Ps transplants at t3 (five replicates each). Aliquots of 0.5 g fresh soil were weighed into 12 ml glass vials. The vials were covered with air-permeable cotton lids and pre-incubated for one week at 4 °C and for 5 days at 15 °C, to minimize the potential influences of the soil manipulation and the sharp increase in temperature in the activity estimates. Soil moisture was equalized to 30–40% g$^{-1}$ DW for all samples, by adding 0.1 ml of sterile milliQ water to the 0.5 g of soil. For the assays, the vials were gas-tight sealed and the concentrations of CO$_2$ (gas) accumulated in the headspace and δ$^{13}$C (gas) values were measured after 89 and 185 h of dark incubation of the soils at 15 °C, using a GasBench II system (Thermo Fisher Scientific). To avoid pseudoreplication, the measurements at 89 and 185 h were performed in two independent aliquots (one for each time point) prepared for the same soil replicate (e.g. 2 time points × 5 replicates of Pn soils = 10 samples). CO$_2$ concentrations were normalized to g$^{-1}$ DW. The increment (Δ) of respired CO$_2$ was calculated for each soil replicate, as $Δ$ CO$_2$ = Δ δ$^{13}$C was calculated in a similar way.

2.7. Chemical and mineralogical properties

Chemical properties were measured for all soils collected in the different years, except for soil organic matter (OM), which was only measured in the t3 transplants. Total carbon (C) and nitrogen (N) were measured for dried (65 °C) and fine-grained soil samples, using an elemental analyzer (NC-2500; CE Instruments, Wigan, UK). Dissolved organic C (DOC) and N (DN) were measured on carbonate-free soil extracts treated with 3 M HCl, using a TOC/DTN analyzer (Sakalar Analytical B.V., Breda, the Netherlands). The soil extracts were prepared in milliQ water (water:soil 10: 1 v/w, shaken over night at room temperature) and filtered through DF 5895-150 ashless paper (Albert LabScience, Dassel, Germany). pH was measured in 0.01 M CaCl$_2$ soil slurries (2:1 v/w). OM content was determined by the weight-loss-on-ignition method (Davies, 1974).

Mineralogical analyses were conducted on rocks (1–5 mm) recovered from the intact P, N and S soils at t0 and the Pn and Ps transplants at t3. The mineralogy of the rock materials was determined by X-ray powder diffraction, as described in Frey et al. (2010) and Kern et al. (2019).

2.8. Data analyses

Statistical analyses were completed using the open-source software R v.4.0.2 with the RStudio v.1.3 environment (RStudio Team, 2020), and results were graphed with the R package ggplot2 (Wickham, 2016). A significance level of 0.05 was considered for all statistical analyses.

Differences in prokaryotic and fungal β-diversity between soil transplantation treatments (Pn and Ps; permafrost warming, Ns; active-layer warming, Sn; active-layer cooling) and controls (intact P at t0; Nn and Ss, disturbance active-layer controls) were estimated from Bray-Curtis dissimilarity values based on OTU relative abundances (Legendre and Gallagher, 2001). Dissimilarities were visualized with principal coordinate analyses (PCoAs) and the statistical significance of observed differences between the soils was tested with permutational analyses of variance (PERMANOVAs, 10$^4$ permutations, function adonis in the vegan package; Anderson, 2001). Multivariate homogeneity of group dispersions was checked prior to the PERMANOVAs to ensure that detected significant differences were associated with the transplantation treatments and not with distinct within-group variability of the soils (betadisper function; Anderson and Walsh, 2013). The microbial α-diversity parameters of richness (number of OTUs), and Shannon’s and Pielou’s evenness indices were calculated on OTU counts, rarefied to the lowest number of sequences per sample (12,900 sequences for prokaryotes and 6170 sequences for fungi) through iteration (mean rarefied values from 100 rounds of rarefaction, function rrarefy, perm, EcolUtils package; Salazar, 2019). To prevent underestimations of α-diversity indices (Cameron et al., 2021), one soil sample containing less than 5000 prokaryotic sequences was removed prior to rarefication.

Overall effects of the transplantation treatments, time and their interaction on the microbial α-diversity parameters were tested with two-way ANOVAs using trimmed means as robust estimators (function t2way, WRS2 package; Mair and Wilcox, 2020), after verifying that the residuals of the data were normally distributed but that the response variables were heteroscedastic. The ANOVAs were applied on the separate subsets of permafrost, and north- and south-facing topsoils. Games-Howell pairwise tests were conducted afterwards to test for differences between soil transplants and controls in each of the years of the experiment. Similar statistics were used to determine the effects of transplantation and time on microbial abundance, microbial activities and the soil chemical properties. Welch tests were used instead of the two-way ANOVAs to evaluate the treatment effects on OM and microbial activities, because these variables were only measured at t3. To reduce heteroscedasticity, all variables were z-score transformed prior to testing, except for microbial abundance and microbial activities, which were log$_{10}$ transformed. Bacterial cells containing multiple copies of the 16S gene in their genomes can cause overestimations of qPCR-based abundance measurements in soils (Angly et al., 2014). To minimize this bias and for each soil sample (e.g. Pn soil, replicate 1), bacterial abundance (16S rRNA gene copies g$^{-1}$ DW) was divided by the weighted average of PICRUSt-predicted 16S copies per genome of the OTUs present in the soil (bacterial abundance of Pn soil, replicate 1 = (relative abundance of OTU i × 16S copies of OTU i) / Pn soil, replicate 1). This calculation was performed for each soil sample using the pmo disbem package (Aperger et al., 2015). Differences in prokaryotic and fungal β-diversity between soil transplantation treatments (Pn and Ps; permafrost warming, Ns; active-layer warming, Sn; active-layer cooling) and controls (intact P at t0; Nn and Ss, disturbance active-layer controls) were estimated from Bray-Curtis dissimilarity values based on OTU relative abundances (Legendre and Gallagher, 2001). Dissimilarities were visualized with principal coordinate analyses (PCoAs) and the statistical significance of observed differences between the soils was tested with permutational analyses of variance (PERMANOVAs, 10$^4$ permutations, function adonis in the vegan package; Anderson, 2001). Multivariate homogeneity of group dispersions was checked prior to the PERMANOVAs to ensure that detected significant differences were associated with the transplantation treatments and not with distinct within-group variability of the soils (betadisper function; Anderson and Walsh, 2013). The microbial α-diversity parameters of richness (number of OTUs), and Shannon’s and Pielou’s evenness indices were calculated on OTU counts, rarefied to the lowest number of sequences per sample (12,900 sequences for prokaryotes and 6170 sequences for fungi) through iteration (mean rarefied values from 100 rounds of rarefaction, function rrarefy, perm, EcolUtils package; Salazar, 2019). To prevent underestimations of α-diversity indices (Cameron et al., 2021), one soil sample containing less than 5000 prokaryotic sequences was removed prior to rarefication.

Overall effects of the transplantation treatments, time and their interaction on the microbial α-diversity parameters were tested with two-way ANOVAs using trimmed means as robust estimators (function t2way, WRS2 package; Mair and Wilcox, 2020), after verifying that the residuals of the data were normally distributed but that the response variables were heteroscedastic. The ANOVAs were applied on the separate subsets of permafrost, and north- and south-facing topsoils. Games-Howell pairwise tests were conducted afterwards to test for differences between soil transplants and controls in each of the years of the experiment. Similar statistics were used to determine the effects of transplantation and time on microbial abundance, microbial activities and the soil chemical properties. Welch tests were used instead of the two-way ANOVAs to evaluate the treatment effects on OM and microbial activities, because these variables were only measured at t3. To reduce heteroscedasticity, all variables were z-score transformed prior to testing, except for microbial abundance and microbial activities, which were log$_{10}$ transformed. Bacterial cells containing multiple copies of the 16S gene in their genomes can cause overestimations of qPCR-based abundance measurements in soils (Angly et al., 2014). To minimize this bias and for each soil sample (e.g. Pn soil, replicate 1), bacterial abundance (16S rRNA gene copies g$^{-1}$ DW) was divided by the weighted average of PICRUSt-predicted 16S copies per genome of the OTUs present in the soil (bacterial abundance of Pn soil, replicate 1 = (relative abundance of OTU i × 16S copies of OTU i) / Pn soil, replicate 1). This calculation followed the rationale of Angly et al. (2014).

DESeq2 analyses were used to identify differentially abundant OTUs between the soil treatments and controls (i.e. responding OTUs). For each DESeq2 comparison of soil treatment vs. control, the log$_{2}$-fold change (LFC) of each OTU was calculated as log$_{2}$ (OTU abundance of control / OTU abundance of treatment). To account for differences in sequencing depth between samples, OTU abundances were normalized with the median of ratios method (Love et al., 2020). The statistical significance of the LFC was checked with Wald tests. p values were adjusted for multiple testing using the Benjamini–Hochberg (BH) method with a false discovery rate threshold of 5%. To lower the false discovery rate, the DESeq2 analyses only included OTUs for which the sum of the sequences over all soil samples was ≥ 10. The pairwise comparisons included all the soil transplants and controls collected in the different years (t0 to t3). The soil transplantation was implemented as the main factor in the DESeq2 tests, controlling for the additional factor of time (−time + soil transplantation) (Love et al., 2020). For the graphical representations, only highly (p < 0.01) differentially abundant OTUs among the 80% most abundant in the subsets of permafrost and north- and south-facing active-layer topsoils were selected. Selected OTUs were classified to the taxonomic levels of “phylum” and “genus”. Only the genera for which all assigned OTUs either increased (LFC > 0) or decreased (LFC < 0) in abundance in the soil treatments were included in the representations.

The 16S rRNA gene copy number per genome was predicted for the soil OTUs, by phylogenetically annotating the centroid sequences against fully sequenced genomes using the tool PICRUSt (Langille et al., 2013). The 16S rRNA gene copy number is an indicator of “copiotrophy”, because copiotrophs usually have > 1 copy of the 16S rRNA gene in their genomes (Lauro et al., 2009; Roller et al., 2016). Only OTUs with NSTI (Nearest Sequenced Taxon Index) scores < 2
were represented, as recommended in the PICRUSt documentation (https://github.com/picrust/picrust2/wiki/q2-picrust2-Tutorial).

To gain ecological information about the fungal taxa responding to the transplantation treatments, the tool FUNGuild (Nguyen et al., 2016) was applied to the differentially fungal OTUs detected in the Pn & Ps vs. P DESeq2 comparisons. The package FUNGuildR (https://rdrr.iogithub/brendanf/FUNGuildR/) was used to apply the FUNGuild tool in the R environment.

2.9. Co-abundance networks

The co-abundance association patterns of the prokaryotic and fungal permafrost OTUs (p < 0.01, among the 80% most abundant) responding to warming were investigated. Correlation matrices were generated by calculating all pairwise Spearman’s rank correlations between the OTUs. Positively correlating OTUs (correlation coefficient > 0.6 and BH adjusted p < 0.05; Ju et al., 2014) were identified and the correlations were represented as networks using the igraph package (Csardi and Nepusz, 2006). In the network representations, OTUs were depicted as nodes and correlations as edges. To gain information about warming-associated alterations in the co-abundance association patterns of the microbial communities, network topological properties of modularity and average degree of connectivity between nodes were calculated, as described in Ju et al. (2014).

3. Results

3.1. Changes in prokaryotic and fungal diversity

Soil transplantation (Pn and Ps: permafrost warming, Ns: active-layer warming, and Sn: active-layer cooling) significantly altered microbial α- and β-diversity (PERMANOVAs, p < 0.05) in all soils (Tables 1 and 2). The soil prokaryotic communities experienced greater structural shifts (F = 28, 68% of explained variation, EV) than the fungal communities (F = 7, 39% EV, Table 2). These shifts were particularly pronounced between the transplanted permafrost (Pn and Ps) and the intact soils (P) (Table 2), where the alterations in prokaryotic β-diversity were coupled to a significant decrease in α-diversity (Fig. 2, Table S2). Diversity changes were stronger for the permafrost transplanted to the S (Ps) than to the N (Pn) topsoil, and mostly occurred during the first year of the experiment but persisted over time (Fig. 2, Tables 2 and S2).

Significant decreases in bacterial and fungal α-diversity were also observed for south-facing active-layer topsoils transplanted to the north-facing slope (active-layer cooling) (Tables 1 and S2). Differences in α- and β-diversity between the intact soils (N, S, and P) and soils transplanted within the same habitats (Ns, Ss, and Ps soils stored at −1.5 °C) indicated influences of soil disturbance in the community assemblages (Table S3).

3.2. Responses of prokaryotic taxa to transplantation

Approximately 3000 prokaryotic OTUs were found to be differentially abundant (i.e. responding OTUs) between transplanted and intact (control) permafrost soils (Ps vs. Pn and Ps vs. P, DESeq2 tests p < 0.05), whereas fewer than 500 responding OTUs were detected for the Ns vs. Nn (active-layer warming vs. disturbance control) and Sn vs. Ss (active-layer cooling vs. disturbance control) comparisons (Table S4). Overall, more OTUs responded negatively (LFC < 0) than positively (LFC > 0) to transplantation (Pn vs. Ps, Ps vs. Nn, and Sn vs. Ss). This trend, which persisted over time (Table S5), was particularly marked in the permafrost, where about 2200 prokaryotic OTUs decreased in abundance and only 600 OTUs increased in the transplanted (Pn and Ps) compared with the intact soils (P).

Taxonomic classification of the significantly (p < 0.01) responding OTUs, among the 80% most abundant in the separate subsets of permafrost and north- and south-facing active-layer soils, showed that, at the phylum level only, the low-abundant (<1% total sequences; Table S6) bacterial phyla Deinococcaceae (genus Deinooccus), Firmicutes and Abditibacteriota, and the archaeal Crenarchaeota all increased with warming (LFC > 0, Pn vs. P, Ps vs. P, and Ns vs. Nn; Fig. 3A, Table S7). The abundant phylum (>1% of all sequences; Table S6) Patescibacteria decreased markedly with simulated warming conditions (704 responding OTUs within 28 uncultured lineages; Fig. 3A, Table S7).

Table 1

| Properties | Permafrost soils | North-facing soils | South-facing soils |
|------------|----------------|-------------------|--------------------|
|            | Treatment Time | Treatment × Time  | Treatment Time     | Treatment × Time  | Treatment Time | Treatment × Time |
| Prokaryotic |                |                    |                    |                    |                |                    |
| α-diversity |                |                    |                    |                    |                |                    |
| richness   | 273.94***      | 0.12               | 4.98               | 1.71               | 0.04           | 0.35               | 8.81***           | 1.92 | 4.76 |
| Shannon     | 205.19***      | 6.79               | 6.74               | 0.47               | 0.82           | 0.18               | 10.66**           | 1.12 | 1.47 |
| Fungi       |                |                    |                    |                    |                |                    |                    |      |      |
| richness   | 133.59***      | 16.08**            | 11.85              | 4.07               | 1.39           | 0.42               | 7.95*             | 1.09 | 0.56 |
| alpha diversity | 4.08     | 9.08               | 18.15*             | 1.34               | 15.30*         | 2.09               | 14.70**           | 50.96*** | 0.80 |
| Microbial abundance | 76.57*** | 12.67*            | 23.18*             | 0.00               | 45.05***       | 0.01               | 1.96              | 4.55 | 3.51 |
| Bacteria    |                |                    |                    |                    |                |                    |                    |      |      |
| Fungi       | 78.25***       | 5.35               | 6.11               | 0.26               | 132.66***      | 0.00               | 0.21              | 12.51* | 3.75 |
| Microbial activities | 11.88      | 4.44***            | 14.61***           | 0.67               | 25.11**        | 1.12               | 4.12              | 1.50 | 0.01 |
| Soil and microbial |              |                    |                    |                    |                |                    |                    |      |      |
| properties  | 25.24***       | 4.54               | 115.82***          | 0.07               | 0.29           | 3.13               | 8.76*             | 3.95 | 0.25 |
| Total       | 354.73***      | 404.78***          | 1392.81***         | 2.21               | 10.78*         | 1.82               | 8.68*             | 6.18 | 0.30 |
| DOC         | 15.48***       | 0.02               | 8.20               | 0.05               | 0.64           | 0.20               | 5.99*             | 2.56 | 0.67 |
| DN          | 47.56***       | 14.97***           | 27.97*             | 0.03               | 17.74*         | 5.92               | 1.91              | 1.20 | 2.87 |
| pH          | 40.82***       | 11.22***           | 36.45**            | 0.02               | 1.24           | 0.21               | 6.70*             | 0.15 | 1.42 |
| OM          | 4.80           | 8.07               |                    | 0.83               | 60.90***       | 0.98               | 7.20              | 45.34*** | 4.18 |
| DNA content | 77.00***       | 4.94               | 15.17              |                    |                |                    |                    |      |      |

Significance of effects were tested with two-way ANOVAs using trimmed means as robust estimators. α = 0.05. The ANOVAs included the intact permafrost at the start of the experiment (2016; controls), and all permafrost and north-facing and south-facing transplanted soils from 2017, 2018 and 2019. Values in bold with superscript points and asterisks indicate marginally significant (p < 0.1) and significant (p < 0.05, ** p < 0.01, *** p < 0.001) differences between the soil groups for the tested factors.

1 F:B ratio: fungal-to-bacterial ratio.

2 Treatment effects on microbial activities and soil OM were evaluated with Welch tests because they were only measured in 2019.

3 Substrate-use rates include the rates of all the substrates used by the microbial communities across the different soils. Soil CO2 respiration was only measured for the permafrost soils, where warming effects were the greatest.

4 Total C: total carbon, Total N: total nitrogen, DOC: dissolved organic carbon, DN: dissolved total nitrogen, OM: organic matter.
Table 2
Effects of the soil-transplantation treatments, time and their interaction on microbial β-diversity.

|                | Prokaryotes | Fungi |
|----------------|-------------|-------|
|                | DF  | SS   | MS  | F  | EV [%] | p   | DF  | SS   | MS  | F  | EV [%] | p   |
| Treatment      | 8   | 16.25| 2.03| 27.89| 68    | <0.001 | 8   | 13.07| 1.63| 7.14| 39    | <0.001 |
| Time           | 2   | 0.81 | 0.40| 5.53 | 3     | <0.001 | 2   | 0.89 | 0.44| 1.94| 3     | <0.001 |
| Treatment × Time | 10  | 1.16 | 0.12| 1.59 | 5     | 0.01  | 10  | 2.00 | 0.20| 0.87| 6     | 0.93   |
| Residuals      | 77  | 5.61 | 0.07| 24   |       |       | 75  | 17.16| 0.23| 52  |       |       |
| Total          | 97  | 23.82|     |      |       |       | 95  | 33.12|     |     |       |       |

Prokaryotes and Fungi Pairwise PERMANOVAs

|                | t1 | t2 | t3 | t1 | t2 | t3 |
|----------------|----|----|----|----|----|----|
|                | F  | p  | F  | p  | F  | p  |
| Pn vs. P       | 9.71 | 0.02 | 27.46 | 0.03 | 15.17 | 0.02 |
| Ps vs. P       | 21.25 | 0.02 | 24.16 | 0.03 | 20.78 | 0.02 |
| Ps vs. Pn      | 3.70 | 0.02 | 4.74 | 0.03 | 3.82 | 0.02 |
| Ns vs. Nn      | 1.99 | 0.03 | 1.75 | 0.06 | 2.73 | 0.01 |
| Ss vs. Sn      | 2.05 | 0.09 | 2.46 | 0.06 | 2.76 | 0.04 |

General effects of treatment and time on β-diversity were assessed using PERMANOVA tests. Differences between pairs of soil treatments and controls were assessed using pairwise PERMANOVAs. α = 0.05.

Pn: permafrost incubated in the north-facing topsoil, Ps: permafrost incubated in the south-facing topsoil, Nn: north-facing active layer incubated in the north-facing topsoil, Ns: north-facing active-layer topsoil incubated in the south-facing topsoil, Ss: south-facing active-layer topsoil incubated in the south-facing topsoil, Sn south-facing active layer incubated in the north-facing topsoil.

1 Tests were performed on Bray-Curtis dissimilarity matrices based on the relative abundance of the prokaryotic or fungal OTUs. The main PERMANOVA tests included the permafrost (P), north-facing active-layer soils (N) and south-facing active-layer soils (S), sequenced at the start of the experiment (2016), and the sequences of all soil transplants (Pn, Ps, Nn, Ns, Ss, Sn) from 2017 (t1), 2018 (t2) and 2019 (t3). Controls correspond to P, Nn and Ss soils.

2 DF: degrees of freedom, SS: sum of squares, F: pseudo-F ratio, EV [%]: % of variation explained by the factors, MS: mean squares, p: p value. p values from pairwise tests were adjusted for multiple comparisons with the Benjamini-Hochberg method. EV = r-squared. Values in bold indicate significant differences between soil groups.

Fig. 2. Prokaryotic (A) and fungal (B) diversity in transplanted soils and controls for the three years of the experiment. Bar plots represent the mean ± SD of the prokaryotic and fungal richness and Shannon index (α-diversity) in the permafrost (P) at the start of the experiment (t0, control) and in the soils transplanted to the north- and south-facing (Pn and Ps) active-layer soils after 1 (t1), 2 (t2) and 3 (t3) years. Symbols indicate significant (* p < 0.05, ** p < 0.01, *** p < 0.001, Games-Howell pairwise tests) differences between the soil groups Pn vs. P and Ps vs. P. Differences in β-diversity between soils are depicted with PCoAs computed on Bray-Curtis dissimilarities based on OTU relative abundances. Capital N and S letters represent the original north-facing (N) and south-facing (S) active layers, whereas lower-case letters represent the destination to which the soils were transplanted.
comprising groups (e.g. Ca. Coliiberbacteria and Ca. Woesebacteria) among the taxa with the strongest abundance reductions in the Pn and Ps soils (Fig. 3B). Numerous (~15) low-abundant (<1% of all sequences) phyla also responded negatively to warming (LFC < 0, Pn vs. P, Ps vs. P and Ps vs. Ns vs. Nn; Fig. 3A, Table S7).

At the fine-resolution taxonomic level of genus, we observed that the abundant (~3% of the total prokaryotic abundance) Noviherbaspirillum and Massilia (γ-Proteobacteria) were among the genera with the greatest increases in abundance in the transplanted permafrost soils (Pn vs. P and Ps vs. P; Fig. 3B, Table S8). Other increasing genera included Jatrophihabitans and Crostella (Actinobacteria), Tundrisphaera and Ca. Nostocoida (Planctomycetota), Granulicella, Ca. Solibacter and Blastocella (Acidobacteria), and Flavisolibacter (Bacteroidota). These groups showed increased abundances in warmer vs. colder soil habitat comparisons (Pn vs. P, Ps vs. Ns vs. Nn) and, conversely, a lower abundance in the cooled S soils (Sn vs. Ss; Fig. 3, Table S8). The above-described patterns of taxa increasing and decreasing with warming did not change when differentially abundant OTUs between the intact permafrost at t0 and the soils stored at −1.5 °C (soil disturbance controls, Table S5) were removed (data not shown). A list of all responding prokaryotic OTUs with their classification and abundance information is provided in Table S9.

PICRUSt analyses suggested the copiotrophic features of the OTUs benefiting from warming. Collectively, the permafrost OTUs responding positively to warming (LFC > 0, Pn vs. P and Ps vs. P) had more 16S rRNA copies in their predicted genomes than the negatively responding OTUs (LFC < 0, Pn vs. P and Ps vs. P) (Fig. S2).

3.3. Responses of fungal taxa to transplantation

DESeq2 comparisons between the transplanted and intact (control) permafrost soils (Ps vs. P and Ps vs. P) showed a larger number (300–400) of differentially abundant fungal OTUs than in the Ns vs. Nn (active-layer warming vs. disturbance control) and Sn vs. Ss (active-layer cooling vs. disturbance control) comparisons (<100; Table S4). Most of the fungal OTUs in the permafrost responded positively to simulated warming, especially for the permafrost transplanted to the S topsoils (Ps, warmest topsoil habitat), where the ratios of positively to negatively responding OTUs was 4:1 for Ps vs. P and ~340:1 for Ps vs. Pn. Similarly, most of the fungal OTUs in the N soils increased in abundance with transplantation from Nn to Sn (active-layer warming), whereas they decreased with transplantation from Sn to Ss (active-layer cooling) (Table S4).

The majority of the fungal OTUs responding (p < 0.01) positively to warming (among the 80% most abundant OTUs in the separate subsets of permafrost and north- and south-facing topsoils) belonged to the Ascomycota phylum (representing >50% total fungal sequences; Table S6), together with Chytridiomycota (Fig. 4, Table S10). Mortierellomycota and Monoblepharomycota decreased with simulated warming, and Basidiomycota showed mixed responses (Fig. 4, Table S10). At the genus level, we observed that mainly ascomycetous...
sapro-pathotrophic taxa (e.g. *Extremus*, *Tetracladium* and *Protomyces*) were overrepresented (LFC > 0) in the transplanted warmer vs. colder soil habitat comparisons (Pn vs. P and Ps vs. Pn), and among the 80% most abundant across the soils were assigned to phyla and genera. Barplots represent the mean ± SD of the log$_2$-fold change (LFC) values of the OTUs belonging to common taxa in Pn & Ps vs. P comparisons. Red and purple colors of genera in B correspond to the phyla in A. Secondary bar colors and symbols provide ecological information about the fungal genera retrieved from the FUNGuild (http://wwwfunguild.org/) (*) databases. Rel. abund (%): sum of the abundance of the OTUs belonging to same taxa expressed as a percentage of the total sequences in the permafrost soils. Same response in Ps vs. Pn: the genera increased (or decreased) in abundance in all Pn vs. P, Ps vs. P and Ps vs. Pn. The same applies for “same response in Ns vs. Nn”. Opposite response in Sn vs. Ss: the genera increased in abundance in Pn vs. P and Ps vs. P, and it decreased in Sn vs. Ss (cooling). Capital N and S letters represent the original north-facing (N) and south-facing (S) active layers, whereas lower-case letters represent the destination to which the soils were transplanted.

3.4. Changes in microbial networks

Co-abundance networks of the positively correlating prokaryotic and fungal OTUs responding to warming (DESeq2 tests $p < 0.01$, 80% most abundant in the permafrost soils) were less complex for the transplanted (Ps and Pn) compared with the intact permafrost (P) at the start of the experiment (Fig. 5). Specially for the OTUs responding negatively to warming (LFC < 0, Pn vs. P and Ps vs. P), networks became increasingly more disconnected between the P, Pn and Ps soils, showing a steep increase in modularity (m) and a decrease in the average degree (ad) of connectivity between the nodes (m = 0.02, 0.34 and 0.92, and ad = 906, 23 and 5 in P, Pn and Ps, respectively; Fig. 5). Associations between taxa were not phylogenetically conserved.

3.5. Changes in microbial abundance

Permafrost transplantation led to an increase in bacterial and fungal abundance, which occurred during the first year of the experiment and persisted over time (Fig. 6, Tables 1 and S12). Increasing fungal-to-bacteria (F:B) ratios in the transplanted permafrost, particularly in the soils transplanted in the south-facing slope (Ps, warmest climate) indicated a trend of more rapid increase in abundance for fungi than for bacteria. Microbial abundance was not significantly affected in the transplanted N topsoils (active-layer warming) and only weakly in the transplanted S topsoils (active-layer cooling), being influenced by time and soil disturbance (Table S12).

3.6. Changes in substrate-use activities and heterotrophic respiration

Biolog assays (at 15 °C) showed that the permafrost transplanted to the N (Pn) and S (Ps) topsoils harbored microbiota capable of growing on a wider range of substrates than the intact permafrost (soils stored at −1.5 °C), and at greater rates (Fig. 6, Table 1). Particularly, the Pn and Ps microbiota metabolized a larger number of amino acids and, to a lesser extent, carboxylate compounds (Fig. 6). Additional assays did not show a significant influence of incubation temperature (4 vs. 15 °C) and initial inoculum (undiluted vs. 1:10 dilution) in the maximum rates of substrate-use activities for the permafrost (data not shown). Substrate-use activities of the N and S active-layer topsoils were only minimally affected by the transplantation (N to S, active-layer warming and S to N, active-layer cooling; Fig. S3). Basal respiration (respired CO$_2$ and δ$^{13}$C values at 15 °C) was similar between the three-year transplanted soils (Pn and Ps) and the one-week thawed intact permafrost (P) (Fig. S4). δ$^{13}$C values (approx. −28) measured in the solid phase were also similar for all permafrost soils.
3.7. Changes in soil properties

Permafrost transplantation to the N and S topsoils was associated with a significant (p < 0.05) increment of up to 54% in total C (Tables 1 and S13), although overall values remained low (≤0.17% C). Total N became measurable in the transplanted permafrost soils (~0.01% N). Dissolved N (DN) significantly increased 6-fold in the transplanted permafrost soils, whereas dissolved organic carbon (DOC) decreased between 10 and 30% (Table S13). Significant differences were also detected for pH and OM content between transplanted and intact permafrost soils (Pn vs. P and Ps vs. P) and between transplanted N active-layer topsoils and disturbance controls (Ns vs. Nn), but values differed minimally between the soil groups (pH ~ 6, OM ranging from 0.9 to 1%).

4. Discussion

4.1. Simulated warming promotes the abundance increase of copiotrophic and metabolically versatile prokaryotic taxa

In agreement with our first hypothesis, the transplantation treatments simulating warming led to augmented microbial abundance in the MBP permafrost soils, and shifted the structure of the microbial communities, particularly the prokaryotic communities, in both the permafrost soil transplanted into the active-layer topsoils and, to a lesser extent, the north-facing active layers transplanted into south-facing active-layer topsoils. As expected, prokaryotic taxa increasing in abundance with simulated warming in the permafrost and active layers, or conversely decreasing with simulated cooling (S to N topsoil transplantation) included abundant bacterial genera with copiotrophic attributes, such as Noviherbaspirillum and Massilia. These genera have been shown to be fast-growing (i.e. forming colonies in less than 7 days of incubation at 25 °C) and they are easily culturable in nutrient-rich artificial media (Ofek et al., 2012; Lin et al., 2013; Baldani et al., 2014). Genera within Actinobacteria and Bacteroidota, which increased in the MBP soils with simulated warming, might behave copiotrophically at elevated concentrations of C and nutrients (Senechkin et al., 2010; Ho et al., 2017). The warming-induced increase in copiotrophy of the MBP soils was further indicated by the greater number of OTUs in the permafrost transplants, which contained multiple 16S rRNA gene copies in their genomes (Lauro et al., 2009; Donhauser et al., 2020).

An increased abundance of copiotrophic prokaryotic taxa, including Noviherbaspirillum and Massilia, has previously been observed in laboratory incubations of MBP soils at elevated temperatures (Lulákóvá et al., 2019; Donhauser et al., 2020) and receiving inputs of root-exudates (Adamczyk et al., 2021), as well as in nutrient-enriched Arctic soils undergoing thawing (Schostag et al., 2019; Adamczyk et al., 2020). Likewise, transplantation of Tibetan topsoils from 3800 to 3200 m a.s.l.
showed an increase in Actinobacterial strains in the transplants under the warmer climate of the lower altitudes (Rui et al., 2015). The thawing temperatures experienced by the MBP permafrost soil transplanted into topsoils would have activated the permafrost microbiota surviving the freezing conditions, here reflected in the increased abundance of spore-forming Firmicutes upon soil transplantation (Coolen and Orsi, 2015; Schostag et al., 2019). The availability of liquid water following thawing and the release of C and nutrients from the previously frozen layers (Abbott et al., 2014; Chen et al., 2016) in the permafrost transplants could have particularly benefited copiotrophic prokaryotic taxa, which adjust quickly to temperature changes and grow faster than other groups with the available nutritional resources (Ho et al., 2017).

The frequent FTCs occurring in the topsoils might have additionally favored copiotrophs in the transplanted permafrost, concuring with previous findings from MBP soil incubations with an increased FTC frequency (Perez-Mon et al., 2020). Crosiella and Deinococcus genera, which are adapted to environments of highly fluctuating temperature (Villa and Cappitelli, 2019), might have also benefited from the FTCs. Particularly the 3 °C warmer climate of the south-facing slope of MBP, with less snow, could have facilitated the expansion of copiotrophic microorganisms in the permafrost, and active layers incubated in the south-facing slope.

In disagreement with our first hypothesis, simulated conditions of warming in the permafrost and overlying active layers led to an abundance increase of under-characterized soil bacterial taxa within Acidobacteriota and Planctomycetota, that a priori would be considered oligotrophic (Fierer et al., 2007; Ho et al., 2017). These included genera such as Tundrisphaera and Granulicella, which contain strains that require nutrient-poor artificial media and several weeks to grow (Rawat et al., 2012; Kulicheyskaya et al., 2017). Tundrisphaera and Granulicella have been described as important litter degraders in Arctic soils (Rawat et al., 2012; Kulicheyskaya et al., 2017; Dedysh and Ivanova, 2019; Klarenberg...
et al., 2020), encoding for a wide variety of hydrolytic enzymes that enable them to utilize lignocellulosic polysaccharides (Rawat et al., 2012; Dedys and Ivanova, 2019).

Metagenomic assessments of MBP soils in a previous study indicated the presence of plant cell debris (e.g. from Poales) in the permafrost layers, and the enhanced functional genetic potential of the permafrost microbiota to degrade polymeric substrates (Perez-Mon et al., 2021). We theorize that the release of preserved biopolymers upon permafrost thawing could have stimulated the growth of the polymer-degrading *Tundrisphaera* and *Granulicella*, explaining their abundance increase in the transplanted MBP permafrost. Our reasoning is in line with GeoChip gene profiling of Tibetan permafrost soils laboratory-incubated at 5 °C for five months (Chen et al., 2021), where an overrepresentation of C-degrading genes suggested the microbial mobilization of biopolymers in the thawed soils. A greater content of plant-derived detritus in the south-facing topsoils of MBP (Perez-Mon et al., 2021) could potentially have contributed to the increased abundance of metabolically versatile prokaryotes capable of utilizing complex polymers in the active layers transplanted in the south-facing slope, through the exchange of material with the surrounding soils.

4.2. Metabolically restricted oligotrophic bacterial taxa are vulnerable to simulated warming

In contrast to the increase in copiotrophs, conditions of warming in the MBP permafrost led to a severe decrease mainly of OTUs belonging to the uncultured Patescibacteria superphylum, as reflected in the loss of prokaryotic α-diversity after transplantation into topsoils. A previous metabarcoding study conducted in the MBP soils showed the overrepresentation of Patescibacteria lineages in the permafrost (Frey et al., 2016). Patescibacteria members feature ultra-small cell sizes (<0.1 μm) and streamlined genomes (~1 Mbp) of reduced metabolic capabilities, likely related to syntrophic lifestyles (Rinke et al., 2013; Brown et al., 2015; Ortiz et al., 2020; Tian et al., 2020; Vigneron et al., 2020). Genome simplicity might be advantageous for the Patescibacterial cells in the oligotrophic permafrost habitats, as these cells would require little energy to grow and divide (Ortiz et al., 2020; Tian et al., 2020). The metabolic restrictions of Patescibacteria would, however, limit their growth upon thawing. Under a warmer mountain climate, alpine permafrost soils might lose their unique prokaryotic populations (Chen et al., 2021), which could be replaced by the copiotrophic and metabolically versatile strains in the soils studied here.

4.3. Permafrost fungi benefited overall from simulated warming

MBP fungal communities profited overall from the simulated warming in the permafrost transplanted into the active-layer topsoils and, to a lesser extent, in the north-facing active layers transplanted into south-facing active layers. The trend of increasing fungal-to-bacterial (ITS:16S) ratios over time, measured in the transplanted permafrost, could be explained by fungi using the increased soil C and nutrients more efficiently than bacteria (Kallenbach et al., 2016). Mainly sapro-pathotrophs, and a few cold-adapted lichenized genera (e.g. *Aspicilia* and *Lecanora*; Singh et al., 2015; Nims et al., 2018; Coleine et al., 2021) increased in abundance with the changing environment. Elevated soil temperatures could have promoted the growth of the sapro-pathotrophs in the transplanted soils, as they are highly adaptable to environmental changes (Schmidt et al., 2012; Selbmann et al., 2013). Further, the exposure to sunlight in the topsoil, which is intensified in the south-facing slopes, could have enabled the hyphal growth of the lichenized fungi together with their algal partners. In contrast, psychrophilic basidiomycetous yeasts (e.g. *Glaciomyces*, *Schizoscella* and *Mrika*) inhabiting the permafrost decreased with warming. The thick cell walls of basidiomycetous yeasts and their ability to form cryoprotective exo-polymeric capsules might explain their prevalence in the frozen permafrost environments (Coleine et al., 2021), but our results suggest that they will be poor competitors against other fungal groups in the future warmer climate.

4.4. Simulated warming loosened the microbial association networks in the permafrost

In agreement with our second hypothesis, the diversity loss and compositional shifts within the permafrost microbial communities transplanted into topsoils resulted in more disconnected microbial networks, with fewer positive associations between the fungal and prokaryotic members. In line with the reasoning of Banerjee et al. (2019) and Morrien et al. (2017), the simpler microbial networks in the MBP transplanted permafrost could potentially reflect a loss of cooperative or trophic interactions between neighboring microorganisms (e.g. syntrophic Patescibacteria obtaining nutrients from their partners), and thereby result in soil microbial communities that are overall more vulnerable to environmental perturbations. This notwithstanding, microbial association networks based on correlations should be interpreted with care, because microbial taxa that do not interact could co-occur in the soils owing to shared habitat preferences. Microbial structural alterations in the transplanted soils, persisting over time after the first year of our experiment, suggest long-lasting effects of the changing soil conditions on the microbiome. Monitoring of the experimental samples in the future is, however, necessary to reveal the longer-term (~5 years) dynamics of the fungal and bacterial populations. Taxonomic shifts within fungal communities, particularly, might occur over longer periods of time than for prokaryotes, owing to lower growth rates of fungi compared with prokaryotic taxa (Rousk and Baath, 2011).

4.5. Three years of simulated warming altered microbial substrate utilization but did not enhance soil respiration

In partial agreement with our third hypothesis, the topsoil-transplanted permafrost microbiota, enriched in fungi and copiotrophic prokaryotes, grew on a broader range of substrates and at enhanced rates in Biolog assays. These observations corroborate the recent findings from GeoChip-based studies in Tibetan permafrost (Chen et al., 2021) and metagenomic profiling of permafrost-affected topsoils in the Swiss Alps (Donhauser et al., 2021), where soil microbial communities at elevated temperatures exhibited greater C-degrading genetic capabilities. Broader microbial activities, particularly for the recycling of N compounds (e.g. microbial necromass, Donhauser et al., 2021), might improve the nutritional status of alpine soils (Metrak et al., 2020), thereby contributing to the ongoing advancement of mountain vegetation towards higher altitudes (Steinbauer et al., 2018; Adamczyk et al., 2019).

In discrepancy with our third hypothesis, soil CO2 emissions rates were similar between three-year thawed permafrost and one-week thawed controls. The MBP permafrost microbiota transplanted into topsoils might feed on necromass or detritus-derived organics in the soils, and it could even benefit from inputs of C and N from the atmosphere (Brankatschk et al., 2011) and the surrounding topsoils. These nutritional sources might support microbial growth after thawing but they might not be enough to sustain high rates of cell metabolic activity. The MBP microbiota might require both elevated temperatures and it could even benefit from inputs of C and N from the atmosphere (Brankatschk et al., 2011) and the surrounding topsoils. These nutritional sources might support microbial growth after thawing but they might not be enough to sustain high rates of cell metabolic activity.
5. Conclusions

Here we demonstrate the fast acclimation of a temperate permafrost microbiota to three-year field-simulated conditions of warming, confirming the indications from short-term laboratory-controlled studies. Our findings suggest that the future warmer climate in mid-latitude mountains lead to the growth of copiotrophic and metabolically versatile bacteria, as well as sapro-pathotrophic fungi within the permafrost, superseding metabolically restricted oligotrophic bacteria (e.g. Patescibacteria) and basidiomycetous yeasts. A warming-induced loosening of microbial association networks could result in permafrost microbial communities being more vulnerable to the changing climate, whereas the growth of microbiota that use a wider range of substrates could alter the C pools in the soil. Three years of simulated warming did not result in greater CO₂ effluxes in the here transplanted permafrost, but the soil microbiota needs high inputs of C and nutrients for their heterotrophic activities to increase substantially. Collectively, our findings provide first insights on the structural and functional acclimations of both the alpine permafrost and active layer microbiomes to in situ warming, which help to improve the prediction of the responses of the mountain soils to the future warmer climate.

CRediT authorship contribution statement

Beat Frey: Study design, Set up field experiment, Sample collection, Writing - Reviewing and Editing
Beat Stierli: Set up field experiment, Sample collection, Laboratory assays,
Michael Plötze: Laboratory assays.
Carla Perez-Mon: Sample collection, Laboratory assays, Data analysis, Writing - Original draft preparation.

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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

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