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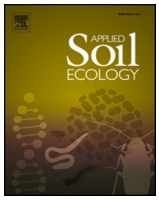

Soil microbial community responses to long-term experimental warming in an alpine *Dryas octopetala* heath in Norway

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ABSTRACT

Over the last century, high-altitude and high-latitude regions have experienced global warming at rates higher than the worldwide average. Climate change influences complex soil-microbe-plant-atmosphere interactions, leading to changes in plant-associated soil microbial diversity and functioning and alterations in nutrient cycling, carbon fluxes, and storage. This study analyzed how two decades of global warming simulated by open-top chambers (OTCs) affected soil bacterial and fungal communities in an alpine dwarf-shrub heath dominated by *Dryas octopetala* in Norway. We collected soil samples from 10 OTCs and 10 control plots and compared their physicochemical properties, microbial biomass, extracellular enzyme activities, and bacterial and fungal community diversity and composition. Warming did not significantly affect the bacterial community despite the tendency to reduce alpha diversity and increase the degree of specialisation. In contrast, two decades of warming significantly affected fungal community composition, which was dominated by ectomycorrhizal *Basidiomycota*. While there was no significant effect on the total fungal community diversity, a significant shift in saprotrophic *Ascomycota* taxa was observed between the warmed and control plots. Their positive correlations with oxidative enzymes and fungal biomass suggest that long-term warming might lead to an increase in fungal biomass and the activity of oxidative enzymes, promoting the decomposition of more recalcitrant biopolymers. This may result in an increase in CO_2 flux into the atmosphere and a decrease in ecosystem C storage.

1. Introduction

Climate change has a significant impact on both species distribution and ecosystem functions worldwide (Kotta et al., 2019; Pörtner et al., 2022). However, the Arctic and alpine ecosystems are particularly vulnerable because warming is amplified at high elevations and northern latitudes compared to the global average (Johnston et al., 2019). Given that cold-adapted species living in these environments may be sensitive to rapid warming, climate change is expected to have a significant effect on cold environments (Qixiang et al., 2018; Mondoni et al., 2022).

The effects of climate change extend to both above ground and below ground processes in terrestrial ecosystems, influencing photosynthesis, biomass accumulation, and overall growth, resulting in significant changes in plant community composition and productivity (Gao et al., 2021). Predicted temperature increase is expected to alter the balance between carbon (C) release and storage by changing the set of intricate relationships between the soil, microbes, plants, and the atmosphere (Callaghan et al., 2004). These relationships are crucial determinants of C cycling, influencing both input and mineralisation processes.

Crowther et al. (2016) compared soil C stocks between warmed and control plots across North America, Europe and Asia sites and revealed differences in C inputs, including plant production, and outputs, such as respiration, in response to warming across sites. These different responses of warming on soil C stocks were variable, with positive, negative, and neutral impacts, reflecting the size of the initial soil C stocks and the extent and duration of warming observed.

Warming effects also extend below ground, where soil microbial

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communities play crucial roles in many processes such as soil organic matter (SOM) decomposition, soil C sequestration, and nutrient cycling (Cavicchioli et al., 2019). Warming can affect soil microorganisms in several ways, either by increasing or decreasing microbial growth and taxonomic and phylogenetic diversity. In particular, a decline in soil moisture due to warmer conditions may decrease microbial diversity (Wu et al., 2022). Warming can also alter the balance between fungi and bacteria (Rinnan et al., 2007; Rousk and Frey, 2015). Furthermore, there is a relationship between the composition of microbial communities and soil extracellular enzyme activity (EEA), and changes in microbial composition may thus affect soil microbial metabolic activity and microbial C use efficiency. In particular, at higher temperatures, dominant populations can metabolise substrates that are not used at lower temperatures (Frey et al., 2008). Despite the highly variable effects of warming on soil enzyme activity across sites, a meta-analysis of 78 studies has shown that experimental warming usually stimulates oxidative enzymes but has variable effects on hydrolytic enzymes (Meng et al., 2020). Shifts in enzymatic activity degrading recalcitrant C sources may increase microbial accessibility to litter and SOM, leading to accelerated soil C loss with prolonged warming (Chen et al., 2020).

Interestingly, a review of 64 field studies revealed that experimental warming had a more pronounced effect on microbial community composition and abundance in colder than in warmer regions (Chen et al., 2020). As such, a five year warming experiment in an Italian Alp site showed a shift in bacterial biomass, changes in community structure, and an increase in oligotrophic taxa, whereas there were no effects on the fungal community (Sannino et al., 2022). D'Alò et al. (2021) reported a shift in the total enzyme activity pattern in response to warming within the same site, with no changes in the ratio of hydrolytic and oxidative enzymes.

Although some responses of Arctic and alpine microbial communities were recorded in individual warming experiments, Jeanbille et al. (2021) detected no general effect of warming on fungal and bacterial abundance in litter and soil when comparing 16 warming experiments at 12 different circumpolar locations. Prior research suggested that one or two decades of warming are required before significant changes in enzyme activity, microbial biomass, and community composition can be observable (Rinnan et al., 2011; Timling and Taylor, 2012). However, there is a scarcity of studies examining the impact of warming over long-term, spanning years to decades (Gao et al., 2021). Timling and Taylor (2012) observed that the effects of warming on low-Arctic microbial communities depended on the duration of the climate manipulation. Notably, the composition and structure of the fungal community remained relatively stable, even after a decade, whereas the diversity of ectomycorrhizal and saprotrophic fungal species increased after 18 years of warming. As the temperature increased, the metabolic activities of these free-living saprotrophs intensified, enhancing the decomposition of organic materials. Initially, this decomposition primarily targeted labile organic compounds, which gradually progressed to more recalcitrant compounds (Van Nuland et al., 2020). Similarly, Rinnan et al. (2011) investigated the growth responses of bacterial community after 7 and 17 years of experimental warming and reported a more pronounced decline in growth after 17 years.

In this study we used a long-term warming experiment using open-top chambers (OTCs) established in a *Dryas octopetala* heath in alpine southern Norway in 2000 as part of the International Tundra Experiment (ITEX). Previous studies have shown only minor changes in plant community composition after 16 years of warming (Hasvik, 2018). This high resistance of the vascular plant community to warming was linked to dry soil conditions, as plant communities in moist sites show stronger responses to warming (Elmendorf et al., 2012).

Since soil microorganisms play a fundamental role in plant resistance, stress tolerance, and soil nutrient availability, and in supporting overall plant growth (Dastagir, 2019), we assessed whether two decades of experimental warming have affected soil microbial communities associated with *D. octopetala,* in terms of diversity, composition, biomass, and enzymatic patterns. Given that soil moisture decreases with warming, we aimed to test the hypothesis that long-term warming decreases bacterial and fungal community diversity and alters its composition. Additionally, we aimed to verify the possible increase in oxidative enzyme activity at the expense of hydrolytic enzymes, which suggests that warming affects chemically complex C substrates (Van Nuland et al., 2020).

2. Materials and methods

2.1. Sampling site and design

The study area was an alpine ITEX site in Finse, near the peak of Mt. Sanddalsnuten (1554 m above sea level) in southern Norway's Scandes mountains, within the 450 km^2 Hallingskarvet National Park (60.626°N; 7.522°E, at approximately 1500 m above sea level). Owing to its calcareous phyllitic bedrock (Olsen and Klanderud, 2014), the study area supports a species-rich alpine community dominated by the dwarf shrub *Dryas octopetala*. The study site is located on a southwest facing sun exposed slope with high wind speeds and little snow accumulation (Klanderud and Totland, 2004). Soils are well drained and thus remain relatively dry for most of the growing season despite high annual precipitation. Other common species are *Bistorta vivipara, Salix herbacea, S. rupestris, Carex vaginata, C. rupestris* (Klanderud and Totland, 2007). The climate warming experiment was established in the year 2000 and, comprised of hexagonal OTCs (1 m in diameter) (Fig. 1). OTCs are commonly used to simulate climate warming by increasing soil and air temperatures (Carey et al., 2016; Hollister et al., 2022). They increased the above ground temperature (~5 cm) by approximately 1.5 °C, and the below-ground temperature (~ − 5 cm) by approximately 1 °C during the summer season (Klanderud and Totland, 2005). Paired untreated plots outside of the OTCs were designated as controls.

Microbes in cold environments are most active during the warmest months of the year, so all soil samples were collected in August 2021 during the peak growing season. We sampled five spots within each of the 10 OTCs and their respective control plots at 0–5 cm depth. A composite sample was obtained for each plot, encompassing local variations. The organic soil was homogenized, and then collected into sterile Falcon tubes. Samples were transported to a laboratory under frozen conditions and stored at −20 °C until analysis.

2.2. Soil properties and fungal biomass

Soil samples were analyzed for total C, organic C, and nitrogen (N) on a FLASH 2000 Analyzer (Thermo Fisher Scientific, USA). Total phosphorus (P) was measured by method described previously by Ohno and Zibilske (1991), which is based on the ionic association of malachite green with phosphomolybdate under acidic conditions of soil samples followed by the measurement of the resulting phosphate ion using spectrophotometry. Soil pH was measured by adding 5 ml of deionized H_2O to 0.5 g of freeze-dried soil in a container, which was vigorously shaken. Subsequently, the soil-water mixture was allowed to settle, and pH was determined in the supernatant using a pH meter. Water content was calculated by freeze-drying soil subsamples. The weight of the wet soil was divided by the weight of the dry soil, and the resulting fraction was multiplied by 100 to get the water content.

Total ergosterol was extracted from 0.5 g of freeze-dried soil with 10 % KOH in methanol and analyzed by high-performance liquid chromatography as described previously by Šnajdr et al. (2008) using a method modified from Bååth (2001).

2.3. DNA extraction and quantitative PCR

Total metagenomic DNA was extracted in duplicates from 200 mg of freeze-dried soil samples using NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany). DNA concentration and purity were assessed using

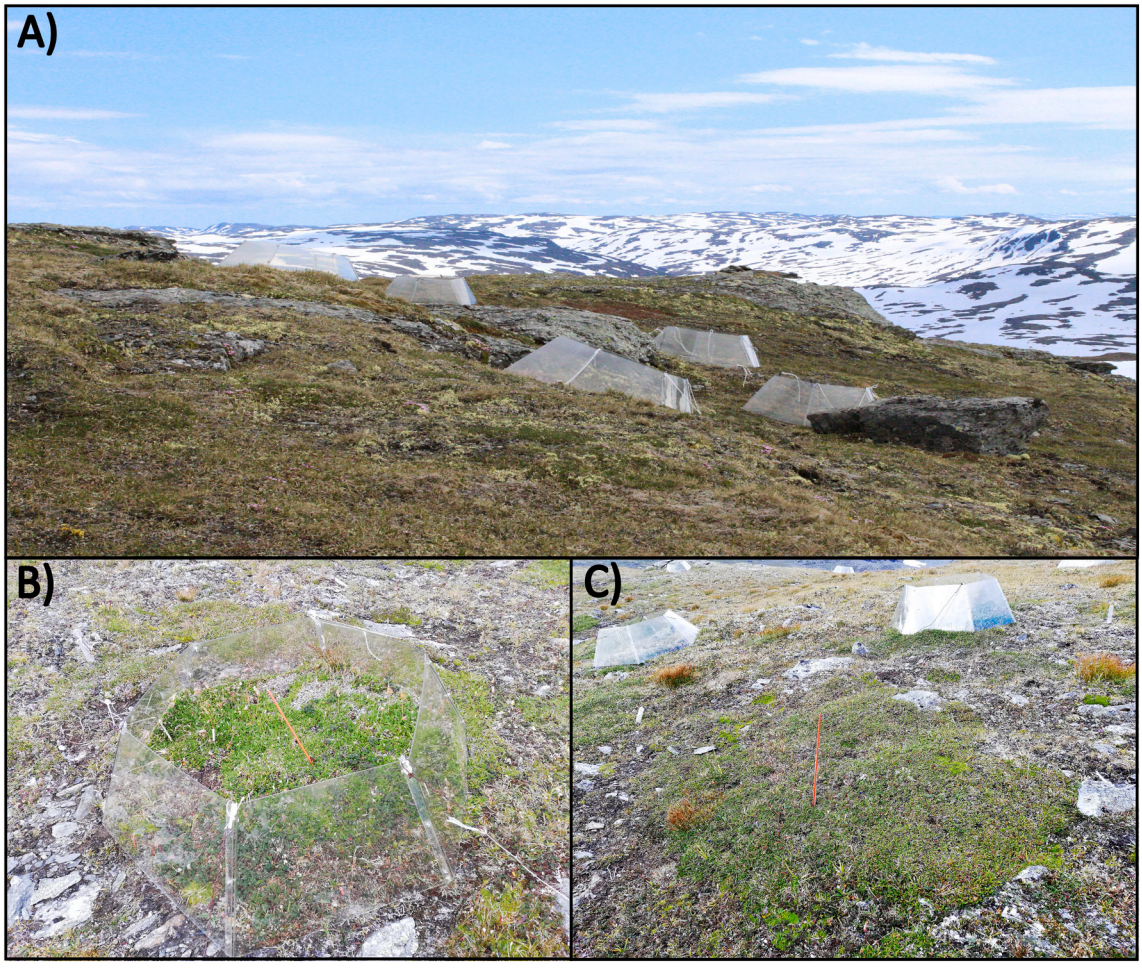

Fig. 1. A) Sampling site in alpine Finse, Norway; B) Open Top Chamber (OTC) – used for simulation of increased temperature; C) control plots – untreated paired plots outside of OTCs.

Qubit (Thermo Fisher Scientific, USA) and NanoDrop ND-100 Spectrophotometer (Thermo Fisher Scientific, USA). DNA concentration of each sample was adjusted to 5 ngμl^{-1}. Bacterial 16S and fungal 18S rDNA copies were quantified by quantitative polymerase chain reaction (qPCR) using 1108f/1132r primers for bacteria (Wilmotte et al., 1993; Amann et al., 1995) and FR1/FF390 primers for fungi (Prévost-Bouré et al., 2011) as described previously (Žifčáková et al., 2016). Briefly, each 20 μl reaction mixture contained 10 μl of SYBR Green Master Mix (Applied Biosystems), 0.9 μl of bovine serum albumin (BSA; 10 mgml^{-1}), 1.35 μl of each primer (0.01 mM), 1.5 μl of DNA template, and 6.1 μl of water. qPCR for each sample was run in triplicates. The qPCR cycling protocol was the same for fungal and bacterial DNA quantification: 56 °C for 2 min, 95 °C for 10 min, 95 °C for 15 s, and 60 °C for 1 min (40 cycles). *Streptomyces lincolnensis* DNS 40335 and *Hypholoma fasciculare* CCBAS281 genomic DNA were used as standards. Fungal and bacterial abundances were expressed as the number of copies of rDNA genes per gram of dry soil.

2.4. Hydrolytic and oxidative enzymes assays

Extracellular enzyme activity was measured as described by Štursová and Baldrian (2011) with some modifications. The activity of the majority of hydrolytic enzymes, including *cellobiohydrolase*, *β-glucosidase*, *β-galactosidase*, *chitinase*, *acid phosphatase*, *β-xylosidase*, *lipase* and *α-glucosidase*, was assessed using 4-methylumbellifore (MUF) fluorescence detection. Activity of enzymes was assayed in soil slurries prepared by mixing 0.25 g of freeze-dried soil with acetate buffer (50 mM, pH 5), then homogenized using UltraTurrax (IKA Labortechnik, Germany). Additionally, the activity of *endocellulase*, *endoxylanase*, and four oxidative enzymes — *laccase*, *oxidase*, *lignin peroxidase*, and *Mn peroxidase* — was assessed using absorbance detection method. Extracts for absorbance detection were prepared by mixing 0.25 g of freeze-dried soil with phosphate buffer (100 mM, pH 7), filtered, and desalted using Sephadex columns (Cytiva). Enzyme activity was expressed in units per g of dry soil (Ug^{1}) where one unit is defined as one μmol of substrate converted per minute. Fluorescence and absorbance were measured using the Infinite microplate reader (TECAN, Austria).

2.5. Illumina amplicon sequencing of fungal and bacterial communities

Amplicon libraries were prepared as described by Voříšková et al. (2019) with some modifications. For bacterial community analysis, primer pair 515F/806R targeting the V4 region of 16S rRNA gene was used (Caporaso et al., 2011). For the fungal community, primers gITS7/ITS4 were used to amplify the ITS2 region of rRNA operon (Ihrmark et al., 2012). Three independent 25 μl PCR reactions were prepared for each DNA sample, containing: 5 μl of 5xQ5 Reaction Buffer (New England Biolabs), 1.5 μl of BSA (10 mgml^{-1}, GeneON), 1 μl of each primer (0.01 mM), 0.5 μl of PCR Nucleotide Mix (10 mM each), 0.25 μl of Q5 High Fidelity DNA polymerase (0.02 Uμl^{-1}, New England Biolabs), 5 μl of 5xQ5 High GC Enhancer (New England Biolabs), 1 μl of template DNA, and 9.75 μl of PCR-grade water. The cycling conditions were 94 °C for 4 min; 25 cycles of 94 °C for 45 s; 50 °C for 1 min; and 72 °C for 75 s, followed by 72 °C for 10 min for primers 515F/806R and 94 °C for 5

min; 35 cycles of 94 °C for 30s; 56 °C for 30s; and 72 °C for 30s, followed by 72 °C for 7 min for primers gITS7/ITS4. The three PCR product replicates were pooled and purified using SPRIselect magnetic beads (Beckman Coulter, USA). The concentration of PCR products was quantified, PCR products were mixed, and Illumina adapter sequences were ligated on amplicons using TruSeq DNA PCR-Free LT Sample Prep Kit (Illumina, San Diego, CA, USA). The amplicon library was subjected to sequencing on an Illumina MiSeq 2 × 250 bp paired-end platform.

2.6. Bioinformatics and statistical analysis

Sequencing outputs containing fungal and bacterial raw sequences, in fastq format, were demultiplexed to aggregate reads belonging to each sample. Then, *Cutadapt* (Martin, 2011) was used to trim primers from both forward and reverse reads. Trimmed fastq files were processed with *DADA2* (Callahan et al., 2016) to filter, denoise, and infer Amplicon Sequence Variants (ASVs).

For bacterial amplicons analysis, *DADA2* parameters were set as truncLen = c(120, 190), maxN = 0, maxEE = c(4, 4), truncQ = 2. Same procedure was used for fungal amplicons analysis with truncLen = c (200,200), maxN = 0, maxEE = c(4, 4), truncQ = 2. Pseudo-pooling was used when performing the sample-inference step to retrieve ASVs. Fungal sequences were further clustered using *DECIPHER* (Wright, 2016), to group ASVs into Operational Taxonomic Units (OTUs), at 97 % of similarity (Estensmo et al., 2021).

Taxonomy was assigned to each retrieved bacterial ASV and each fungal OTU using the classify-consensus-blast function implemented by *QIIME2* (Bolyen et al., 2019), version 2021.8. *Silva* database (Quast et al., 2012) 99 v138.1 was used to assign taxonomy to bacterial ASVs, *UNITE* (Nilsson et al., 2019) database version 8.99 10-05-2021 was used to assign taxonomy to fungal OTUs. Only taxa with consensus score equal or higher than 0.7, and classified as either Bacteria or Fungi in the respective datasets were considered for subsequent analyses. In addition, functional guilds were assigned to fungal OTUs using the *FungalTraits* database version 1.2 16-12-2020 (Põlme et al., 2020).

Richness, Shannon and Simpson diversity indices were computed using *PAST* software version 4.11 (Natural History Museum, University of Oslo, Norway).

All the other analyses were performed using R statistical software (R Core Team, 2023). ASVs and OTUs abundances, taxonomy, and metadata were managed using *Phyloseq* (McMurdie and Holmes, 2013). Raw counts obtained using *DADA2* were transformed using the *vegan* (Oksanen et al., 2022) with the Hellinger transformation. A combination of *vegan* and *phyloseq* was used to perform a PERMANOVA test to evaluate the effect of treatment on the community composition. Same packages were used for the NMDS ordination, to visualise how samples were organised according to the first two main axes of variation, and the partial RDA, used to test the effect of a mixed effect model on the community and to visualise how samples were organised according to the first two main axes of variation. To visualise the community composition for both bacterial and fungal amplicons, relative abundances were computed. The differential abundance (DA) analysis, performed using the *ANCOM-BC* package (Lin et al., 2021), was used to identify statistically significant differences between individual microbial taxa when comparing controls *versus* OTCs ($p < 0.05$). The algorithm required raw data, so no transformation was applied to the counts.

Partial RDA and DA analysis were performed using a linear mixed effect model that included two variables. The model was defined as Abundance ~ Treatment + Plot. Treatment was considered a fixed effect while Plot was considered a random effect.

Statistical tests for soil parameters, enzymes activity, bacterial and fungal abundances by qPCR, and alpha-diversity indices between warmed and control plots were performed using two-tailed paired Wilcoxon test ($p < 0.05$), and *p*-values were adjusted using the Benjamini-Hochberg method (false discovery rate). Normality of the data was tested using Shapiro–Wilk's test. These analyses were performed to test whether the treatment had an effect on any of the listed parameters.

All plots were obtained using *ggplot2* (Wickham, 2016) version 3.4.3. Software and packages that were used, with the corresponding version, are: *cutadapt* version 4.3, R version 4.3.1 Beagle Scouts, vegan version 2.6–4, *phyloseq* version 1.46, DADA2 version 1.30, *DECIPHER* version 2.30.0, and *ANCOM-BC* version 2.4.0.

3. Results

3.1. Soil properties, microbial biomass and extracellular enzyme activity

The warming treatment had no significant effect on the physico-chemical parameters, microbial biomass, or ergosterol content, but all these parameters tended to increase in the OTC plots, except for soil pH (Table 1).

In general, bacterial mean abundance (4.53E+10 rRNA gene copies g^{-1} dry soil) was higher than fungal abundance (~4.35E+09 rRNA gene copies g^{-1} dry soil) based on the qPCR approach (Table 1). Although bacterial and fungal abundances did not differ significantly between control and warming treatment, fungal biomass tended to increase in the OTCs, as evidenced by increases in both the fungal rDNA gene copy numbers and ergosterol content (Table 1).

Enzyme patterns did not differ significantly between treatment and control samples (PERMANOVA, $p = 0.28$) (Fig. 2). When the enzymes

Table 1
Soil properties, extracellular enzymes activities and microbial biomass in warming and control plots.

	Control	Warming	p-value	Adjusted p-value
Water content (%)	36.8 ± 4.06	39.4 ± 14.3	0.91	0.97
pH	5.92 ± 0.10	5.79 ± 0.23	0.15	0.87
C (%)	9.02 ± 2.10	11.3 ± 8.11	0.91	0.97
N (%)	0.57 ± 0.12	0.72 ± 0.50	0.85	0.97
TOC (%)	7.04 ± 1.79	9.27 ± 7.23	0.97	0.97
P (%)	0.71 ± 0.22	0.86 ± 0.35	0.39	0.94
Ergosterol (μg/g)	16.4 ± 6.77	24.9 ± 12.8	0.12	0.87
Bacteria-qPCR (rDNA gene copies numbers/g dry soil)	4.97E+10 ± 1.53E+10	4.08E+10 ± 1.06E+10	0.22	0.97
Fungi-qPCR (rDNA gene copies numbers/g dry soil)	3.79E+09 ± 2.32E+09	4.91E+09 ± 3.92E+10	0.97	0.70
Laccase (mU/g) (Oxidative)	15.4 ± 9.74	22.4 ± 18.8	0.47	0.75
Cellulase (mU/g) (Hydrolytic)	45.3 ± 9.30	38.9 ± 17.0	0.35	0.75
Xylanase (mU/g) (Hydrolytic)	16.5 ± 11.3	21.6 ± 8.54	0.31	0.75
Oxidase (mU/g) (Oxidative)	2.04 ± 2.07	3.50 ± 3.16	0.25	0.75
Peroxidase (mU/g) (Oxidative)	4.98 ± 4.32	15.2 ± 22.6	0.68	0.80
Mn peroxidase (mU/g) (Oxidative)	9.67 ± 5.35	8.17 ± 4.45	0.82	0.82
Beta-glucosidase (U/g) (Hydrolytic)	5.78 ± 2.42	9.34 ± 7.24	0.22	0.75
Acid phosphatase (U/g) (Hydrolytic)	0.29 ± 0.21	0.52 ± 0.82	0.74	0.80
Beta-xylosidase (U/g) (Hydrolytic)	1.59 ± 0.59	2.11 ± 1.85	0.68	0.80
Chitinase (U/g) (Hydrolytic)	1.30 ± 0.63	2.74 ± 3.40	0.14	0.75
Cellobiohydrolase (U/g) (Hydrolytic)	1.93 ± 0.84	2.76 ± 2.45	0.48	0.75
Beta-galactosidase (U/g) (Hydrolytic)	1.35 ± 0.51	2.08 ± 1.87	0.28	0.75
Alpha-glucosidase (U/g) (Hydrolytic)	0.37 ± 0.24	0.60 ± 0.67	0.74	0.80
Lipase (U/g) (Hydrolytic)	67.7 ± 16.9	82.1 ± 57.7	0.48	0.75

Values represent means ± standard errors; adjusted *p*-values indicate significant difference ($p < 0.05$).

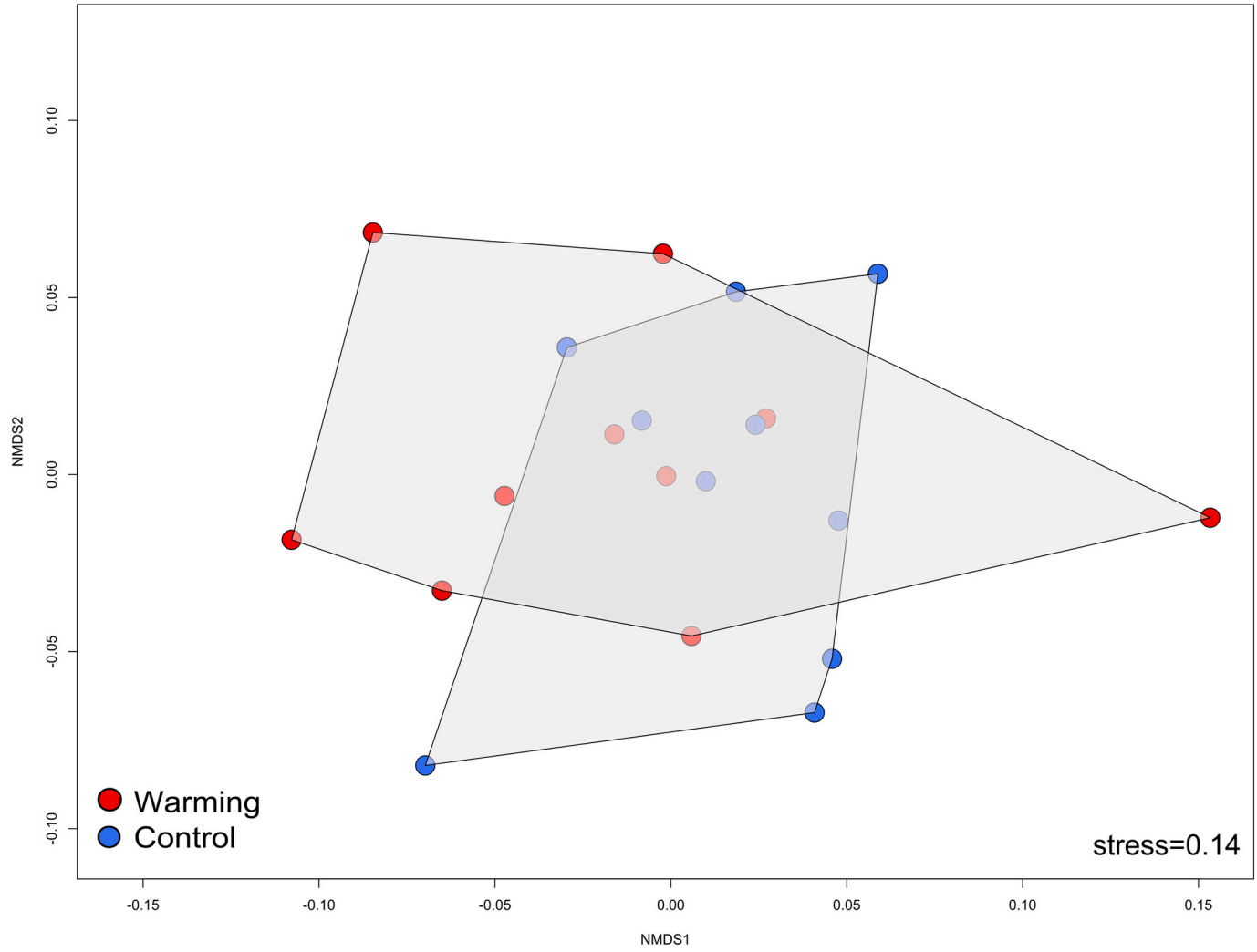

Fig. 2. Non-metric Multidimensional Scaling (NMDS) ordination plot of differences (Euclidean distance) in enzyme activity patterns between warming (red circles) and control plots (blue circles).

were examined individually (Table 1), OTC plots tended to have increased activity of the majority of both hydrolytic and oxidative enzymes compared to the controls, but these differences were not statistically significant. For example, the measured activity of *β-glucosidase* was 5.78 ± 2.42 (Ug^{-1}) in control plots and 9.34 ± 7.24 (Ug^{-1}) in warmed plots ($p = 0.16$).

3.2. Bacterial communities

A total of 492,944 bacterial raw reads was obtained (Supplementary Materials **Table 2S**, for more details regarding the number of reads used for the analysis). The reads were clustered into 2407 ASVs, assigned to 36 different bacterial phyla. Alpha-diversity and evenness tended to decrease under warming, as highlighted by lower Richness, Shannon and Simpson indices values (Table 1S, Fig. 1S).

Partial RDA showed that bacterial community composition was not affected by warming (PERMANOVA, $R^2 = 0.04$, $F = 0.80$, $p < 0.777$) (Fig. 3a, Fig. 1S).

The most abundant bacterial phyla (Fig. 4a), for both OTCs and control plots, were *Proteobacteria* (~29 %), *Actinobacteriota* (~26 %), and *Acidobacteriota* (~15 %). The most abundant orders were *Burkholderiales* (~12 %) and *Rhizobiales* (~11 %). DA analysis of the bacterial community at different taxonomic levels did not identify any taxa as significantly affected by warming (data not shown). Relative abundance of the 30 most abundant bacterial ASVs from warming and control plots were reported in **Table 3S**.

3.3. Fungal communities

A total of 738,755 fungal raw reads was obtained (see **Table 4S** Supplementary Materials for more details regarding the number of reads used for the analysis). Reads were clustered into 833 OTUs, assigned to 10 different fungal phyla. Opposite to bacteria, the fungal alpha-diversity and evenness tended to increase in OTC samples compared to control plots (Table 1S, Fig. 1S). Partial RDA showed a shift in the fungal community composition, suggesting a response to warming (Fig. 3b, Fig. 1S), with treatment being significant as a fixed explanatory factor (PERMANOVA, $R^2 = 0.08$, $F = 1.58$, $p < 0.002$). Additionally, community composition in warmed plots was significantly correlated with higher values of water, C, N, and TOC contents.

At phylum level (Fig. 4b), *Basidiomycota* (~63 %) dominated the community in both control and OTCs plots, followed by *Ascomycota* (~34 %) and *Mortierellomycota* (~2 %). *Ascomycota* tended to increase under warming (~39 %) compared to controls (~29 %). The most abundant fungal order was *Agaricales* (~25 %), followed by *Russulales* (~14 %), *Sebacinales* (~12 %), *Thelephorales* (~8 %), and *Helotiales* (~8 %) (Fig. 4b). Except for *Helotiales*, which belong to the *Ascomycota* phylum, the listed orders belong to the phylum *Basidiomycota*. Among

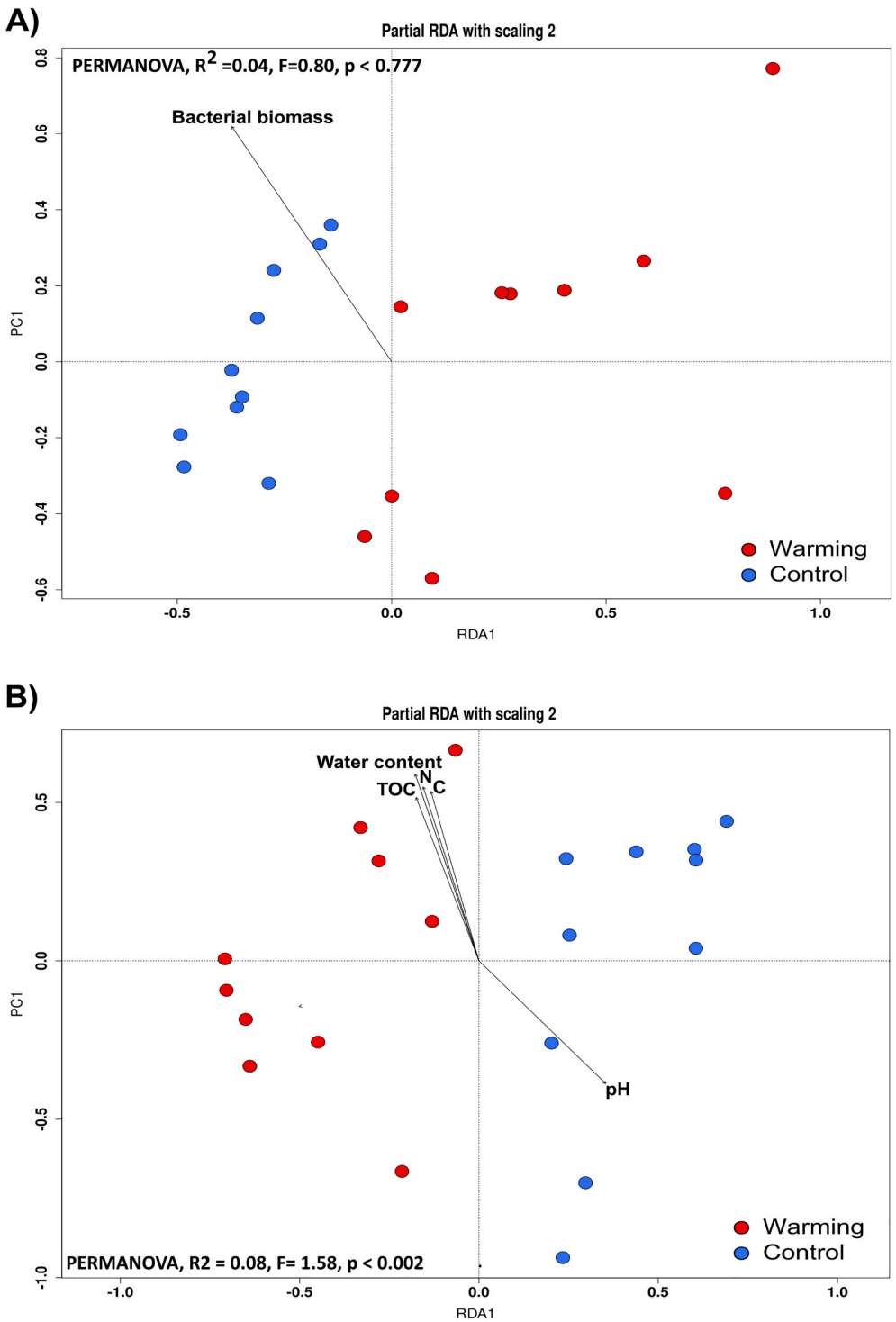

Fig. 3. Partial redundancy analysis (*pRDA*) biplots of bacterial (A) and fungal (B) beta-diversity with treatment (control or warming) used as covariate. Only significant edaphic variables and microbial biomass ($p < 0.05$) have been fitted in the ordinations. TOC = Total Organic Carbon; C = Carbon content; N = Nitrogen content.

them, *Agaricales*, *Thelephorales*, and *Helotiales* tended to increase under warming compared to controls. Relative abundance of fungal functional groups did not significantly ($p < 0.05$) differ between controls and OTCs. Relative abundances of the 30 most abundant fungal ASVs from warming and control plots are reported in **Table 5S**.

Ectomycorrhizal fungi were the most abundant and homogeneous group in both OTC and control plots (~27 %), while root endophytes and saprotrophs tended to increase in OTCs (Fig. 4c, Fig. 1S). DA analysis of the fungal community at different taxonomic levels showed a significant impact on several fungal taxa which increased their abundance in response to warming. In particular, order *Eurotiales* ($p = 7.45E-04$), genera *Penicillium* ($p = 6.36E-04$) and *Cladosporium* ($p = 6.01E-10$), and two ASVs belonging to the *Penicillium* genus (ASV1, $p = 6.31E-06$ and ASV2, $p = 9.50E-05$) were found to have increased in abundance (Fig. 5, Fig. 1S). Spearman's correlation between the above-mentioned fungal taxa and soil properties, microbial biomass (qPCR), and EEAs

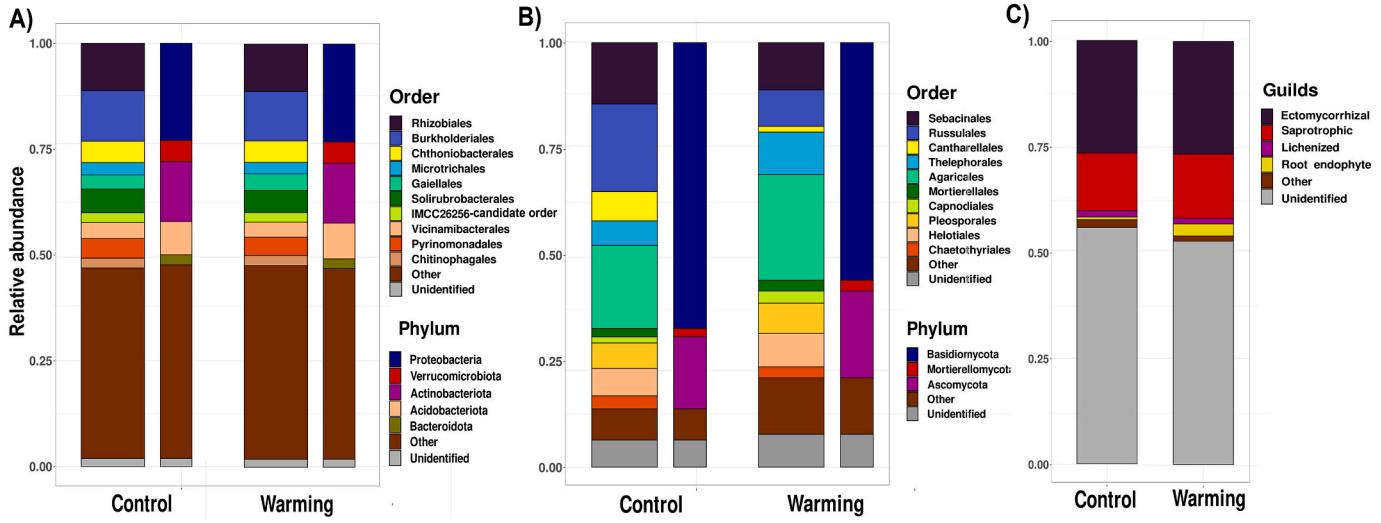

Fig. 4. Relative abundance of bacterial (A) and fungal (B) taxa in warming and control plots at phylum and order levels. Fungal functional guilds relative abundances (C) in warming and control plots. The data represent the mean abundance for treatment type (n = 10).

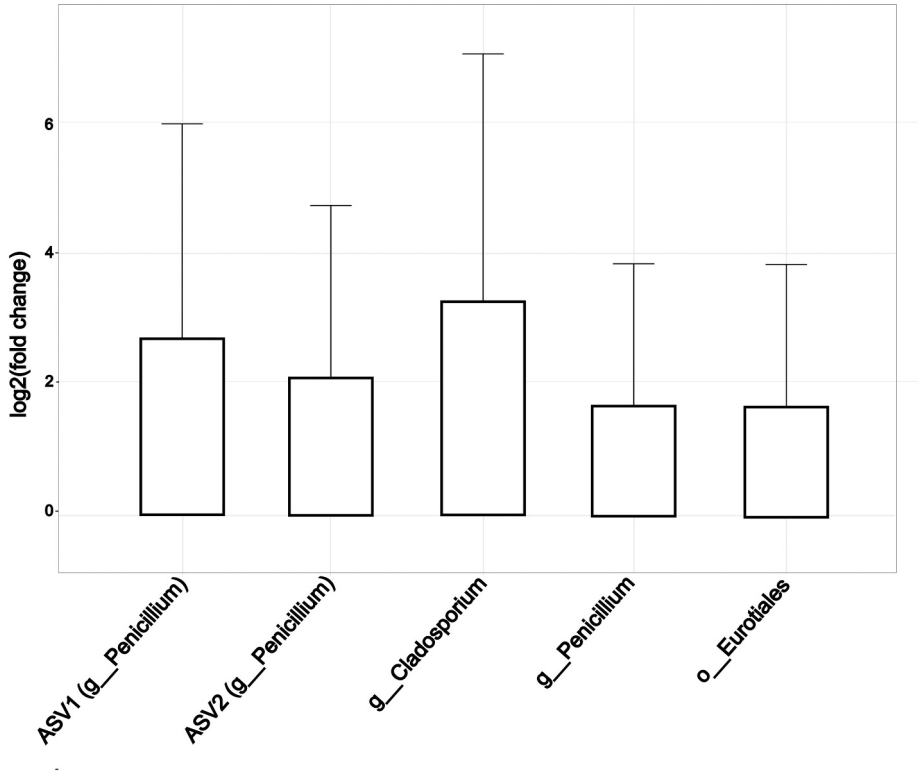

Fig. 5. Differential abundance of fungal taxa (log2 fold change) with statistically significant difference between warming and controls (ANCOM-BC, $p < 0.05$). Positive values represent taxa that are significantly more abundant in warming-treated samples compared to controls. g_ = genus level; o_ = order level.

showed that *Eurotiales*, as well as *Penicillium* and *Cladosporium*, were significantly correlated with ergosterol content and negatively correlated with *cellulase* activity (Fig. 6, Fig. 1S). All taxa tended to be positively correlated with oxidative enzymes (*Mn peroxidase* and *oxidase*), but not with hydrolytic enzymes.

4. Discussion

The current study is one of the first to combine the analysis of soil bacterial and fungal communities, enzyme activity, and microbial biomass in response to two decades of experimental warming associated with the dwarf shrub *D. octopetala*. Given its wide distribution in Arctic-alpine tundra, *D. octopetala* is significant in climate change research and an essential component in the studies of Arctic phylo- and bio-geography (Skrede et al., 2006), landscape ecology (Eichel et al., 2016, 2017), and microbial community ecology (Bjorbækmo et al., 2010; Brunner et al., 2017). *D. octopetala* stands out for its dominance in alpine and Arctic heaths and its adaptation to low soil moisture, nutrient availability, and low soil and air temperatures. Previous studies have also reported the resistance of alpine and Arctic *Dryas* to long-term warming and related this to the relatively dry environment (Hasvik, 2018, Jonsdottir et al., 2023). Our study showed no effects of long-term warming on any of the

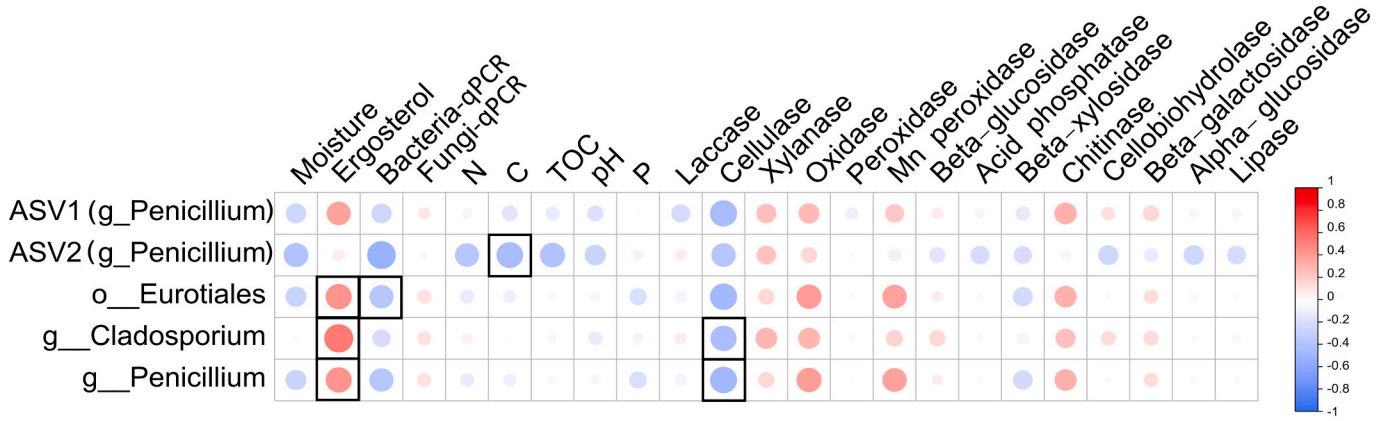

Fig. 6. Spearman correlations of differential abundance taxa with soil properties, microbial biomass and extracellular enzymes. The colours and sizes of circles denote the signs and the levels of significance of the correlations, respectively; marked squares indicate significant correlations ($p < 0.05$). g_ = genus level; o_ = order level.

associated soil parameters. This is in line with previous studies that did not observe any changes in soil chemical parameters after three years of warming in an alpine grassland on the Qinghai-Tibet Plateau (Ding et al., 2019) and after 13 years of warming in an upland Atlantic shrubland dominated by *Calluna vulgaris* (Domínguez et al., 2017). Thus, we hypothesize that the remarkable resilience of the *D. octopetala* heath community to long-term warming is somehow linked to the stability of the soil chemical parameters.

The *Dryas*-associated microbial community composition and activity only marginally responded to long-term warming. Our results showed that long-term warming significantly affected the fungal community composition, but had no significant effect on bacteria. The processes driven by fungi that decompose organic matter and by other essential mycorrhizal symbionts of woody plants are intricately connected to the composition, activity, and diversity of fungal communities (Ehrlich, 2006; Clemmensen et al., 2015). These processes are anticipated to be sensitive to alterations in both biomass and relative proportions of fungal functional groups, which normally arise in response to environmental changes (Solly et al., 2017). Although fungal richness and evenness tended to increase in response to warming, we observed decreasing trends in bacterial richness and evenness. These findings may suggest that bacterial communities respond to simulated warming by increasing their degree of specialisation, resulting in a community dominated by a few abundant species and a moderate occurrence of rare species. This may diminish functional redundancy and compromise the overall stability of the soil ecosystem in case of further perturbations (Deslippe et al., 2012; Walters and Martiny, 2020; Sannino et al., 2022). On the other hand, the tendence of fungal diversity to increase, and particularly that of their saprotrophic members, suggests that alpine fungal communities may develop enhanced degradation capacities under warming conditions (Newsham et al., 2022).

The most abundant bacterial phyla were *Proteobacteria*, *Actinobacteriota,* and *Acidobacteriota*. Bacterial taxa belonging to *Proteobacteria* are commonly considered copiotrophic organisms, with their abundance typically increasing after fertilization experiments, likely due to greater organic matter input from vascular plants (Ramirez et al., 2010; Koyama et al., 2014).

Actinobacteriota, usually associated with plant roots, may play a crucial role in regulating the decomposition and synthesis of SOM, thereby significantly improve the fertility of soils in harsh environments (Liu et al., 2017). *Acidobacteriota*, on the other hand, are generally oligotrophic with low growth rates. They possess anabolic capabilities, efficiently converting nutrients into biomass, exhibiting remarkable adaptability to nutrient-poor conditions, thus contribute to ecosystem stability (Canini et al., 2020).

The lack of a detectable effect of warming on the bacterial taxonomic composition is consistent with the findings of Gao et al. (2021), who examined the impact of long-term warming (14 years) on the bacterial taxonomic composition. They attributed the resistance of bacterial communities to warming treatments to several mechanisms, including the smaller magnitude of warming at the time of peak plant biomass compared with the intra-annual fluctuations in temperature. However, the fungal community's response has not yet been investigated.

With respect to the fungal kingdom, *Ascomycota* is usually the dominant phylum in soils worldwide and expresses a high number of genes related to stress tolerance, competitive abilities, and resource uptake, allowing members of this group to colonise a wide range of environments (Egidi et al., 2019). However, species belonging to *Basidiomycota* are frequently detected in association with *D. octopetala* in Arctic and alpine environments. This was also observed at our study site, where *Basidiomycota* clearly dominated the fungal community. This diverse fungal phylum includes numerous mycorrhizal species that are involved in symbiotic relationships. Mycorrhizal associations are particularly beneficial to plants under low nutrient conditions as they facilitate nutrient uptake, especially essential elements such as P and N (Ryberg et al., 2009; Bjorbækmo et al., 2010; Geml et al., 2012).

The taxonomic groups that were most frequently detected in this study, were basidiomycetous ectomycorrhizal (ECM) genera that are known to produce an extensive external mycelium, including species such as *Sebacina (Sebacianales), Inocybe (Agaricales)*, *Tomentella (Thelephorales*), and *Cortinarius* (*Agaricales*) (Bjorbækmo et al., 2010; Wang and Han, 2022). We observed an increase in the abundance of *Thelephorales* under warming conditions. Indeed, components of this group generally have melanized hyphal cell walls, which are often associated with adaptation to high temperatures and drought (Bjorbækmo et al., 2010), which can occur inside OTCs during warm and dry summers.

We found significant changes in the abundance of some *Ascomycota* taxa, which is consistent with Semenova et al. (2015), who found effects of long-term (18 years) warming on community composition and richness of soil ascomycetes in dry and moist tundra. The abundance of the order *Eurotiales*, which encompasses highly sporulating and stress-tolerant species (Dijksterhuis et al., 2018), as well as the saprotrophic ascomycetous genera *Penicillium* (*Eurotiales*) and *Cladosporium* (Capnodiales), significantly increased in the warming treatment. The positive correlation observed between the increased abundance of *Ascomycota* taxa and both fungal biomass and oxidative enzyme activity, coupled with their negative correlation with *cellulase* activity, suggests a pivotal role for these *Ascomycota* taxa in enhancing the capacity of alpine ecosystems to decompose organic matter and cycle nutrients, thereby influencing ecosystem functioning. As suggested by Crowther and Bradford (2013), these correlations could be linked to alterations in soil C dynamics, potentially resulting in soil C losses and a decline in the

abundance of recalcitrant C compounds (Six et al., 2006; Amelung et al., 2008; Feng et al., 2007; Deslippe et al., 2012; D'Alò et al., 2022).Indeed, previous studies showing that experimental warming can increase fungal biomass, given that fungi act as a significant source for the production of oxidative enzymes (De Gonzalo et al., 2016; Kinnunen et al., 2017). The initial depletion of readily hydrolysable substrates under warming can constrain *cellulase* activity, prompting a shift towards utilizing more recalcitrant C pools to sustain microbial metabolic activity through oxidative enzymes (Chen et al., 2018).

Additionally, concerning the functionality of fungal taxa, warming tended to increase the abundance of root endophytes and saprotrophs. The increased abundance of saprotrophic fungi suggests that rising temperatures may decrease the relative proportions of fungal mutualists and free-living decomposers, thereby accelerating soil C loss (Talbot et al., 2008; Phillips et al., 2013; Van Nuland et al., 2020).

5. Conclusion

In the studied alpine heath, where *D. octopetala* demonstrated notable resistance to prolonged warming, we found no significant differences in soil physico-chemical parameters in response to increasing temperatures. Consequently, only minor changes occurred in the associated microbial communities. However, warming affected the fungal community composition, which was mainly dominated by the ECM *Basidiomycota*. A significant shift in *Ascomycota* taxa was observed between the warmed and control plots. Their positive correlations with oxidative enzymes and fungal biomass suggest that rising temperatures may result in a soil microbial community that produces more oxidative enzymes that primarily utilise recalcitrant C. This may result in an increase in CO_2 flux into the atmosphere and a decrease in ecosystem C storage. However, two decades of warming did not have any effect on bacterial communities, despite a tendency in reduced alpha diversity and increased degree of specialisation. This trend may lead to an increase in bacterial vulnerability in a warmer world, which should be explored further.

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CRediT authorship contribution statement

Federica D'Alò: Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Gabriele Tosadori:** Writing – review & editing, Visualization, Methodology, Investigation. **Laura Zucconi:** Writing – original draft, Supervision, Conceptualization. **Silvano Onofri:** Writing – review & editing, Supervision. **Fabiana Canini:** Writing – review & editing, Validation. **Ruben E. Roos:** Writing – review & editing, Methodology. **Kari Klanderud:** Writing – review & editing, Methodology. **Jana Voříšková:** Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: D Alo F. reports financial support was provided by under the European Union H2020 Grant Agreement. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All raw sequences have been submitted to the NCBI Sequence Read Archive (SRA) database under the BioProject number PRJNA1008075.

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