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Root exudates increase soil respiration and alter microbial community structure in alpine permafrost and active layer soils

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Original - Significance Statement

It is of great importance to assess how belowground microbial communities respond to climate change due to their key role in soil carbon dynamics. The findings here are highly relevant in that increased plant growth in alpine areas due to climate warming will increase carbon and nutrient inputs to the soil, which may stimulate respiration and carbon loss in permafrost. The novelty of the present research is in demonstrating how microbial communities respond to such inputs in high alpine ecosystems.

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Summary

Due to climate warming alpine ecosystems are changing rapidly. Ongoing upward migrations of plants and thus an increase of easily decomposable substrates will strongly affect the soil microbiome. To understand how belowground communities will respond to such changes, we set up an incubation experiment with permafrost and active soil layers from northern (NW) and southern (SE) slopes of a mountain ridge on Muot da Barba Peider in the Swiss Alps and incubated them with or without artificial root exudates (AREs) at two temperatures, 4°C or 15°C. The addition of AREs resulted in elevated respiration across all soil types. Bacterial and fungal alpha diversity decreased significantly, coinciding with strong shifts in microbial community structure in ARE-treated soils. These shifts in bacterial community structure were driven by an increased abundance of fast-growing copiotrophic taxa. Fungal communities were predominantly affected by AREs in SE active layer soils and shifted towards fast-growing opportunistic yeast. In contrast, in the colder NW facing active layer and permafrost soils fungal communities were more influenced by temperature changes. These findings demonstrate the sensitivity of soil microbial communities in high alpine ecosystems to climate change and how shifts in these communities may lead to functional changes impacting biogeochemical processes.

Introduction

High mountain systems such as the European Alps are particularly vulnerable to human-induced climate change. Mean annual temperatures increased by about 2°C since the late 19th century until the end of the 20th century in the European Alps (Auer *et al.*, 2007) and are projected to rise further by 2 – 4°C by the middle of this century (CH2018, 2018).

Although alpine environments provide harsh conditions for life, such as high rates of precipitation and UV radiation, and large fluctuations of solar radiation and temperatures (Donhauser and Frey, 2018), high-alpine soils harbour a diverse and largely unknown microbial life that is able to thrive and metabolise (Rime *et al.*, 2015; Frey *et al.*, 2016). Permafrost regions occur at extreme latitudes, but also frequently found at high elevations and lower latitudes such as the European Alps (Donhauser and Frey, 2018). Permafrost is defined as ground material that remains below 0°C for two or more consecutive years and is overlain by seasonally unfrozen soil called the active layer (Margesin, 2009). Warming-induced thawing of permafrost promotes microbial activity, often resulting in enhanced greenhouse gas (CO_2, CH_4, N_2O) emissions due to enhanced microbial activity (Schuur *et al.*, 2015).

Evidence from various mountain regions across the world shows that as global temperatures are rising, plants are migrating upwards (Pauli *et al.*, 2007, 2012; Steinbauer *et al.*, 2018), a phenomenon which has been shown to correlate with disappearing alpine permafrost (Parolo and Rossi, 2008). This upward migration of plants leads to increased primary production and therefore will influence the carbon stock in high alpine soils (Hagedorn *et al.*, 2010). An increase in plant-derived material will likely affect the microbial communities belowground, as microbial communities are often closely linked to alpine plants (Peay *et al.*, 2013; Yashiro *et al.*, 2016). This becomes particularly apparent at higher elevations and in nutrient-poor soils (Porazinska *et al.*, 2018; Adamczyk *et al.*, 2019). In addition, it was suggested that deep plant roots will invade newly thawed permafrost (Blume-Werry *et al.*, 2019) and interact with the detached permafrost microorganisms which are no longer detached from plant processes upon thaw. The secretion of root exudates may lead to the stimulation of microbial activity resulting in enhanced mineralisation of organic material stored in permafrost, thus

likely leading to a soil carbon loss. This process is known as the priming effect (Fontaine *et al.*, 2003, 2007; Pegoraro *et al.*, 2019).

An immediate consequence of rising temperatures and the advancement of plants to higher elevations will be the increased nutrient availability in the form of low molecular weight carbon (LMW-C) compounds to the normally oligotrophic soils in alpine regions. These LMW-C compounds are released into the soil through root exudation and via leaching of litter, and can be readily metabolised by soil microbial communities (Eilers *et al.*, 2010). Studies have shown that an increase in readily available carbon leads to shifts in taxonomic and functional traits of soil microbial communities (Cheng and Kuzyakov, 2005; Eilers *et al.*, 2010; Koyama *et al.*, 2014; Ridl *et al.*, 2016; Adamczyk *et al.*, 2020). Copiotrophic traits such as low resource efficiency, fast growth and metabolic versatility allow microorganisms to respond quickly to an increase in labile substrates and be more resilient to disturbances. In contrast, microbes with an oligotrophic lifestyle are better adapted to nutrient-poor environments as they exhibit slower growth, higher resource efficiency and more stress tolerance allowing them to be more resistant to climate change related disturbances (Koch, 2001; Fierer *et al.*, 2007; Lauro *et al.*, 2009; De Vries and Shade, 2013; Donhauser *et al.*, 2020). Further research suggests that at a coarse scale, microbial groups function differently in decomposition processes. Bacteria use predominantly labile substrates, whereas fungi are better adapted to utilise more complex organic material (Wardle *et al.*, 2004; De Graaff *et al.*, 2010). This is further associated with longer turnover times of fungi compared with bacteria (Rousk and Bååth, 2011).

In the present study we explore the effects of increased LMW-C compounds in the form of root exudates on microbial communities in active layer and permafrost soils from a mountain ridge in Switzerland. In doing so, we aim to gain a better understanding of how microbial communities will respond to elevated carbon and nutrient availability and, more generally, how alpine soil ecosystems will be affected by a warming climate. As the soil microbiome plays an integral role in soil ecosystem functioning, such as transformation and mineralisation of SOM, we hypothesise that in response to increased inputs of LMW-C compounds, microbial activity and thus respiration rates will increase

considerably. Furthermore, we predict that microbial community structure, particularly bacterial, will change markedly, shifting towards fast-growing copiotrophic taxa.

To mimic the photosynthetic products that are normally secreted by plants through their roots, we performed an incubation experiment using artificial root exudates (AREs) comprising simple carbohydrates, amino acids and organic acids. We performed a mesocosm experiment under controlled conditions with three different soils from a high-alpine mountain ridge on Muot da Barba Peider in the Swiss Alps (Frey *et al*., 2016). Soils were taken from the active layer of colder north-facing (NW) and warmer south-facing slopes (SE) as well as from 12,000-year-old permafrost (PF) at a soil depth of 160 cm. After an acclimatisation period to either 4°C or 15°C, AREs were periodically applied to three soil types over one month. Microbial communities were analysed by sequencing bacterial 16S rRNA and fungal ITS amplicons using the Illumina MiSeq platform.

Results

Changes in soil respiration after the addition of AREs

During the acclimatisation period we observed at the onset of the measurements a spike in respiration followed by an overall decrease over time (Fig. 1). We observed the highest respiration rates in south-east (SE) facing active layer soils (range: 0.006 - 0.116 µmol C-CO_2 g^{-1} d^{-1}), followed by north-west (NW) facing active layer soils (0 - 0.097 µmol C-CO_2 g^{-1} d^{-1}) and the lowest for permafrost (PF) soils (0 - 0.01 µmol C-CO_2 g^{-1} d^{-1}). In general, soils incubated at 15°C exhibited a higher respiration rate than soils incubated at 4°C. Respiration rates were strongly influenced by soil type ($F_{(2, 522)} = 69.4$, $p < 0.001$) and to a lesser extent by temperature ($F_{(1, 522)} = 4.8$, $p < 0.05$; Table S1). However, the interaction of these two factors significantly contributed more to the respiration ($F_{(2, 522)} = 14.9$, $p < 0.001$) than temperature alone (Table S1).

After the temperature acclimatisation, AREs were added continuously to the soils. In all ARE-treated soils, mean respiration rates increased strongly compared to control samples (Fig. 1). We observed the greatest relative changes in respiration rates between ARE-treated and control soils in PF soils that

were incubated at 15°C (562-fold; ARE: ≤ 0.6 µmol C-CO_2 g^{-1} d^{-1}; Control: ≤ 0.008 µmol C-CO_2 g^{-1} d^{-1}) followed by 4°C incubated PF soils (272-fold; ARE: ≤ 0.32 µmol C-CO_2 g^{-1} d^{-1}; Control: ≤ 0.005 µmol C-CO_2 g^{-1} d^{-1}). Compared with the active layer soils, these soils exhibited a delayed response to ARE treatment, in particular those incubated at 4°C. ARE-treated active layer NW soils incubated at 4°C had a 92-fold increase in respiration rates in comparison to control soils, whereas in 15°C incubated NW soils, the increase was 52-fold. We found the smallest relative changes in respiration rates of ARE-treated and control soils among the active layer SE soils, 18-fold at 4°C (ARE: ≤ 0.79 µmol C-CO_2 g^{-1} d^{-1}; Control: ≤ 0.03 µmol C-CO_2 g^{-1} d^{-1}) and 28-fold at 15°C (ARE: ≤ 1.59 µmol C-CO_2 g^{-1} d^{-1}; Control: ≤ 0.05 µmol C-CO_2 g^{-1} d^{-1}), respectively. Over the measured time period, the driver that by far contributed the most to respiration rates was the artificial root exudate treatment ($F_{(1, 420)}$ = 460.1, $p < 0.001$; Table S1). Soil type as well as temperature still remained significant factors driving the respiration rates, however the interaction of soil type and temperature did not contribute significantly ($F_{(2, 420)}$ = 1.1, $p > 0.05$; Table S1).

Microbial substrate utilisation

We further tested if microbial communities of the different soil types and treatments had different substrate utilisation. There was a clear trend of soil microbial communities with ARE addition utilised more carbon sources (Fig. S1). Cyclodextrin, phenylethylamine and glycyl-L-glutamic acid have only been assimilated by PF microbial communities indicating a different substrate utilisation pattern with ARE compared to NW and SE soils. In contrast, SE control microbial communities used the least substrates.

Influence of AREs on microbial abundance

Overall, we observed an increase of total DNA content as well as 16S and ITS copy numbers in ARE-treated soils compared to control soils in all three soil types (Fig. 2). Furthermore, we found that the abundance of fungi relative to bacteria (F:B ratio) increased about 8-fold with the addition of AREs (control F:B ratio = 0.15; ARE F:B ratio = 1.31).

ANOVA was used to test to what extent the observed changes in DNA and gene copy numbers were driven by the addition of AREs, temperature and their interaction (Table 1). DNA content was significantly affected in both active layers SE and NW by AREs, and for the latter also by temperature and the interaction between ARE and temperature. For PF soils we did not observe a significant relationship in the increase of DNA content and the different treatments. Bacterial 16S copy numbers in all soil types were significantly related to ARE treatment, and in SE and PF soils also to temperature. Similarly, fungal ITS copy numbers exhibited a significant statistical relationship with ARE treatment in both active layer soils NW and SE. For PF soils we were not able to examine the relationship between ITS copy numbers and ARE and temperature treatment as ITS copy numbers for control soils were below the detection limit of the instrument (2.7×10^3 ITS copy numbers). Incubation temperature influenced ITS copy numbers significantly only in SE soils.

Changes in microbial alpha diversity

ARE treatment significantly influenced bacterial alpha diversity, whereas temperature alone or the interaction of the two factors had little or no influence (Table 2). Both active layer soils, NW and SE, exhibited a decrease in bacterial richness and to a lesser extent in bacterial Shannon diversity upon ARE treatment (Fig. 3A, 3C). PF soils were least affected by the addition of AREs - we only observed a significant decrease in Shannon diversity.

Fungi exhibited similar patterns between richness and Shannon diversity (Fig. 3B, 3D). Richness and Shannon diversity were significantly affected by ARE treatment in all soils (Table 2). We observed a decline in fungal alpha diversity in both active layers (Fig. 3B, 3D). In PF soils fungal richness and Shannon diversity were only affected in 15°C-incubated soils by ARE treatment, however, instead of a decline, these soils exhibited an increase of both fungal richness and Shannon diversity (Fig. 3B, 3D; Table 2). Temperature alone had a particularly large impact on Shannon diversity in the colder NW-facing active layer and PF soils (Table 2).

Shifts in microbial community structure

PERMANOVA analysis revealed that differences in microbial beta diversity were predominantly attributed to soil type (bacteria: 48.9%; fungi: 45.2%; Table S2), confirmed by principle coordinate analysis (PCoA) based on the Bray-Curtis dissimilarities (Fig. 3E and 3F). For bacterial communities, the PCoA also depicted a clear clustering based on treatment (ARE vs control), in particular for both active layer soils NW and SE. In fact, for active layer soils, the highest percentage of total variation in bacterial beta diversity was explained by ARE treatment (49.4% NW; 36.9% SE; Table 3) for individually analysed soils. Additionally, temperature contributed to the variation in the bacterial community structure in both active layer soils to a similar extent (19.2% NW; 18% SE). In PF soils, shifts in bacterial beta diversity could be attributed almost evenly to ARE treatment (13.6%), temperature (14.9%) and the interaction of the two (14.5%).

In contrast to bacteria, AREs most strongly influenced fungal communities in the active layer SE, explaining 65.4% of the total variation in beta diversity (Table 3). For the active layer NW, we found temperature primarily contributing to changes in fungal community structure (44.5%), whereas only 29% were attributed to AREs. In PF soils the interaction of ARE and temperature mainly influenced fungal communities (27.5%), and to a lesser extent by AREs alone (22%). However, we could observe a clear separation between control and ARE-treated PF soils (Fig. 3F).

Differential abundance of the most dominant microbial phyla in response to AREs

To examine the observed shifts in microbial community structure in more detail, we analysed the differential abundance of microbial phyla in response to AREs. For this, we calculated their $\log_2$ ratios in control and ARE-treated soils (Fig. 4A-C and 5A-C). The bacterial phyla Actinobacteria, Bacteroidetes and Proteobacteria exhibited positive $\log_2$ fold changes in response to AREs in both active layer soils NW and SE (Fig. 4A, B). In contrast, the phylum Patescibacteria showed opposite responses to AREs in these soils - a positive $\log_2$ fold change in SE and a negative $\log_2$ fold change in NW soils. In the same soils Acidobacteria, Planctomycetes, Gemmatimonadetes and WPS-2 (or Eremiobacterota) had small but negative $\log_2$ fold changes. In PF soils only significant negative $\log_2$ changes were observed for Cyanobacteria, WPS-2, Planctomycetes and Chloroflexi (Fig. 4C).

Within the fungal kingdom (Fig. 5A-C), the majority of phyla that demonstrated a significant response were in the warmer SE-facing active layer. The phyla Ascomycota, Mucoromycota and Basidiomycota all exhibited positive $\log_2$ fold change in response to AREs. In NW soils the only phylum responding to ARE treatment was Mortierellomycota, which exhibited a large negative $\log_2$ change and in PF soils Mucoromycota had a slight positive $\log_2$ fold change.

Differential abundance of the most dominant microbial genera in response to AREs

As bacterial and fungal phyla consist of various heterogeneous groups, we also investigated the changes in the differential abundance of the ten most common bacterial and fungal genera in response to ARE treatment among the three soil types (Fig. 4D, E and 5D-F). For bacteria, the majority of genera in all soils that predominantly had positive $\log_2$ fold changes upon ARE treatment, belonged to the phylum Proteobacteria (Fig. 4D, E). Within this phylum, the genus *Undibacterium* exhibited the strongest positive response to AREs in SE soils. In NW soils, *Brevundimonas* showed the largest positive $\log_2$ fold change. The genus *Massilia* exhibited an increase in both active layer soils. The abundance of genera of the phylum Actinobacteria such as *Pseudarthrobacter* (SE and NW soil), *Mycobacterium* (SE soil) *Cryobacterium*, *Paenarthrobacter* and *Phyciococcus* (all in NW soils) increased. *Pedobacter*, a genus belonging the phylum Bacteroidetes, exhibited a large $\log_2$ fold change in response to AREs. In fact, in SE soils the ten most abundant genera all showed a significant positive $\log_2$ fold change. We observed no significant responses of individual genera to AREs in PF soils.

Within the fungal kingdom, the majority of genera that exhibited either positive or negative $\log_2$ fold changes were members of the phyla Ascomycota and Basidiomycota (Fig. 5D-F). Genera belonging to the phylum Basidiomycota (*Mrakia*, *Soliccozyma*, *Goffeauzyma*, and *Rhodotorula*) only had positive $\log_2$ fold changes in response to AREs in SE soils, whereas all those belonging to Ascomycota (*Coniochaeta*, *Neobulgaria*, *Tetracladium*) exhibited negative $\log_2$ changes in the same soil, apart from the abundant *Pseudogymnoascus*. In NW soils, genera mainly decreased in abundance in response to AREs, of which the majority belong to the phylum Ascomycota such as *Mycosphaerella*, *Polysporina*, *Acarospora*, *Verrucaria* and *Pseudogymnoascus*. In contrast, in PF soils the majority of

ascomycete genera increased, with psychrophilic *Rhynchosporium* exhibiting a particularly strong increase in abundance. The basidiomycete genus *Naganishia* exhibited a large increase in response to AREs in PF soils. We further found the genus *Mortierella*, a member of the phylum Mortierellomycota, becoming significantly less abundant in the active layer NW upon treatment with AREs and two genera belonging to Mucoromycota, *Umbelopsis* (in SE soils) and *Mucor* (in PF soils), increasing.

Discussion

Root exudates lead to greater microbial abundance and respiration

Overall, permafrost soils showed lowest absolute respiration rates compared to active layer soils which can be attributed to labile carbon pool depletion in permafrost soils similar as in another study with the same permafrost soil (Lulakova *et al*., 2019). As anticipated, we found that the addition of AREs to the soils led to highly increased respiration rates. PF soils were found to have the lowest average respiration rate and active layer SE the highest. These differing responses of the soil types are likely due to the overall higher total DNA content in the warmer SE soils compared with NW or PF soils. However, the relative change of respiration between ARE-treated and control soils was much higher for permafrost than both active layer soils, presumably because PF soils exhibit conditions of higher carbon starvation and energy limitation for microorganisms (Fontaine *et al.*, 2007) compared with the active layer. The physico-chemical properties of soils from the same site have been previously described by Frey et al. (2016), who found that, in direct contrast to PF soils, the active layer SE contained the highest carbon and nitrogen contents as well as the lowest pH and mineral nutrients.

In PF soils with ARE and elevated temperature (15°C) there was a pronounced peak in respiration during initial stage of the ARE application. Exudate-induced soil C loss is commonly attributed to a priming effect that is, a short-term increase in microbial mineralization of native soil C as a result of fresh carbon inputs to the soil (Kuzyakov *et al*., 2000). Exudates may enhance the accessibility of C previously protected in mineral–organic associations and microorganisms are alleviated from energy

limitation and increase decomposition activity (Keiluweit *et al*., 2015). At a later stage, however, respiration rates decreased over time, likely due to the depletion of N, P and other nutrients in these nutrient-poor PF soils (Blagodatskaya and Kuzyakov, 2008). Rapidly increasing respiration rates after the amendment of labile carbon sources with further decrease over time have similarly been observed in arctic permafrost (Adamczyk *et al*., 2020) and in nutrient-poor soils of an alpine glacier forefield (Zumsteg *et al*., 2013; Rime *et al*., 2016b). The rapid increase in heterotrophic respiration might be the result of an increase in opportunistic microorganisms that are specialised in decomposing readily available substrates, as previously reported by De Graaff *et al.* (2010). Hence, constant inputs of easily available carbon in the form of root exudates and via leaching of litter increase respiration, and will likely alter the belowground carbon dynamics. Such findings have already been shown for other ecosystems such as Arctic glacier forelands (Yoshitake *et al.*, 2007), Arctic tundra (Lynch *et al.*, 2018) and forests and grasslands (Eilers *et al.*, 2010), but not yet for high alpine environments. Furthermore, increased microbial activity could be the result of a priming effect, potentially leading to increased SOM decomposition (Fontaine *et al.*, 2007; Pegoraro *et al.*, 2019). This means that microbes readily utilise root exudates as an energy and carbon source to produce exoenzymes, helping to degrade SOM resulting in a potential carbon loss to the atmosphere (Schimel and Weintraub, 2003).

In response to AREs, we further found an increase in microbial abundance, with fungal abundance exhibiting a greater increase relative to bacterial abundance (F:B ratio). Some studies have suggested that bacterial groups are favoured by easily available substrates (Wardle *et al.*, 2002, 2004; De Graaff *et al.*, 2010), while others have also observed an increase in fungal growth in addition to bacterial growth (Bååth *et al.*, 1978; Meidute *et al.*, 2008). It has further been shown that fungal growth is closely correlated to changes in fungal biomass (Rousk and Bååth, 2007). The increase in fungi was proposed to be driven by fast-growing opportunistic fungi, such as yeasts (Meidute *et al.*, 2008). Fungi may use the added substrates more efficiently by incorporating them into biomass (Bailey *et al.*, 2002), which may explain the relative increase of fungal abundance compared with bacteria observed in the current study. Another potential explanation is that saprotrophic fungi were feeding on bacterial cell debris (Miltner *et al.*, 2012) due to a higher bacterial turnover (Wang et al., 2019). One could

further speculate that the higher F:B ratio was also the result of predation of bacteria (Rousk and Bååth, 2011), in particular protozoa grazing on slow-growing bacterial taxa (Schostag *et al.*, 2019).

Root exudates decrease microbial diversity and alter microbial community structure

The addition of AREs resulted in a decrease in bacterial alpha diversity in particular in both active layer soils. This drop in alpha diversity was further accompanied by significant shifts in bacterial beta diversity. The changes in bacterial alpha and beta diversity were likely the result of an expansion of fast-growing copiotrophic bacteria and the competitive exclusion of others (Fierer *et al.*, 2007; Adamczyk *et al*., 2020). Strong shifts in bacterial community structure due to readily available nutrients have been already reported in various studies (Brant *et al.*, 2006; Cleveland *et al.*, 2007; Eilers *et al.*, 2010; Goldfarb *et al.*, 2011). Similar to bacteria, fungal richness and Shannon diversity decreased with ARE addition in NW and SE soils. In NW soils, however, temperature had the strongest effect on fungal Shannon diversity leading to greater diversity in 15°C-incubated samples. Thus, rising temperatures might strongly alter fungal diversity, and in particular the fungal diversity in cold soils may increase as a first response to higher temperatures as was also shown in other terrestrial cryoenvironments (Deslippe *et al.*, 2012; Newsham *et al.*, 2016). In warmer active layer soils the decline of fungal richness in response to AREs suggests that fast-growing opportunistic fungi, like yeast, became more dominant as they were favoured by more nutrient-rich conditions (Meidute *et al.*, 2008). Coinciding with these findings, fungal beta diversity was strongly shaped by temperature in NW soils, whereas ARE addition mainly altered fungal communities in SE soils.

PF microbial communities were, in general, less responsive to elevated temperatures and ARE than those in NW and SE soils. We expected to find the opposite pattern because microbial communities in the active layer soils, in particular those from the SE exposed slope, experience plant input and larger temperature fluctuations under field conditions making these microbial communities more tolerant to elevated temperature and root exudates. In PF soils, however, bacterial communities were surprisingly resistant to increases in temperature and ARE. A similar pattern of diversity and community structure with elevated temperature (15°C-incubated samples) has been observed previously based on 16S

rRNA amplicon sequencing in other mountain soils (Donhauser *et al*., 2020). One possibility that permafrost soils responded to elevated temperature only weakly might be a deficiency of energy and nutrients available for enzymatic degradation. Capek *et al*., (2015) found that the degradation of autochthonous soil carbon by the permafrost microbiome was not sufficient to induce an increase in the microbial biomass of Siberian permafrost.

Fungal alpha-diversity in PF soils was increased by the addition of ARE in 15°C-incubated soils compared to both active layer soils. Increased labile carbon inputs can trigger the activation of dormant microorganisms by alleviation of their carbon limitation (Blagodatskaya and Kuzyakov, 2013). Thus, the increase in fungal diversity in PF soils might be the result of reactivated dormant fungal spores in response to ARE and elevated soil temperature (Moore-Landecker, 2011; Lulakova *et al*., 2019) and/or of an increase of opportunistic fungal taxa with enhanced resource utilisation in response to ARE. Metatranscriptomic profiling also revealed that fungal activities quickly increased after permafrost thaw (Coolen and Orsi, 2015).

PF soils also contained a microbial community with a different substrate utilisation pattern than in both active layer soils. Interestingly, PF microbial communities were able to assimilate more carbon sources than SE microbial communities. We thus suggest that PF soils contained a highly metabolic versatile microbial community which is becoming active at elevated temperature which is in line with previous findings of permafrost soils (Ernakovich and Wallenstein, 2015; Lulakova *et al*., 2019). These taxonomic changes could be coupled to the increase in microbial C decomposition, as suggested in genomic surveys of Arctic and alpine soils undergoing warming (Coolen and Orsi, 2015; Feng *et al*., 2020; Donhauser *et al*., 2021).

Copiotrophic bacteria and yeast increase in response to root exudates

The ARE-stimulated CO_2 fluxes were accompanied by profound shifts in the abundance of certain members of the microbial community. We hypothesised that copiotrophic taxa would increase with the addition of AREs. However, the copiotrophic/oligotrophic framework has to be considered carefully to avoid misinterpretations, in particular at higher taxonomic levels, as physiological traits of

microorganisms are context-dependent (Senechkin *et al.*, 2010; Morrissey *et al.*, 2016; Hartmann *et al.*, 2017). We nevertheless apply this framework as it has been proven useful in describing general trends linking microbial structure and function in soil ecosystems (Ali *et al.*, 2018; Schostag *et al.*, 2019).

The increased abundance in SE- and NW-facing active layer soils of the phyla Proteobacteria, Bacteroidetes and Actinobacteria in our study can be attributed to a mainly copiotrophic lifestyle (Fierer *et al.*, 2007, 2012). However, it is important to note that not all members of the aforementioned phyla are strict copiotrophs (Ho *et al.*, 2017). Among Proteobacteria, the genus *Brevundimonas* showed the greatest increase in response to AREs in the colder NW soils. *Brevundimonas* has been found in cold deserts of the north-western Himalayas and has been characterised to consist of psychrotrophic bacteria with plant growth promoting attributes at low temperatures (Yadav *et al.*, 2015). This group might therefore aid the advancement of plants to higher elevations. The genus *Massilia* (Proteobacteria) increased in active layer soils of both aspects. This genus, commonly found in terrestrial cryoenvironments (Rime *et al.*, 2016b; Adamczyk *et al.*, 2020; Perez-Mon *et al.*, 2020), has been described to consist of mainly copiotrophic bacteria which might be more competitive than others in using labile substrates for microbial growth (Rime *et al*., 2016b; Su *et al.*, 2020). The phylum Bacteroidetes increased significantly in both active layers NW and SE in response to AREs, particularly the genus *Pedobacter* in NW soils. Although, members of *Pedobacter* have been reported to be cellulolytic bacteria (López-Mondéjar *et al.*, 2016), and thus likely playing an important role in carbon cycling of more complex substrates, the addition of AREs may have stimulated their growth, as members of this genus have been shown to also utilise glucose (Pankratov *et al.*, 2007; Wilkins *et al.*, 2014). An increase in vegetation in high alpine environments will likely benefit this taxon. Most genera of the highly abundant phylum Actinobacteria increased with the addition of AREs. We have found in cultivation experiments *Pseudarthrobacter* strains isolated from permafrost soils and incubated at low temperatures (between -1°C and 4°C) to be among the fastest growing microbes (unpublished data), indicating that this genus composes a copiotrophic population.

In contrast, the abundance of Acidobacteria, Chloroflexi, Gemmatimonadetes and Planctomycetes declined with ARE addition. Of these phyla, in particular the phylum Chloroflexi is consistently associated with oligotrophic environments as it predominantly can be found in carbon poor soils (Tebo *et al.*, 2015). Chloroflexi strongly decreased in the normally nutrient-poor PF soils in response to AREs. This phylum has previously already been found to be highly abundant in both active layer and permafrost soils of the same study site (Frey *et al.*, 2016), but also at high elevations in carbon poor alpine environments (Costello and Schmidt, 2006; Rime *et al.*, 2016a; Adamczyk *et al.*, 2019). Chloroflexi is a diverse phylum including autotrophic, heterotrophic and mixotrophic taxa (Hanada *et al.*, 2002; Bennett *et al.*, 2020). Thus, different physiological strategies might be responsible for them coping with the harsh environments in soils at higher elevations (Adamczyk *et al.*, 2019). Overall these findings indicate that with warmer temperatures and more nutrient inputs to high alpine soils, the abundance of bacterial oligotrophic taxa will likely initially decrease.

Among fungal phyla, Basidiomycota increased with ARE addition in SE soils. Considering that many members of this phylum have been reported to have oligotrophic features (Ho *et al.*, 2017; Yao *et al.*, 2017; She *et al.*, 2018), the general increase in Basidiomycota in response to AREs shows how heterogenic this fungal group is and, as such, generalisations at the phylum level should be made with care. At a lower taxonomic level, the increase in Basidiomycota could be explained by an increase in yeast or yeast-like organisms, such as the genera *Mrakia*, *Solicoccozyma*, *Goffeauzyma* and *Rhodotorula*, as a result of AREs being the major carbon and energy sources. *Mrakia* also showed a significant increase in the colder NW-facing active layer soils. In PF soils it could be that the basidiomycete yeast *Naganashia*, often found in extreme cold and dry environments (Schmidt *et al.*, 2017) as well as in alpine soils at high elevation (Adamczyk *et al.*, 2019), profited from the addition of labile carbon substrates. *Naganashia* includes psychrophilic species earlier placed in *Cryptococcus* (Turchetti *et al.*, 2020). Ascomycete genera exhibited varied responses. For instance, ascomycete yeasts (*Nakazawaea* and *Coniochaeta*) increased, while slow-growing lichenised fungi (*Verrucaria, Acarospora, Polysporina*; Ahmadjian, 1963; McDonald, Gaya, & Lutzoni, 2013) decreased in active layer NW soils. In PF soils *Rhynchosporium* showed a strong increase in abundance with ARE.

Rhynchosporium has recently been found in permafrost areas on the Qinghai-Tibet exhibiting psychrophilic features (Wang *et al*., 2015). The phylum Mortierellomycota declined in SE and particularly strong in NW soils. Members within this phylum are commonly found in alpine and Arctic habitats and contain psychrophilic or psychrotolerant species (Frisvad, 2008; Brunner *et al.*, 2011; Dresch *et al.*, 2019). The genus *Mortierella* also responded negatively to AREs in NW soils. *Mortierella* species are known to be cold-loving opportunistic alpine "snow moulds" (Schmidt *et al.*, 2008, 2012), and are also proposed to decompose simple organic compounds (Newsham *et al.*, 2018). Increasing vegetation in former bare alpine environments as a result of warmer temperatures is leading to a decline in abundance of psychrophililc *Mortierella* species (Schmidt *et al.*, 2008). Overall, rising temperatures in high alpine environments and the resulting increase in vegetation, will likely result in a decrease of the cold-adapted fungi.

Conclusion

In this study, we found that artificial root exudates strongly affected soil respiration, microbial diversity and community structure in active layer and permafrost soils. The increase in heterotrophic soil respiration appeared to be mainly driven by shifts in community structure towards fast-growing, copiotrophic taxa, particularly among bacteria. Shifts in fungal community structure were dominated by the increased abundance of yeasts. Fungal communities in the colder soils, in particular in the NW-facing active layer soils, were predominantly affected by changes in temperature, whereas in SE soils the addition of ARE was the major driver shaping these communities. These shifts in both fungal and bacterial community structure towards fast-growing taxa coincided with a strong decrease in microbial alpha diversity. As our incubation experiments were conducted over a relatively short time span, such responses may prove to be the result of transient effects. Longer-term experiments will likely shed more light on the response of slow-growing taxa to increased plant input. Higher input of carbon and nutrients in high alpine soils as the result of upward migration of plants, will change the soil microbiome considerably, with copiotrophic taxa likely dominating at the expense of slow-growing and oligotrophic taxa. Being important mediators of biogeochemical processes, these shifts in bacterial

and fungal community structure will inevitably have impacts on high alpine soil ecosystem functions, including belowground carbon dynamics. With globally rising temperatures, warmer conditions will further promote microbial activity, thereby likely accelerating SOM decomposition and possibly enhancing carbon loss to the atmosphere. However, to better predict the consequences of such changes, more research is needed to assess how the functional changes observed in laboratory incubations translate into actual ecosystem functioning.

Experimental Procedures

Site and soil description

Soils were collected on a ridge of the mountain 'Muot da Barba Peider' in the eastern Swiss Alps in autumn 2016, as previously described in Frey et al. (2016). Top soils (active layer) were taken on the NW flank of the ridge (46.49634° N, 9.93145° E; 2979 m a.s.l.) and on its SE flank (46.49587° N, 9.93226° E; 2979 m a.s.l.). Bulk soils (approx. 200 g of fresh soil) were collected using a soil corer (10 cm deep; 10 cm diameter) and put into a plastic bag. On each flank four independent soil samples (with a distance of 5 m from each) were taken (in total 8 samples: 4 samples x 2 slopes). The NW slope has continuous permafrost (PF) below 1 m depth, whereas the SE slope is only seasonally frozen. Three soil profiles of approx. 2 m^2 and separated by approx. 5 m were excavated with shovels on the NW slope, down to a depth of 160 cm. Bulk soils were collected at a depth of 160 cm from each profile, producing a total of three independent permafrost samples. Ethanol-sterilised materials were used for all steps of sample collection and processing. The permafrost samples were kept frozen through sampling, transportation and storage. Soil samples from the active layers were transported in cooled boxes to the laboratory. The annual mean soil temperature at 5 cm soil depth (GeoPrecision sensors; GeoPrecision GmbH, Ettlingen, Germany) was -1.6°C for NW top soils and +1.7°C for SE top soils. Minimum and maximum soil temperatures between 2017 and 2018 were -12.0°C and +16.6°C for the NW slope and -3.5°C and +29.6°C for the SE slope. Temperature in permafrost soils at 150 cm soil depth was recorded to be -0.8°C in 'Muot da Barba Peider' (Zenklusen Mutter *et al.*,

2010). The regional mean annual precipitation fluctuates around 1500 mm (meteoswiss). Vegetation was equally scarce on both sides of the ridge, with barren soil and some rare individual occurrences of plants, in particular *Poa*, *Cerastium* and *Jacobea* spp. (Frey *et al.*, 2016). In order to evaluate the microbial community response to LMW-C compounds, an incubation experiment was set up with soils from the three different origins. Fresh top soil samples were kept at 4°C and permafrost samples were kept at -1.5°C for one week until the incubation experiment started. Two days before the start of the experiment, soils were sieved through a 2 mm mesh (after thawing the PF samples at 4°C) to remove coarse gravel.

Mesocosm preparation and substrate addition

An 85-day long incubation experiment (corresponding to the growing season at this elevation) was set up comprising two stages: (i) acclimatisation of the soils to temperatures of either 4°C or 15°C, followed by (ii) artificial root exudate application, during which the soils were kept at the same temperatures and in the dark as the first stage. As experimental containers we used 100 ml Erlenmeyer flasks with permeable cotton wool lids to allow for aeration. 60 g of fresh soil was added to the individual flasks, each container receiving a single soil type (NW, SE and PF). There were four independent replicates for each soil type, temperature and treatment (apart from one permafrost sample which was a composite sample comprising the three independent PF samples), resulting in a total of 48 flasks (3 soil types x 2 temperatures x 2 treatments x 4 replicates). During the acclimatisation stage, half of the soils were incubated at 4°C and the other half at 15°C for 46 days. Artificial root exudates were prepared according to Baudoin et al. (2003) comprising the following carbon sources (stock-solution): glucose (26.7 mM), fructose (26.7 mM), sucrose (13.3 mM), citric acid monohydrate (13.3 mM), sodium acetate (40.05 mM), malic acid (19.8 mM), glycine (19.95 mM), serine (13.3 mM) and glutamic acid (7.8 mM). These substrate classes represent LMW-C compounds that are known to be utilised by permafrost microorganisms (Ernakovich and Wallenstein, 2015). The ARE stock-solution was filter-sterilised into an autoclaved glass jar. A total volume of 40 µl AREs per g of dry soil (equal to 300 µg C per g dry soil) was applied approx. once a week over a

period of 29 days, starting after the acclimatisation stage. During the same period, 40 µl of sterilised Milli-Q water was added to control flasks. Each time after ARE or water addition, CO_2 measurements were made. Soil moisture in all flasks was monitored gravimetrically during the whole experiment and soils were watered using Milli-Q water after each measurement series. After the last application of AREs, the soil samples remained at the given incubation temperatures for another ten days until harvest. 5 g of soil (dry weight equivalent) from each sample was taken for the community-level metabolism and substrate use using EcoPlate assays. All remaining soil samples were stored at -80°C before DNA extraction was performed for further analyses.

Soil basal respiration

Soil basal respiration was determined using an EGM-4 Environmental Gas Monitor (PP System, Amesbury, MA, USA). CO_2 measurements started 11 days after initial acclimatisation over a period of 30 days. During the acclimatisation stage, five measurement series were performed, each approx. one week apart. During each series, measurements were made at three different time points (0 h, ~24 h and ~48 h) to calculate respiration rates. In the second stage during which AREs were applied, also five series of measurements were performed, each approx. once a week over 29 days. During each series CO_2 measurements were taken each time after ARE application at three different time points (0 h, ~4 h and ~8 h). At the start of each measurement series, each flask was sealed with butyl rubber stoppers and immediately measured after sealing. The flasks were then returned to a given incubation temperature remaining sealed until the measurement series was completed. To express respiration rates per soil dry weight, the final respiration rates were converted from the headspace CO_2 change in ppm to µmol C-CO_2 g^{-1} day^{-1}.

Community-level physiological profiles (CLPP)

CLPPs were determined using Biolog EcoPlates (Biolog Inc., Hayward, CA, USA). Each Biolog EcoPlate contains 31 different kinds of carbon sources in triplicate (seven types of carbohydrates, nine carboxylic acids, four polymers, six amino acids, two amines/amides and three miscellaneous types).

Soil suspensions for each soil type (merged samples for each soil type and treatment; 12 samples in total) 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 (Lulakova *et al*., 2019). NaCl extracts were allowed to settle for 10 min to clear the supernatant, which was diluted tenfold to obtain a final dilution of 10^{-2}. A 125 µl aliquot was dispensed into each well of the EcoPlates. Plates were placed into 15°C incubators inside lidded boxes containing water to maintain humidity during the assay. The temperature of 15°C was frequently measured during summer on top soils at "Muot da Barba Peider", and short-term incubations of the alpine soils at this temperature have only shown minor effects on the soil bacterial community (Donhauser *et al.*, 2020). The optical density (OD) in each well was read at 595 nm with an INFINITE M200 reader (TECAN, Männedorf, Switzerland) 24, 48, 72 and 96 h after inoculation of the plates. After subtracting the OD of a control well, the mean across 31 different substrates was calculated as the average well colour development (AWCD). AWCD thus represents the carbon use capacities of the 31 substrates and was used to calculate functional diversity (Lulakova *et al*., 2019).

DNA extraction and quantification of bacterial and fungal ribosomal markers

DNA was extracted from approximately 1 – 2.5 g soil of each individual sample using the DNeasy PowerSoil Pro kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. For permafrost samples up to four DNA extractions per sample were made to obtain sufficient DNA for downstream analysis. DNA was quantified using the high sensitivity Qubit assay (Thermo Fisher Scientific). Relative abundances of the bacterial 16S rRNA genes and fungal ITS were determined by quantitative PCR (qPCR) using ABI7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The same primers (without barcodes) and cycling conditions as for the sequencing approach were used. For qPCR analyses 2.5 ng DNA in a total volume of 25 µl containing 0.5 µM of each primer, 0.2 mg of BSA ml^{-1} and 12.5 µl of QuantiTect SYBR Green PCR master mix (Qiagen, Hilden, Germany) were used. Three standard curves per target region (correlations $\geq$ 0.997) were obtained using tenfold serial dilutions (10^{-1} to 10^{-9} copies) of plasmids generated from cloned targets (Frey *et al.*, 2011). Data was converted to represent average copy number of targets per µg DNA.

High throughput sequencing of bacterial and fungal ribosomal markers

The V3 – V4 region of the bacterial small-subunit (16S) rRNA gene and the internal transcribed spacer region 2 (ITS2) of the eukaryotic (fungal groups, some groups of protists and green algae) ribosomal operon were PCR amplified from 5 ng of DNA template, using primers and conditions previously described by Frey et al. (2016). PCRs were run in triplicates, pooled and purified using Agencourt Ampure XP (Beckman Coulter, Beverly, USA). Bacterial and fungal amplicon pools were sent to the Genome Quebec Innovation Centre at McGill University (Montreal, Canada) for barcoding using the Fluidigm Access Array technology and paired-end sequencing on the Illumina MiSeq v3 platform (Illumina Inc., San Diego, CA, USA). Raw sequences were deposited in the NCBI Sequence Read Archive under the BioProject accession number PRJNA673329.

Sequence quality control, OTU clustering and taxonomic assignments

Quality filtering, clustering into operational taxonomic units (OTUs) and taxonomic assignments were performed as previously described in Frey et al. (2016). In brief, a customised pipeline largely based on UPARSE (Edgar, 2013; Edgar and Flyvbjerg, 2015) implemented in USEARCH (v. 9.2; Edgar, 2010) was used. Filtered reads were de-replicated and singleton reads removed prior to clustering. Sequences were clustered into OTUs at 97% sequence identity (Edgar, 2013). For taxonomic classification of the OTUs, corresponding centroid sequences were queried against selected reference databases using the naïve Bayesian classifier (Wang, Garrity, Tiedje, & Cole, 2007) implemented in MOTHUR (Schloss *et al.*, 2009), and a minimum bootstrap support of 80%. Prokaryotic sequences were queried against the SILVA database (v. 132; Quast et al., 2013). Eukaryotic ITS2 sequences were first queried against a custom-made ITS2 reference database retrieved from NCBI GenBank, and sequences assigned to fungi were subsequently queried against the fungal ITS database UNITE (v. 8.0; Abarenkov et al., 2010). Prokaryotic centroid sequences identified as originating from organelles (chloroplast, mitochondria), as well as eukaryotic centroid sequences identified as originating from soil animals (Metazoa), plants (Viridiplantae, except green algae), or of unknown eukaryotic origin,

were removed prior to data analysis. A brief description of the overall microbial community composition as well as complete lists of all archaeal, bacterial and fungal OTUs including taxonomic assignment, the number of sequences and abundance information can be found in Supplementary Data S1.

Data analysis

All statistical analyses were performed using R (v. 3.6.0; R Core Team, 2019) and all graphs were generated with the *ggplot2* package (v. 3.2.0; Wickham, 2016), unless specified otherwise. Main and interactive effects of soil type, temperature and ARE treatment on respired CO_2 before and during the treatment with AREs were tested by analysis of variance (ANOVA). Similarly, the main and interactive effects of ARE treatment and temperature on DNA content as well as 16S and ITS copy numbers for each soil type were tested using ANOVA.

For analysis of microbial alpha diversity, observed richness (number of OTUs) and Shannon diversity index were estimated based on OTU abundance matrices rarefied to the lowest number of sequences (bacteria: 10,092; fungi: 8,677) using the R package *phyloseq* (v. 1.28.0; McMurdie & Holmes, 2013). To assess the main and interactive effects of ARE treatment and temperature on alpha diversity in each soil type a two-way ANOVA was performed. Pairwise comparisons of significant effects were conducted using Tukey's HSD post-hoc tests. Assumptions for homoscedasticity and normality of residuals were tested and, where necessary, transformation (Tukey's ladder of powers) of response variables was performed.

Bray-Curtis dissimilarities were calculated based on square root transformed relative abundances of OTUs. The effect of soil type on microbial community structure (beta diversity) was assessed by conducting a permutational ANOVA (PERMANOVA, number of permutations = 9,999) with the function 'adonis' implemented in the *vegan* package (v. 2.5.5; Oksanen *et al.*, 2019). Similarly, for each soil type the main and interactive effects of AREs and temperature on community structure were

analysed. Principal coordinate analysis (PCoA) ordinations of microbial community structure were calculated using the 'ordinate' function implemented in the R package *phyloseq*.

To identify microbial phyla and genera that were significantly different between ARE and control samples we first agglomerated OTUs to the phylum and genus level, respectively, and generated subsets for each soil type. To avoid problems that can arise from analysing compositional data, we performed differential abundance analysis by applying a negative binomial generalised linear model to the OTU count data using the *DESeq2* package (v. 1.24.0; Love, Huber, & Anders, 2014). The effect of AREs on microbial taxa was assessed by controlling first for temperature. We applied normal shrinkage to $\log_2$ fold changes using 'lfcShrink' function implemented in *DESeq2*. Phyla and genera were considered significantly different (Wald test) between ARE-treated and control samples if the false discovery rate (adjusted p-value) was < 0.05. Only the ten most abundant phyla and genera with significant $\log_2$ fold changes were plotted.

Respiration data, microbial abundance (16S and ITS copy numbers) as well as DNA data can be found in Supplementary Data S2.

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Conflict of Interest

Authors declare no conflict of interest.

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Author Contributions

B.F. designed the study; J.R. performed lab experiments and data collection; M.A. performed data analysis; M.A. with the contribution of B.F. wrote the study.

Figure Captions

Figure 1. Respiration rates of active layer SE and NW as well as permafrost soils during the acclimatisation stage to temperatures at 4°C or 15°C and during the ARE stage. ARE added for the first time is indicated by the vertical red dashed line. Respiration measurements were performed after each ARE application. After the last measurement, the soil samples remained at the given incubation temperatures for another nine days (216 hours) until harvest. Mean values with standard errors of four replicates are shown for each measurement series. Abbreviations: SE: south-east; NW: north-west; ARE: artificial root exudates.

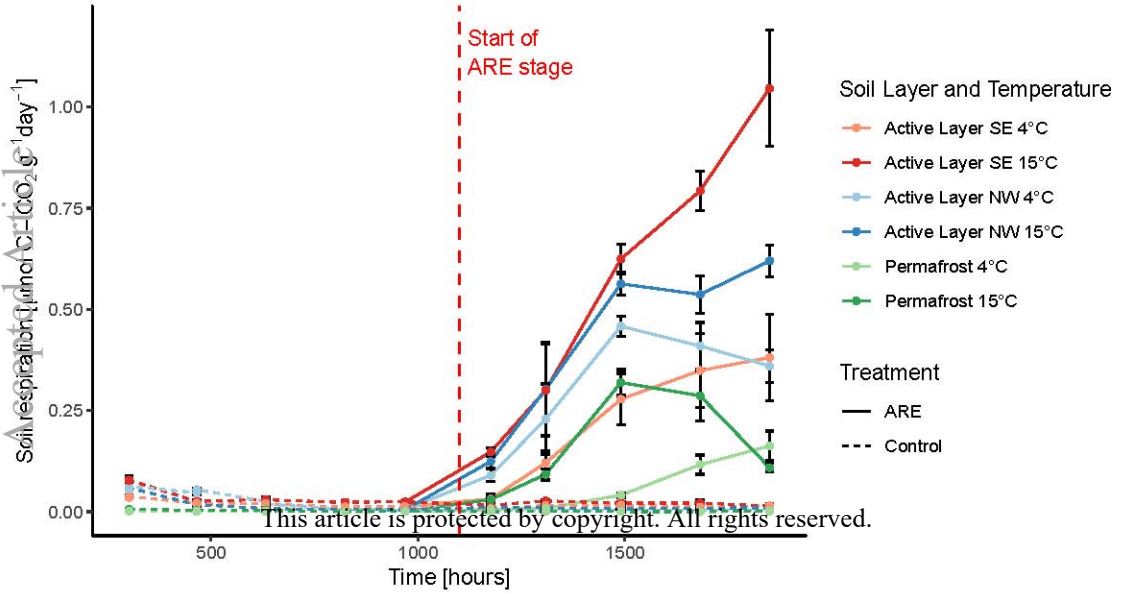
Figure 2. Bar plots representing (A) DNA content, (B) bacterial 16S copy numbers and (C) fungal ITS copy numbers in active layer SE, active layer NW and permafrost soils of control and ARE-treated samples incubated either at 4° or 15°C. Shown are mean values with standard errors of four replicates (except for NW 4°C 16S and ITS copy numbers only three replicates). Logarithmic scale used for DNA plot. The ITS copy numbers in PF soils were below the detection limit (2.7×10^3 ITS copy numbers per µl). Abbreviations: SE: south-east; NW: north-west; PF: permafrost; ARE: artificial root exudates.

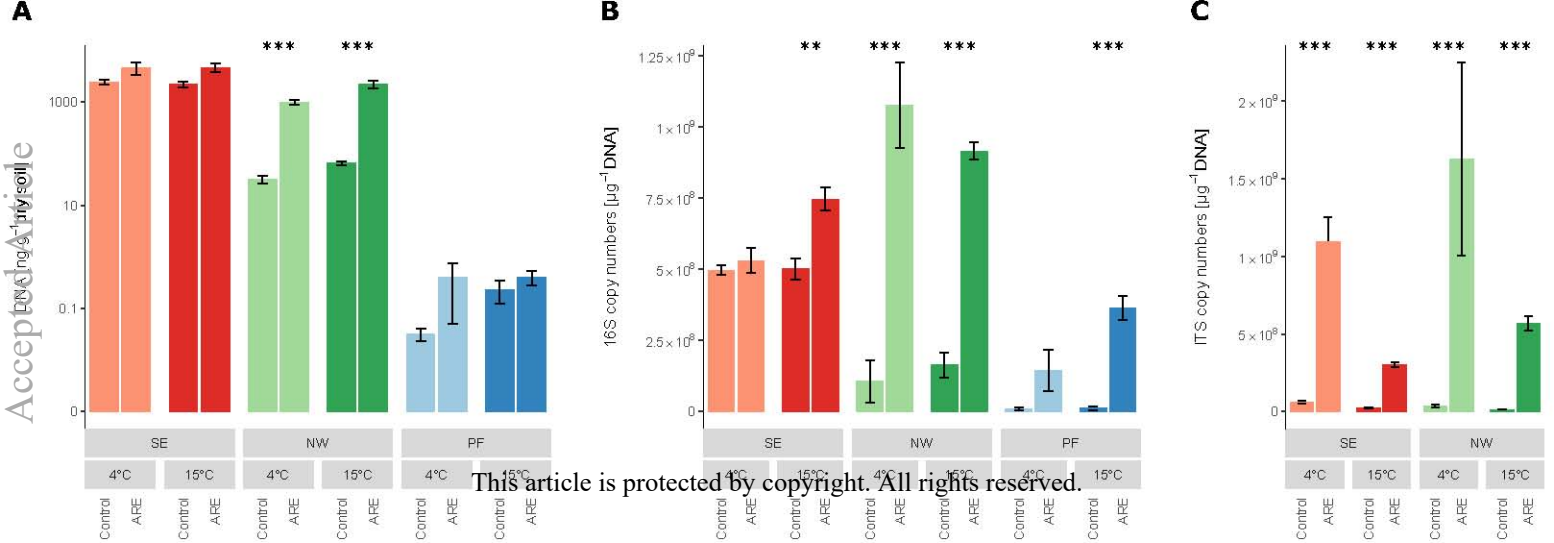
Figure 3. Variation in (A – D) microbial alpha diversity and (E and F) community structure in active layer soils SE and NW, as well as in permafrost soils of ARE-treated and control samples incubated either at 4°C or 15°C. Shown are (A) bacterial and (B) fungal richness, as well as (C) bacterial and (D) fungal Shannon diversity. Mean values (with standard errors) of four replicates are shown (except for SE 4°C ARE, PF 4°C ARE and PF 15°C ARE samples and the bacterial NW 15°C control sample, which contain only three replicates). E and F show principal coordinate analysis (PCoA) of (E) bacterial and (F) fungal community structure based on Bray-Curtis distance matrices. Distances between symbols on the ordination plots reflect relative dissimilarities in the community structure. The

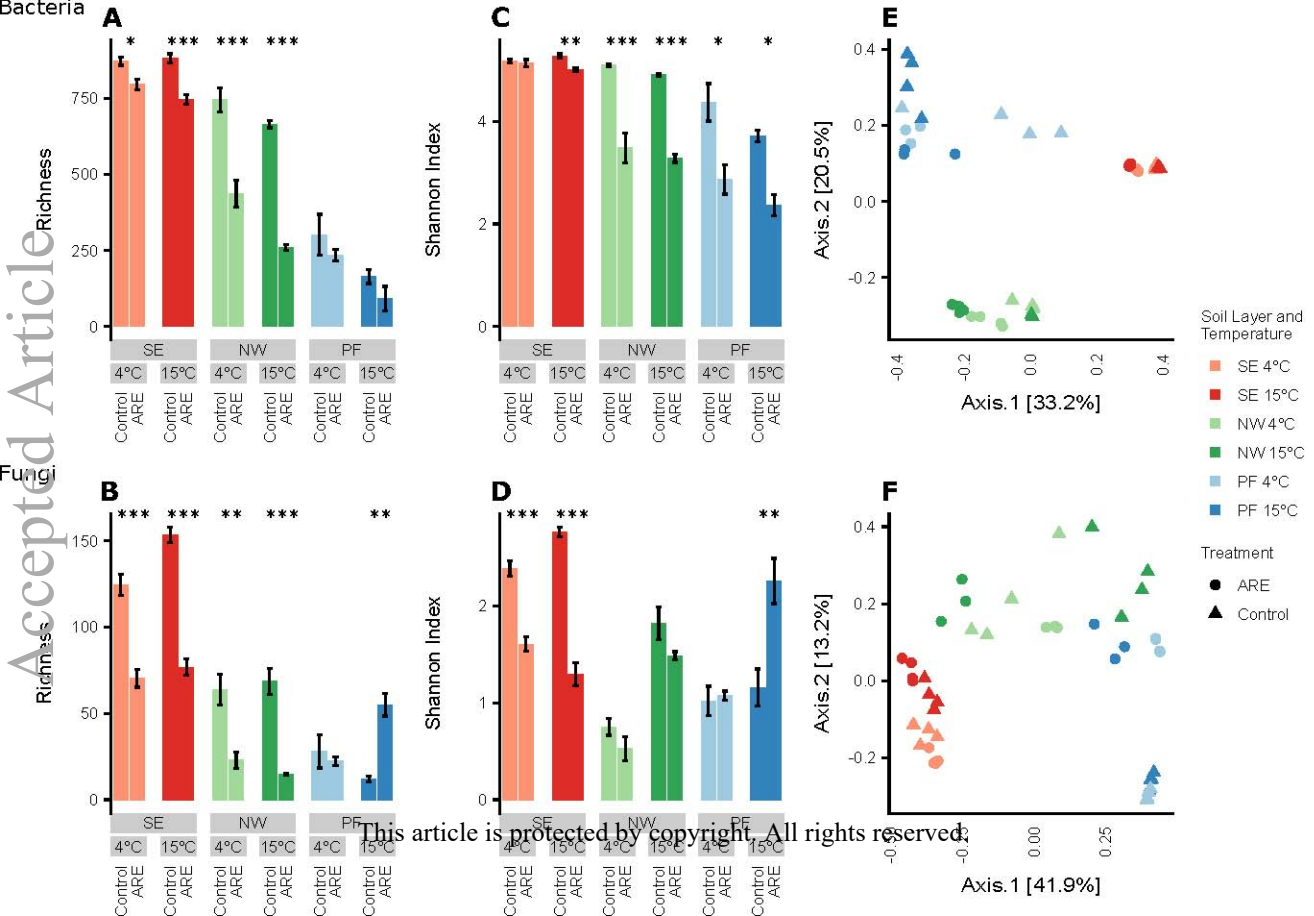
variation in microbial community structure explained by each PCoA axis is given in parentheses and the number of samples is the same as for A-D. Abbreviations: SE: south-east; NW: north-west; ARE: artificial root exudates.

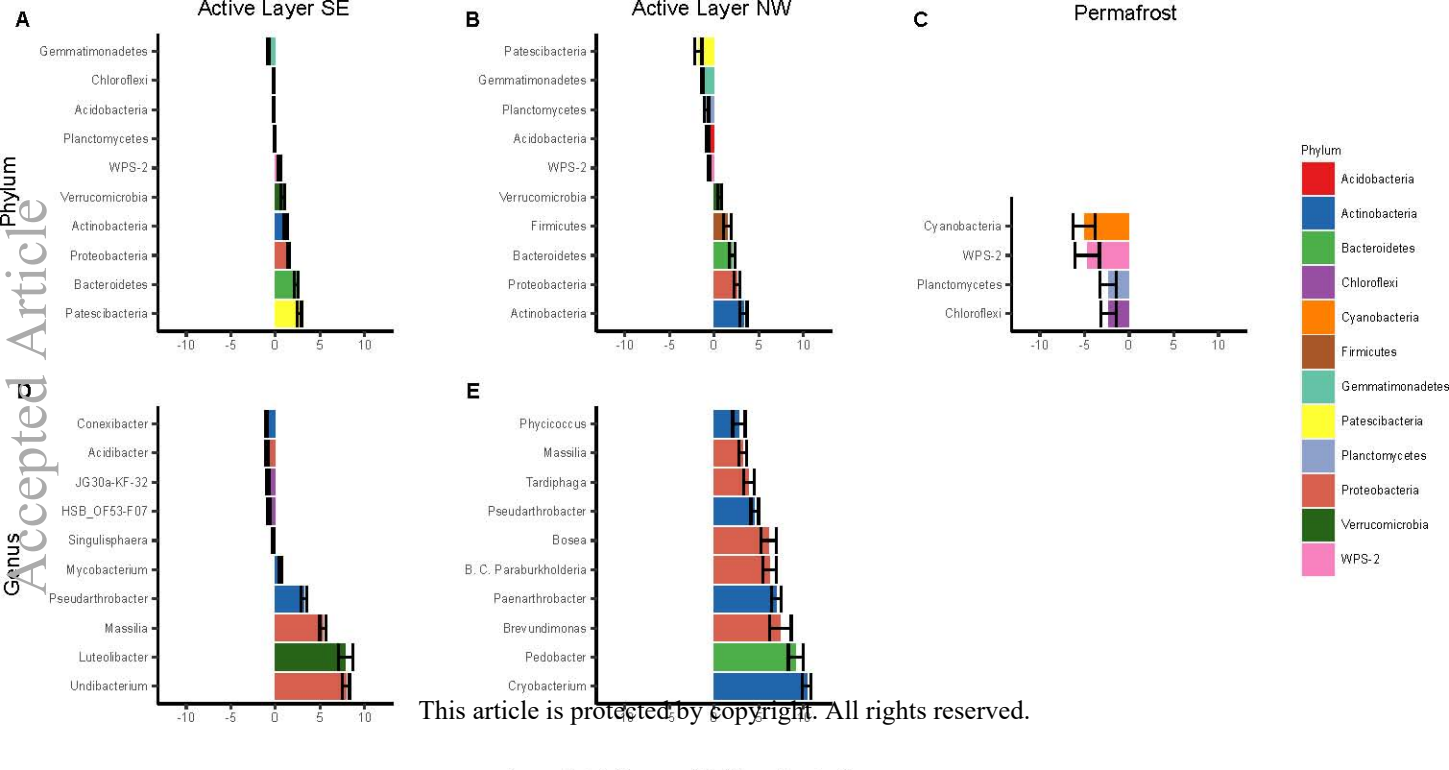
Figure 4. Differential abundant (A – C) bacterial phyla and (D, E) genera in response to artificial root exudates (AREs) in active layer SE, active layer NW and permafrost soils. Shown are only significant log_2 fold changes of the ten most abundant phyla and genera based on a significance level of $p < 0.05$ after false discovery rate correction. Error bars represent the standard error. Abbreviations: SE: south-east; NW: north-west. The genus *Burkholderia-Caballeronia-Paraburkholderia* was shortened to B.C. Paraburkholderia displaying purposes.

Figure 5. Differential abundant (A – C) fungal phyla and (D – F) genera in response to artificial root exudates (AREs) in active layer SE, active layer NW and permafrost soils. Shown are only significant log_2 fold changes of the ten most abundant phyla and genera based on a significance level of $p < 0.05$ after false discovery rate correction. Error bars represent the standard error. Abbreviations: SE: south-east; NW: north-west.









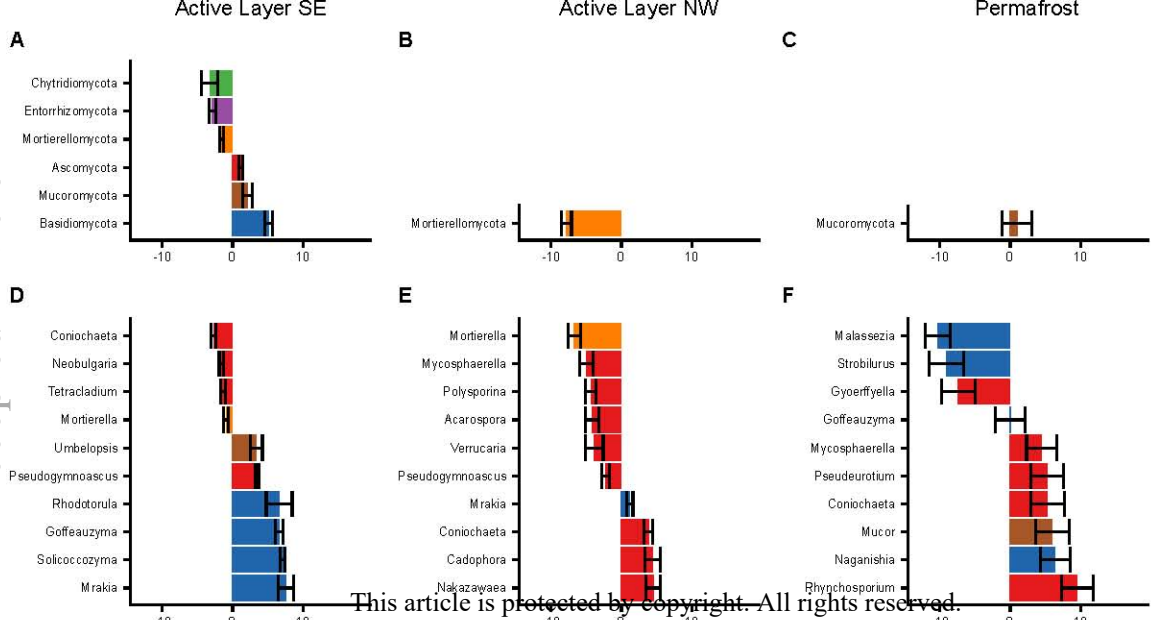


Table 1. Main and interactive effects of artificial root exudates (AREs) and temperature on total DNA content, and 16S and ITS copy numbers in active layer and permafrost soils.

	Active Layer SE	**Active Layer NW**	**Permafrost**
Total DNA			
ARE	$F_{(1, 10)} = 10.5$ **	$F_{(1, 11)} = 268.5$ ***	$F_{(1, 10)} = 3.3$ n.s.
Temperature	$F_{(1, 10)} = 0.0$ n.s.	$F_{(1, 11)} = 21.9$ ***	$F_{(1, 10)} = 1.9$ n.s.
ARE × Temperature	$F_{(1, 10)} = 0.0$ n.s.	$F_{(1, 11)} = 9.6$ *	$F_{(1, 10)} = 0.0$ n.s.
16S copy numbers			
ARE	$F_{(1, 10)} = 17.4$ **	$F_{(1, 10)} = 91.9$ ***	$F_{(1, 10)} = 47.8$ ***
Temperature	$F_{(1, 10)} = 8.7$ *	$F_{(1, 10)} = 0.1$ n.s.	$F_{(1, 10)} = 7.5$ *
ARE × Temperature	$F_{(1, 10)} = 8.1$ *	$F_{(1, 10)} = 1.6$ n.s.	$F_{(1, 10)} = 9.4$ *
ITS copy numbers			
ARE	$F_{(1, 10)} = 870.8$ ***	$F_{(1, 10)} = 78.7$ **	ND
Temperature	$F_{(1, 10)} = 119.6$ ***	$F_{(1, 10)} = 4.7$ n.s.	ND
ARE × Temperature	$F_{(1, 10)} = 2.6$ n.s.	$F_{(1, 10)} = 0.4$ n.s.	ND

*Shown is the F-statistic of ANOVA results. Subscripts denote the degrees of freedom and residuals for each factor. Significant levels: n.s. (not significant), * p < 0.05, *** p < 0.01, *** p < 0.001. Abbreviations: SE: south-east; NW: north-west. ND = not detected; ITS copy numbers in PF soils were below the detection limit (2.7 x 10^3 ITS copy numbers)*

Table 2. Main and interactive effects of artificial root exudates (AREs) and temperature on bacterial and fungal alpha diversity (richness and Shannon index) in active layer and permafrost soils.

	Bacteria		**Fungi**	
	Richness	**Shannon**	**Richness**	**Shannon**
Active Layer SE				
ARE	$F_{(1, 10)} = 64.0$ ***	$F_{(1, 10)} = 12.9$ **	$F_{(1, 11)} = 156.5$ ***	$F_{(1, 11)} = 170.1$ ***
Temperature	$F_{(1, 10)} = 3.8$ n.s.	$F_{(1, 10)} = 0.5$ n.s.	$F_{(1, 11)} = 12.7$ **	$F_{(1, 11)} = 0.4$ n.s.
ARE × Temperature	$F_{(1, 10)} = 2.6$ n.s.	$F_{(1, 10)} = 6.4$ *	$F_{(1, 11)} = 4.7$ n.s.	$F_{(1, 11)} = 15.2$ **
Active Layer NW				
ARE	$F_{(1, 11)} = 118.6$ ***	$F_{(1, 11)} = 91.5$ ***	$F_{(1, 12)} = 55.8$ ***	$F_{(1, 12)} = 6.1$ *
Temperature	$F_{(1, 11)} = 15.4$ **	$F_{(1, 11)} = 1.5$ n.s.	$F_{(1, 12)} = 0.1$ n.s.	$F_{(1, 12)} = 79.4$ ***
ARE × Temperature	$F_{(1, 11)} = 2.3$ n.s.	$F_{(1, 11)} = 0.0$ n.s.	$F_{(1, 12)} = 1.1$ n.s.	$F_{(1, 12)} = 0.6$ n.s.
Permafrost				
ARE	$F_{(1, 10)} = 2.2$ n.s.	$F_{(1, 10)} = 27.4$ ***	$F_{(1, 10)} = 8.4$ *	$F_{(1, 10)} = 10.9$ **
Temperature	$F_{(1, 10)} = 9.7$ *	$F_{(1, 10)} = 4.9$ n.s.	$F_{(1, 10)} = 0.6$ n.s.	$F_{(1, 10)} = 11.4$ **
ARE × Temperature	$F_{(1, 10)} = 0.0$ n.s.	$F_{(1, 10)} = 0.1$ n.s.	$F_{(1, 10)} = 14.2$ **	$F_{(1, 10)} = 8.9$ *

*Shown is the F-statistic of ANOVA results. Subscripts denote the degrees of freedom and residuals for each factor. Significant levels: n.s. (not significant), * p < 0.05, *** p < 0.01, *** p < 0.001. Abbreviations: SE: south-east; NW: north-west.*

	Bacteria		Fungi	
Active layer SE				
Temperature	$F_{(1, 11)}$ = 5.7 **	R^2 = 18.0%	$F_{(1, 11)}$ = 4.9 *	R^2 = 8.4%
ARE	$F_{(1, 11)}$ = 11.7 ***	R^2 = 36.9%	$F_{(1, 11)}$ = 38.1***	R^2 = 65.4%
Temperature × ARE	$F_{(1, 11)}$ = 3.3 *	R^2 = 10.4%	$F_{(1, 11)}$ = 4.3 *	R^2 = 7.3%
Active Layer NW				
Temperature	$F_{(1, 11)}$ = 10.2 ***	R^2 = 19.2%	$F_{(1, 12)}$ = 28.5 ***	R^2 = 44.5%
ARE	$F_{(1, 11)}$ = 26.4 ***	R^2 = 49.4%	$F_{(1, 12)}$ = 18.6 ***	R^2 = 29.0%
Temperature × ARE	$F_{(1, 11)}$ = 5.8 **	R^2 = 10.9%	$F_{(1, 12)}$ = 5.0 *	R^2 = 7.8%
Permafrost				
Temperature	$F_{(1, 10)}$ = 2.6 ***	R^2 = 14.9%	$F_{(1, 10)}$ = 3.1 **	R^2 = 11.9%
ARE	$F_{(1, 10)}$ = 2.4 ***	R^2 = 13.6%	$F_{(1, 10)}$ = 5.7 ***	R^2 = 22.0%
Temperature × ARE	$F_{(1, 10)}$ = 2.5 ***	R^2 = 14.5%	$F_{(1, 10)}$ = 7.1 ***	R^2 = 27.5%

Table 3. Main and interactive effects of artificial root exudates (AREs) and temperature on bacterial and fungal community structure in active layer and permafrost soils.

*Shown is the pseudo F-statistic of PERMANOVA results (number of permutations 9,999). Subscripts denote the degrees of freedom and residuals for each factor. Significant levels: n.s. (not significant), * p < 0.05, *** p < 0.01, *** p < 0.001. Abbreviations: SE: south-east; NW: north-west.*