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Community adaptation to temperature explains abrupt soil bacterial community shift along a geothermal gradient on Iceland

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ABSTRACT

Understanding how and why soil microbial communities respond to temperature changes is important for understanding the drivers of microbial distribution and abundance. Studying soil microbe responses to warming is often made difficult by concurrent warming effects on soil and vegetation and by a limited number of warming levels preventing the detection of non-linear effects. A unique area in Iceland, where soil temperatures have recently increased due to geothermic activity, created a stable warming gradient in both grassland (dominated by *Agrostis capillaris*) and forest (*Picea sitchensis*) vegetation. By sampling soils which had been subjected to four years of temperature elevation (ambient (MAT 5.2 °C) to +40 °C), we investigated the shape of the response of soil bacterial communities to warming, and their associated community temperature adaptation. We used 16S rRNA amplicon sequencing to profile bacterial communities, and bacterial growth-based assays (^{3}H-Leu incorporation) to characterize community adaptation using a temperature sensitivity index (SI, log (growth at 40 °C/4 °C)). Despite highly dissimilar bacterial community composition between the grassland and forest, they adapted similarly to warming. SI was 0.6 (equivalent to a minimum temperature for growth of between −6 and −7 °C) in both control plots. Both diversity and community composition, as well as SI, showed similar threshold dynamics along the soil temperature gradient. There were no significant changes up to soil warming of 6–9 °C above ambient, beyond which all indices shifted in parallel, with SI increasing from 0.6 to 1.5. The consistency of these responses provide evidence for an important role for temperature as a direct driver of bacterial community shifts along soil temperature gradients.

1. Introduction

Soil microbial communities and their response to changing temperatures have been the focus of extensive research in the past decade (Zhou et al., 2016; Oliverio et al., 2017). Moreover, it has been proposed that including microbial dynamics in ecosystem models will improve our ability to predict responses of biogeochemical cycles to changing climate conditions (Todd-Brown et al., 2012; Wieder et al., 2013, 2015), even though the putative link between community composition *per se* and ecological function remains elusive in most soil environments (Prosser, 2012; Bier et al., 2015). Thus, community responses to temperature and other climate-related variables is a fundamental ecological process which must be understood to fully explain the drivers of microbial distribution and abundance (Zhou et al., 2016; Delgado-Baquerizo et al.,

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2018) and the potential consequences for biogeochemical cycles.

For fundamental ecological understanding, as well as for applications to ecosystem modelling of carbon cycling, sub-arctic and arctic environments are considered particularly important for the study of the relation between soil microbes and temperature (Wieder et al., 2015, 2019). Current and future climate warming is greater than in other parts of the globe (Post et al., 2019), and temperature sensitivity of soil microbial activity is higher at low temperatures (Kirschbaum, 1995, 2000; Nottingham et al., 2019). Moreover, microbial responses to climate may affect biogeochemical cycling, including climate feedbacks (Jenkinson et al., 1991; Cavicchioli et al., 2019). The potential impact of such feedbacks is considerable, due to the large amount of carbon currently present as soil organic matter in sub-arctic and arctic soils (Tarnocai et al., 2009).

A range of techniques have been used to study the relationship between soil microbial communities and temperature, including laboratory incubations (Oliverio et al., 2017), sampling along climatic gradients (Yergeau et al., 2007; Nottingham et al., 2018), and *in-situ* climate manipulation experiments (Weedon et al., 2017). Although providing relevant insights, each of these approaches have a number of limitations. The large spatial scales necessary for gradient studies may introduce additional confounding factors related to differences in soil and vegetation. In experimental warming studies, practical and financial considerations compel investigators to choose a small number of (usually small) temperature steps (De Boeck et al., 2015).

Studying temperature responses of soil communities along geothermal gradients at local-scales (10–100s of metres) overcomes these limitations (O'Gorman et al., 2014). Potential biogeographic and large-scale edaphic confounders are held constant; and a range of temperatures can be studied allowing for the study of dynamics not possible with traditional experimental set-ups with only one or two warming levels. One such geothermal system is the ForHot site in SW Iceland (Sigurdsson et al., 2016). This system has been used to study the effects of warming on a wide range of processes in soil ecology and biogeochemistry (Walker et al., 2018, 2020; Marañón-Jiménez et al., 2019; Poeplau et al., 2020; Zhang et al., 2020; Verbrigghe et al., 2022). Focussing on the microbial community composition, Radujković et al. (2018) found that bacterial and fungal communities changed only at warming levels exceeding +6–8 °C above ambient. However, the authors could not decisively conclude whether this change was a direct effect of temperature or indirect effects due to, for example, effects on vegetation growth and phenology (Leblans et al., 2017), reduced soil organic matter concentration, or differences in soil texture (Poeplau et al., 2017; Verbrigghe et al., 2022). It is difficult to distinguish between potential drivers using community data alone, given that the relationship between specific taxonomic groups and ecological functions is largely unknown (Prosser, 2012).

A possible method for more precisely evaluating the direct effects of temperature on microbial communities is to directly characterize their physiological adaptation to temperature, as an aggregate community trait (Pietikäinen et al., 2005; Rinnan et al., 2009). It has been shown that soil bacterial communities adapt to the thermal environment, shifting measurable aspects of their aggregated temperature response (e.g. optimal [T_{opt}], and apparent minimal temperatures [T_{min}] for growth and activity) with changing temperature (Rinnan et al., 2009; Rousk et al., 2012; Bååth, 2018; Nottingham et al., 2019; Li et al., 2021). The use of the apparent minimum temperature for growth (T_{min}) provides an easily interpretable index of temperature sensitivity (Pietikäinen et al., 2005; Bååth, 2018). In pure culture studies, differences in T_{min} can be used to differentiate between psychrophiles, mesophiles and thermophiles (Ratkowsky et al., 2005; Corkrey et al., 2016). In an incubation study of alpine soils it was recently shown that such shifts in T_{min} occurred concurrently with a shift in bacterial community composition (Donhauser et al., 2020), but only when the imposed temperature treatment exceeded the measured T_{opt} of the *in situ* community. This result shows how targeted physiological assays can be used to complement community profiling methods to better understand the predominant mechanisms of microbial community variation (Hicks et al., 2022). Precise estimation of T_{min} requires a relatively large number of assay temperatures. If only relative changes in community adaptation to temperature are of interest, a more efficient method is to measure growth at two different assay temperatures, chosen to span a large part of the range between T_{min} and T_{max} (ideally making an interval that includes T_{opt}), and to calculate a temperature sensitivity index (SI) as the log of the ratio of growth rates at high/low temperatures (Ranneklev and Bååth, 2001; Rinnan et al., 2009; Nottingham et al., 2019, 2021).

In this study, we combined measures of temperature adaptation of bacterial growth with taxonomic characterization of bacterial communities in order to test whether the previously observed shifts in bacterial community composition along the same geothermal warming gradient (Radujković et al., 2018) were related to community adaptation to temperature. We studied two temperature gradients, both with a gradient of ambient up to +40 °C, but with very different vegetation community composition (a forest and grassland site). We hypothesized that (1) in the part of the gradient with no difference in community composition there would be no temperature adaptation of the bacterial community, while a community change would be concomitant with increased adaptation to higher temperatures; and (2) the same responses would be found in the two different vegetation types, despite having different original community composition. Thus, we expected temperature adaptation to be correlated with the temperature increase to the same degree in both vegetation types.

2. Materials and methods

2.1. Site description and sampling

The study area is the ForHot research site in the Hengill geothermal area, 40 km east of Reykjavik, Iceland. Mean annual temperature (MAT) is 5.2 °C with the mean temperature of the coldest and warmest month (December and July) being −0.1 °C and 12.2 °C, respectively (Synoptic Station, Iceland Meteorological Office, 2016). There is usually no permanent snow cover during winter due to the mild oceanic climate, but the soil may freeze for at least 2 months during mid-winter. In May 2008 an earthquake shifted geothermal systems in the area surrounding the research site, resulting in hot groundwater and steam that penetrates to the surface through fissures in the bedrock. Heat is subsequently conducted perpendicular to the fissures, resulting in transects with soil temperatures declining with distance to the fissure. This study focused on temperature gradients ranging from un-warmed controls to +40 °C compared to the ambient temperature. Sampling was concentrated in two vegetation types: an unmanaged grassland (in the area denoted "GN" in Sigurdsson et al., 2016) dominated by *Agrostis capillaris* L., *Ranunculus acris* L. and *Equisetum pratense* Her.,1784; and a planted *Picea sitchensis* (Bong.) Carr. forest, with no significant understorey vegetation ("FN" in Sigurdsson et al., 2016). The soils of the area are Silandic Andosols with a silt loam texture. In the topsoil (5–10 cm depth) the average total C content is 7.1 and 5.4% in the forest and grassland vegetation types, respectively. Corresponding total N concentrations are approximately 0.48% for both vegetation types and soil pH ranges between 5.0 and 5.7 at the grassland vegetation type, and between 5.6 and 6.3 under the forest vegetation. The area is described in more detail by Sigurdsson et al. (2016).

Soils were sampled in May 2012, that is 4 years after the beginning of warming. At each vegetation type four replicate transects were established, each consisting of 10 (forest) or 9 (grassland) sampling plots (1 × 1 m) on each transect. The position of plots was chosen to span a range of soil temperature elevations from ambient to approximately +40 °C (relative to the ambient plot for each transect) based on soil temperature measurements made on several occasions in the spring of 2012 using a handheld temperature probe inserted to 10 cm depth. Due to the non-

linear increase in temperature elevation towards the warmed bedrock fissure, and the need to keep adequate spacing between plots, a higher number of temperatures, with smaller step sizes between temperature levels were studied in areas with soil temperature < +10 °C. Sampling plots were separated by between 3 and 10m. Subsequent monitoring of the soil temperature in each plot revealed some divergence from the original temperatures used to select plot locations. Therefore, for all analyses below we use the plot-level averages of temperature offset relative to the temperature in ambient soils of the corresponding transect, measured on 4–10 different occasions in the period from October 2011 to July 2013. Note that although located in the same area, the plots we sampled are not the same as those forming the permanent transects established in 2013 and described in several recent papers (Sigurdsson et al., 2016; Marañón-Jiménez et al., 2018; Radujković et al., 2018; Walker et al., 2018, 2020). For bacterial community analyses, three soil cores were taken at each plot with a 3 cm diameter soil corer and the soil from 5 to 10 cm depth were retained for further analyses. Soil samples for community temperature adaptation measurements were taken 7 days later from the same plots using the same sampling method. This sampling occasion also included an additional set of eight plots (two on each transect) in the grassland located at approximately +25 °C in order to have more coverage of this temperature level.

2.2. Community temperature adaptation measurements

Soil samples were stored at 17 °C until analysed (within 2 months). This higher storage temperature (not the more commonly used refrigerator temperature of 4 °C) was used in order to not affect temperature adaptation in samples taken from the soils with the highest temperatures. This temperature has been shown not to affect temperature adaptation during this time period (Bárcenas-Moreno et al., 2009; Birgander et al., 2013). Adaptation of the bacterial community to temperature was measured using a growth based index, where bacterial growth was estimated as leucine (Leu) incorporation (Bååth, 1994; Bååth et al., 2001). Bacterial growth was determined at two temperatures, 40 °C and 4 °C, and the log ratio (defined as log (growth at 40 °C/4 °C)) was used as sensitivity index (SI) of temperature adaptation of the bacterial community. A high value indicates adaptation to high temperatures, a low value to low temperature conditions (see Fig. S1 in supplementary information, also Rinnan et al., 2009; Nottingham et al., 2019). The index correlates to the more commonly used T_{min} (apparent minimum temperature for growth) to express temperature adaptation (Nottingham et al., 2019). Due to the large range of *in situ* temperatures, a larger difference in the high and low incubation temperature was used compared to earlier studies (Rinnan et al., 2009; Nottingham et al., 2019).

For each replicate, bacteria were extracted from soil (1 g soil in 25 mL water) by shaking, followed by a low speed centrifugation (1000×*g* for 10 min). The bacterial suspension was then distributed in microcentrifugation vials (1.5 ml in each) and 2 μl ^{3}H-Leu (37 MBq mL^{-1} and 5.74 TBq mmol^{-1}, PerkinElmer, USA) and unlabeled Leu (resulting in a final Leu concentration of 275 nmol L^{-1}) was added after 30 min. Growth was terminated by adding trichloroacetic acid after incubating for 2.5 h at 40 °C and 24 h at 4 °C. Incubation times were chosen in order to achieve more similar total Leu incorporation at the two temperatures. Due to logistic reasons, measurements at the different temperatures were made at different days. Subsequent washing steps and measurement of radioactivity were performed following the procedure described by Bååth et al. (2001).

Samples from ambient soils were also subjected to growth analysis at a range of temperatures (5 °C intervals between 0 °C and 50 °C) in order to determine T_{min} and T_{opt} for bacterial growth for communities subject to ambient conditions. T_{min} was calculated by the Ratkowsky equation (Ratkowsky et al., 1982) on square root transformed data at temperatures below T_{opt} (≤25 °C, see Fig. 1). A relationship between T_{min} and SI was found in a gradient spanning 20 °C in mean annual temperature

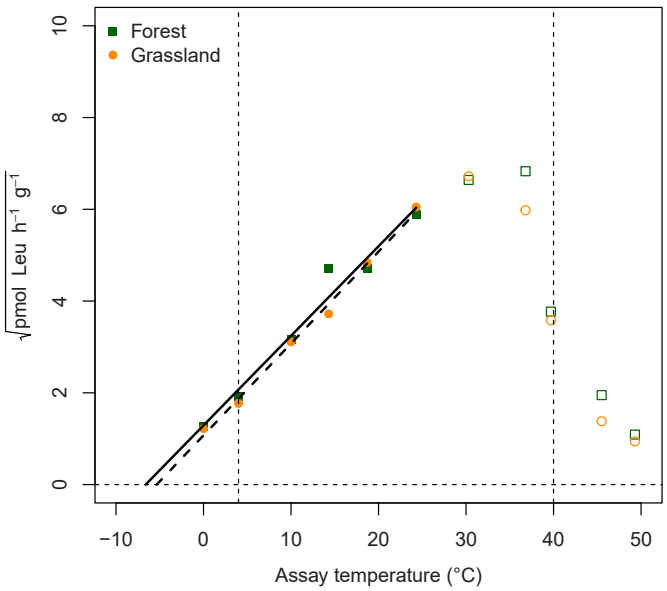


Fig. 1. Temperature dependence of growth (Leu uptake) from bacterial communities sampled from soils with ambient temperatures (MAT 5.2 °C) in the grassland (GN, squares and solid line) and forest (FN, circles and dashed line) habitats. The data are plotted using square root transformation, with filled symbols included in calculations using the square root (Ratkowsky) equation. T_{min} was −6.6 °C and −5.4 °C in GN and FN, respectively. Thin vertical lines indicate temperatures used for the Sensitivity index (SI, log(growth at 40 °C/ at 4 °C).

(Nottingham et al., 2019). This was used to calculate corresponding values of T_{min} from SI, assuming that SI at the elevation gradient and the present site corresponds to the same T_{min}.

2.3. DNA extraction, amplification and sequencing

Soil samples were stored at 4 °C within 2 h of sampling, and processed in the following 48 h. Soils were sieved and mixed, and DNA extracted using MoBio PowerSoil DNA kit following the manufacturer's protocol. DNA extracts were checked for quality and quantity using a Nanodrop spectrophotometer and prepared for Illumina sequencing of the V3 region of the bacterial 16S rRNA gene using the primers in Bartram et al. (2011) following the procedures described in Weedon et al. (2017). Libraries were sequenced on an Illumina MiSeq using 2 × 150 cycle paired-end sequencing (V2 chemistry) at the VUmc Clinical Genetics sequencing facility (Amsterdam, The Netherlands).

2.4. Bioinformatics

Initial sequence processing and Operational taxonomic unit (OTU) clustering was performed using the USEARCH software (Edgar, 2013). Paired-end sequences were assembled with a maximum of 3 mismatches allowed in the overlapping region (77% of raw-reads retained). This was followed by quality filtering with maximum expected errors set at 0.05 which removed an additional 23% of the successfully merged reads. OTUs were then defined using the UPARSE algorithm with 97% minimum similarity (Edgar, 2013), after removing all singleton reads. Chimeric sequences were removed with UCHIME (Edgar et al., 2011). A set containing representative sequences for each OTU was aligned using PyNAST (J Gregory Caporaso et al., 2010) using as a reference alignment the Green Genes version 13_8 (DeSantis et al., 2006) 'core-set' as distributed with QIIME version 1.7.0 (J. G. Caporaso et al., 2010). Sequences belonging to OTUs that failed to align with at least 75% sequence similarity, were most likely chimerical sequences or sequencing errors, and were removed from the dataset (178 OTUs

representing 0.6% of successfully assembled reads). All original reads were mapped back onto the resulting OTUs to produce an OTU table. For phylogenetic distance measures, we generated a phylogenetic tree based on the aligned representative set using FastTree (Price et al., 2009). Lastly, we assigned all OTUs to a taxonomic classification using the Ribosomal Database Project Bayesian classifier (Wang et al., 2007) with a threshold minimum confidence of 80%. Raw sequences are deposited in the NCBI Sequence Read Archive (accessible via BioProject accession PRJNA891345).

2.5. Statistics

Alpha diversity (Shannon index) was computed for each bacterial community sample. Pairwise weighted Unifrac distances (Lozupone et al., 2006) were computed for all sample combinations and visualized using principle coordinates analysis (PCoA). The first PCoA axis (explaining 25% of the dataset variation) was subsequently used as a univariate proxy of community composition. The significance and relative magnitude of vegetation type and temperature elevation effects on total community composition was analysed with a permutational multivariate analysis of variance (Anderson, 2001) on the Unifrac distance matrix. Read counts were normalized to 1154 reads per sample for all alpha- and beta-diversity analyses.

Alpha diversity, PCoA scores and SI were each modelled as a function of temperature elevation, separately for each vegetation type. Initial visualization indicated a step-wise pattern for all responses at both vegetation types. This was further investigated by fitting regression trees to each dataset and comparing the resulting break-point model with normal linear regression using AIC values. We used a parametric bootstrap approach to estimate the uncertainty associated with the break-point in the tree regression for each response x vegetation type combination. Bootstrap distributions of the breakpoint were generated by generating 1000 sets of temperature points created by adding Gaussian distributed noise to each of the temperature values, and refitting the regression tree model. The standard deviations of the added noise (1.73 and 2.14 °C for grassland and forest plots, respectively) were based on the observed variation in soil temperature elevation measurements between October 2011 and July 2013. To directly test for a relation between community temperature adaptation and composition, separate linear regressions were performed between PCoA1 scores and SI for each vegetation type.

To identify bacterial OTUs response to warming we performed a differential abundance analysis using the ANCOMBC R-package (Lin and Peddada, 2020). For both sites, we filtered samples to a minimal sequencing depth of 3750 reads, and filtered OTUs on an abundance above 0.001% and minimal occurrence in 3 samples. We ran ANCOMBC for soil bacterial community data of both sites separately, using the increase in soil temperature as independent variable and transect as a covariate, with the Bonferroni method for false discovery rate correction.

3. Results

3.1. Community adaptation to temperature

Bacterial growth in control soils at ambient temperature from both vegetation types closely followed the Ratkowsky model with the square root of growth increasing linearly with temperature below T_{opt} (Fig. 1). T_{min} of bacterial growth was similar in the two vegetation types, −6.6 °C and −5.4 °C in the grassland and forest, respectively. T_{opt} in both vegetation types was around 30 °C. Above T_{opt} bacterial growth decreased rapidly with increasing temperature. In the ambient soils, SI (log ratio of growth at 40 and 4 °C, see Fig. 1) was 0.59 and 0.61 in the grassland and forest, respectively.

Increasing soil temperatures resulted in increased SI in both the forest and the grassland (Fig. 5a and b), indicating growth adaptation to higher temperatures of the bacterial community along the geothermal gradient at both sites. SI increased from around 0.6 in both ambient sites to around more than 1.5 in the warmest plots. Recalculating these changes in SI to approximate changes in T_{min} resulted in an increase from a T_{min} of around −6.6 °C in ambient sites to around −3.5 °C in the sites with >+15 °C above ambient temperatures (MAT increasing from +5 °C to >20 °C). Thus, T_{min} increased around 3 °C with an increase in MAT of >15 °C.

3.2. Bacterial community composition

In ambient soils, the bacterial communities of both grassland and forest were dominated by OTUs assigned to the phyla Proteobacteria (35% of reads in the forest, 31% in the grassland), Actinobacteria (15% and 21%) and Acidobacteria (26% and 20%). Other phyla contributing between 1 and 8% of reads were Chloroflexi, Firmicutes, Bacteroidetes, Verrucomicrobia, Nitrospirae, and Gemmatimonadetes. Despite this similarity in dominant phyla and the fact that 44% of OTUs were detected in both vegetation types, bacterial community composition was significantly different between the two vegetation types (Weighted Unifrac PERMANOVA $P = 0.004$, $R^2 = 0.27$, $n = 13$).

The bacterial community was significantly affected by increasing soil temperatures at both vegetation types. This was visible at the level of alpha diversity (Fig. 2, breakpoint regression $P < 0.05$), overall community composition (Fig. 3, weighted Unifrac PERMANOVA temperature effect: $P = 0.001$, $R^2 = 0.12$), and the large number of differentially abundant OTUs (Fig. 4; ANCOMBC; $P < 0.05$).

Alpha diversity (Shannon index) decreased from around 6.5 to 7 in ambient plots to between 5 and 6 in the warmer soils (Fig. 2). This decrease in diversity was paralleled with a changing community composition, with the forest soil mainly changing along PCoA1 (explaining 24.5% of the variation), while temperature effects were detected in both PCoA1 and PCoA2 (11.0% of the variation) for the grassland community composition (Fig. 3). The temperature effect was of roughly the same importance as vegetation type differences in explaining variation in the bacterial community composition (weighted

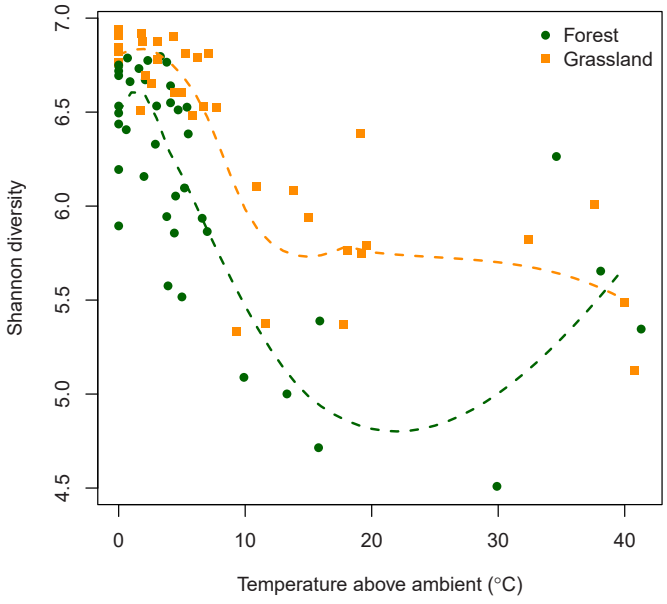


Fig. 2. Alpha diversity of the bacterial community (Shannon index) versus soil temperature above ambient (MAT 5.2 °C) along two geothermal soil temperature gradients (grassland,GN, yellow squares and forest, FN, green circles). Dashed lines represent a loess smoothing function added to summarize the main patterns of change in the response along the temperature gradient. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

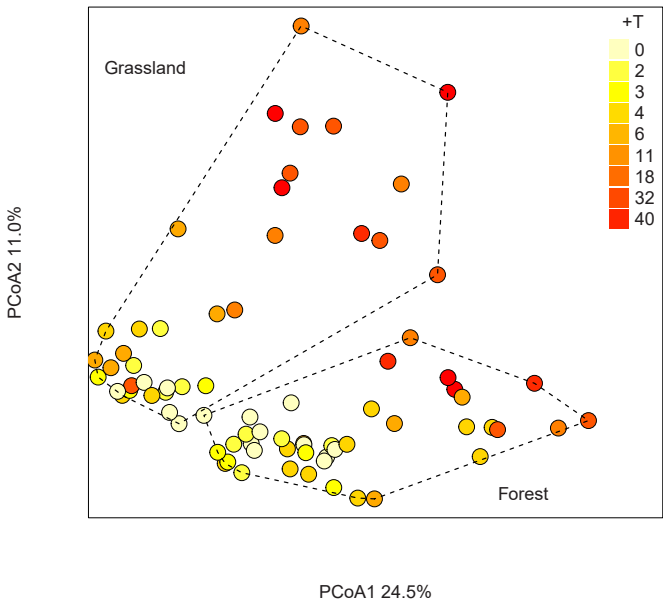


Fig. 3. Principal coordinates analysis ordination (PCoA) of bacterial community profiles from two geothermal soil temperature gradients (grassland, GN, and forest, FN). Ordinations are based on weighted-Unifrac distances computed from 16S rRNA gene amplicon data and colour coded according to temperature elevation (in °C) above ambient soils (MAT 5.2 °C). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Unifrac PERMANOVA: temperature effect: $P = 0.001$, $R^2 = 0.12$, site effect: $P = 0.001$, $R^2 = 0.15$).

The overall shifts in community composition were made up of changes in relative abundance of a large number of OTUs. In total, 863 out of 2986 OTUs for the forest site and 407 out of 3144 OTUs for the grassland site showed differential abundance across the soil warming gradient. Only 141 OTUs were differentially abundant in both vegetation types, of which most OTUs belonged to the phyla Proteobacteria, Acidobacteria, Actinobacteria, and Verrucomicrobia (Fig. 4, Table 1). In the forests soils, the 185 OTUs that increased in abundance went from contributing 0.7% of the total reads in the ambient soil samples to 48.6% in the soils warmed above 6 °C. In total, 678 OTUs decreased significantly in abundance, their share of the total reads reduced from 52.7 to 15.5%. In grassland soils, 190 OTUs increased in abundance from 4.3% of the total reads in the ambient soil to 29.0% in the soils warmed above 6 °C. In total 217 OTUs decreased significantly in abundance, reducing their contribution to total reads from 18.8 to 4.1%.

3.3. Comparing temperature effects on bacterial community composition and growth adaptation

There appeared not to be a gradual effect of temperature along the geothermal gradient on the soil microbial community. Instead, for all of the aforementioned measures (community profile (PCoA1), Shannon diversity, growth adaptation to temperature (SI)), a break point model as a function of soil temperature was a closer fit to the observed data than either simple linear regression or a null model (Table 2, all models $P < 0.05$). There was therefore a threshold temperature, below which there appeared to be no effect on the bacterial community. Median threshold temperature was 8.1, 6.4 and 8.8 °C for community profile, SI and Shannon diversity in the forest site, with corresponding values for the grassland being 9.0, 8.7 and 8.8 °C. However, when accounting for uncertainty in soil temperature measurements, the range of estimated threshold temperatures spanned 3.4–11.8 °C (end points of bootstrap 95% confidence intervals) with relatively more uncertainty for the forest.

As noted above, the observed shifts in bacterial community composition and SI with temperature followed very similar dynamics in both vegetation types (Fig. 5a and b). Accordingly, there were significant linear correlations between these two measures at both sites ($R^2 = 0.78$ and 0.74, for forest and grassland, respectively (Fig. 5c)).

4. Discussion

We characterized bacterial community composition and temperature adaptation of sub-arctic soils sampled along a 40 °C soil warming gradient under two vegetation types. We confirmed previous observations that bacterial community profiles only changed relative to ambient conditions when subjected to more than approximately 6–9 °C warming (Radujković et al., 2018; Walker et al., 2018). Crucially, we provide evidence that this community shift is driven by direct responses to temperature, by showing that the community change coincided closely with increases in temperature adaptation of the community. Moreover, this overlapping of community trait and compositional measures was observed in both grassland and forest soils, despite their distinct bacterial community profiles at both ambient and warmed conditions, suggesting that this result could potentially be generalized to a variety of soil and vegetation types harbouring divergent bacterial communities.

4.1. Bacterial community composition responses to warming

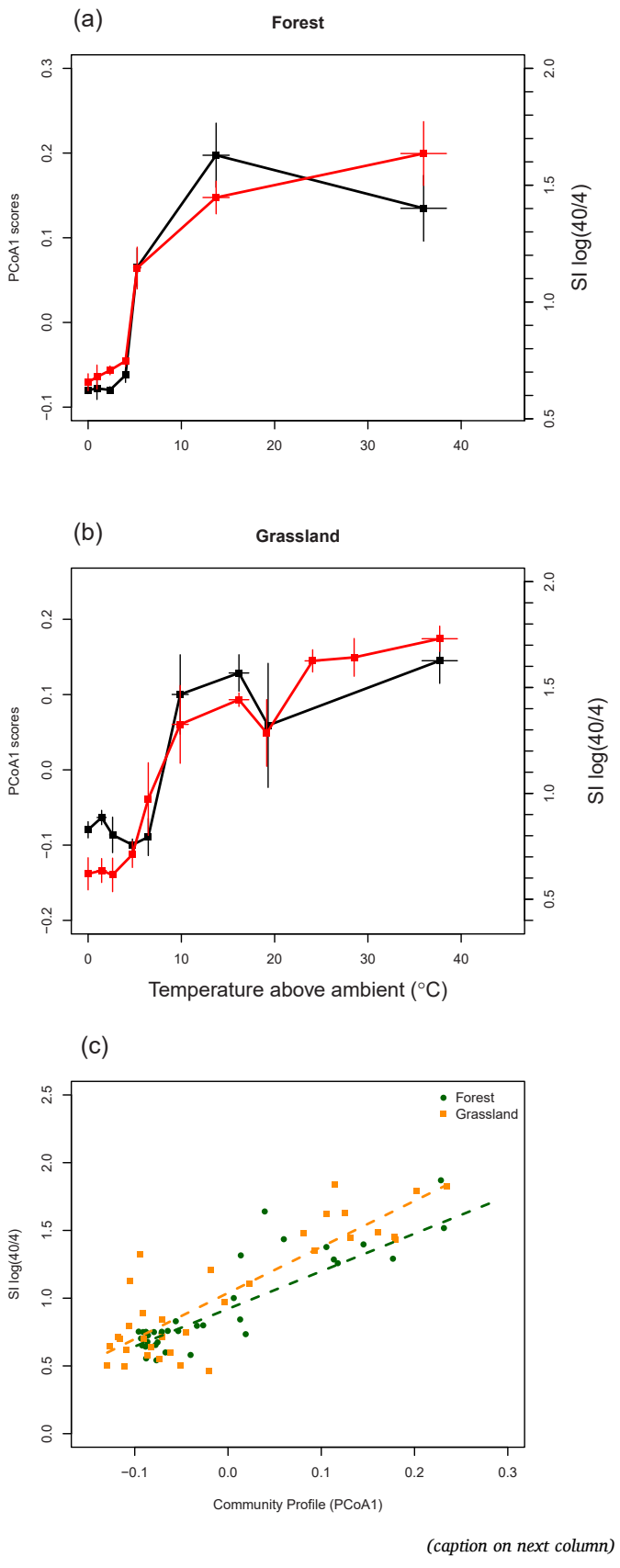
The bacterial community composition in both grassland and forest soils shifted in response to increasing soil temperatures (Fig. 3). The shift was characterized by a decrease in alpha diversity, with more OTUs declining in abundance over the warming gradient than OTUs increasing (Table 1). This is contrary to studies finding increasing diversity with increasing temperature, for example in elevation gradients (Nottingham et al., 2018; Ji et al., 2022). On the one hand, this discrepancy could suggest different effects of temperature in response to long-term (gradients) versus short-term warming (4 years in the present temperature gradient). On the other hand, the relatively high temperatures at the upper end of the gradient in the present study may have led to conditions outside the typical temperature tolerance ranges for the majority of the local soil bacterial taxa. A decreasing diversity was also found in a soil warming study in Panama, where temperatures became much higher than the normal soil temperature (Nottingham et al., 2022).

The warm responders made up a small fraction of the bacterial community (0.7% in forest and 4.3% in grassland) in the ambient soil but increased to 48.6 and 29.0% of the total community >6 °C warming in the forest and grassland, respectively. A similar (partial) community turnover was also observed in an incubation of alpine soils, where increasing temperatures lead to a community dominated by presumably warm-adapted taxa (Donhauser et al., 2020). Community-level responses to warming, either experimentally imposed, or measured along climatic gradients, have been observed for bacteria in a number of soils (Yergeau and Kowalchuk, 2008; DeAngelis et al., 2015; Oliverio et al., 2017; Monteux et al., 2018; Radujković et al., 2018; Moinet et al., 2021), although they are not always present (Weedon et al., 2017). The responding OTUs appeared to be fairly evenly distributed amongst the major phyla, and there was no over-representation of known thermophilic groups. Within each phylum, and indeed order, there were typically OTUs that both increased and decreased with soil warming. This emphasizes that temperature niche, at least within the range of temperatures covered by our gradient, seems to be largely decoupled from high-level taxonomic identity (Oliverio et al., 2017; Radujković et al., 2018), supporting the idea that temperature preference of bacteria is a shallowly conserved trait (Martiny et al., 2015).

4.2. Bacterial community adaptation to temperature

The bacterial community adaptation to temperature, estimated as SI, increased over the gradient, showing that communities had adapted to

Fig. 4. Abundance shifts of the OTUs that were differentially abundant ($P < 0.05$) across the warming gradient for both Forest and Grassland vegetation types, expressed in estimated log_2-fold changes and grouped by Order rank on the y-axis. Each point represents a distinct OTU. Colors indicate Phylum rank. See also Table 1 for numbers of responding OTUs at each vegetation type for the 10 most abundant phyla. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



(caption on next column)

Fig. 5. Relationships between bacterial community composition and community adaptation to temperature along soil temperature gradients. (a) and (b) bacterial community expressed as axis scores of PCoA ordinations of UniFrac distances (black points and line) and community growth adaptation as SI (log growth at 40 °C/at 4 °C) (red points and line) as a function of soil temperature elevation above ambient. For visualization, lines join mean values (large points) computed for groups of samples with similar measured temperature elevations (7–10 groups); error bars are standard errors of the mean for both the response and the soil temperature elevation (n = 3–9 samples per group). Actual statistical modelling (see Table 1) was performed on plot-level observations. Values of SI of 0.5 and 1.5 correspond to T_{min} of approximately −7 and −4 respectively, using relationships from Nottingham et al. (2019) (c) Relationships between plot-level bacterial community composition (PCoA1 scores) and growth adaptation to temperature as a Sensitivity Index (SI) separated by vegetation type (grassland, GN, orange squares, and forest, FN, green circles). Lines represent linear regressions, in both cases $P < 0.05$, $R^2 = 0.78$ (forest), 0.74 (grassland). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the higher temperature regimes. Most previous studies of the relationship between soil temperature and temperature adaptation of bacterial community growth expressed the results in terms of T_{min} (the theoretical minimum temperature for growth) derived from fitting the Ratkowsky square root model to temperature-growth data (Bååth, 2018). Converting SI to T_{min} showed that T_{min} would increase from around −6.5 °C to −3.5 °C at temperatures >15 °C above ambient, an increase of 0.2 °C per 1 °C increase in temperature. A T_{min} of −6 to −7 °C with a MAT of +5 °C is within the expected range for this sub-arctic oceanic climate (Bååth, 2018). The increase due to the soil warming is comparable to earlier estimations based on climatic gradients (Rinnan et al., 2009; Nottingham et al., 2019), where an increase of 0.2–0.5 °C per degree increase in MAT was typically found. In summary, the increase in T_{min}/degree increase in MAT appears always to be < 1. Bååth (2018) suggested, based on the studies above, a value for a T_{min} increase of 0.3 °C/degree increase in MAT, which is similar to our results.

An alternative to SI and T_{min} is to compare Q_{10} values (the fold change per 10 °C warming of a biological process) measured over a defined interval, where higher Q_{10} values, calculated over the same temperature span, will be found for a more warm-adapted community with higher T_{min} (Bååth, 2018; Nottingham et al., 2019). Thus, a T_{min} of −6 to −7 °C, found at ambient conditions, is equivalent to a Q_{10} between 10 and 20 °C of around 2.6, while a T_{min} of −3.5 °C, found at temperatures >15 °C, corresponds to a Q_{10} of 3.0 (calculated according to Bååth, 2018). Previous studies of respiration at the ForHot grassland site reporting no significant Q_{10} changes in warmed areas (Marañón-Jiménez et al., 2018; Walker et al., 2018). While Walker et al. (2018) only used soils with warming up to 6 °C above ambient temperature (i.e. with results compatible to the present study), Marañón-Jiménez et al. (2018) studied respiration of grasslands soils with warming levels up to Amb+15.9 °C. A different sensitivity of temperature adaptation for soil respiration compared to bacterial growth may explain this difference, although similar changes in temperature adaptation at different MAT for both variables have previously been reported (Bååth, 2018; Li et al., 2021). An alternative explanation is the relative large variability in the respiration data in combination with fewer soils from plots with >+8 °C above ambient (only 2 plots). A closer inspection of their results (Table 2, Marañón-Jiménez et al. (2018)) reveals that plots with Amb+<8 °C had Q_{10} values of 2.29–2.83 (similar to the value of 2.6 we found for bacterial growth), while the plots with Amb+>8 °C had higher Q_{10} for respiration, 3.09 and 4.77 (as expected of a warm-adapted community, Bååth, 2018), suggesting a lack of statistical power may have led to the conclusion of no change in temperature sensitivity. Instead, a threshold in temperature adaptation similar to the one described in the present study may also be present for soil respiration.

Table 1
Distribution of bacterial OTUs across phyla (only top 10 most abundant phyla shown). Total number of OTUs as well as numbers observed to significantly differ (positively or negatively) between samples above or below 6 °C in one or both sampling sites. Significant differences were calculated using ANCOM-BC ($P < 0.05$, after Bonferroni false discovery correction).

Phylum	Total # OTUs	Increasing indicators Grassland	Decreasing indicators Grassland	Increasing indicators Forest	Decreasing indicators Forest	Increasing indicators both sites	Decreasing indicators both sites
Acidobacteria	1013	31	41	21	156	8	24
Actinobacteria	763	27	15	13	84	2	8
Bacteroidetes	585	5	16	9	33	0	7
Chloroflexi	456	6	5	13	28	2	1
Firmicutes	62	6	0	1	5	0	0
Gemmatimonadetes	158	1	3	1	8	0	2
Nitrospirae	71	2	4	3	6	0	3
Proteobacteria	1884	45	46	35	145	12	13
Verrucomicrobia	426	12	24	3	59	0	13
WS3	66	0	4	0	3	0	1

Table 2
Model selection metrics (AIC values) comparing linear regression and single breakpoint stepwise function models for each of community profile (PCoA axis 1 scores), temperature sensitivity index (SI, log (growth at 40 °C/4 °C)), and bacterial alpha diversity (Shannon) for both vegetation types. Bold numbers indicate the selected best model (lowest AIC).

		Linear Model ($k = 3$)	Breakpoint model ($k = 3$)
Forest	Community profile ($n = 41$)	−83	**−98**
	SI ($n = 35$)	2.3	**−2.7**
	Alpha diversity ($n = 41$)	63	**47**
Grassland	Community profile ($n = 35$)	−72	**−93**
	SI ($n = 42$)	1.1	**−7.2**
	Alpha diversity ($n = 35$)	31	**7**

4.3. Mechanisms behind threshold dynamics

The clear evidence of a threshold dynamic in the response of community composition for both vegetation types to increasing soil temperature confirms previous observations from this geothermic area (Radujković et al., 2018; De Jonge et al., 2019). However, our study provides evidence that this compositional shift is accompanied by a change in the temperature adaptation of the bacterial community. We do not know whether the abrupt shift of bacterial communities we observed is unique to our study site, or reflects a warming response in a wider range of soils and ecological contexts. If the pattern is indeed more common, it may explain some of the divergence in results of warming experiments. Studies that show non-effects of warming on soil bacterial communities may have involved experimental warming treatments that fail to surpass the critical threshold temperature. Given that our best estimates of the threshold temperature for warming effects is in the range 6–9 °C, this would explain the relatively minor effects of open top chamber treatments (typical warming effects 0.5–3 °C, Marion et al., 1997) and even soil heating cables (typical warming effects ~ 5 °C, Rustad et al., 2001). It is important to note that the exact level of warming at which the temperature threshold occurs is not particularly well constrained by our data, due to uncertainty in the precise level of temperature elevation at each site, and relatively sparser sampling along the gradient above +9 °C.

Threshold dynamics in community changes with warming could arise as the result of an ecophysiological 'tipping point' that is common to bacterial communities. It has been suggested that warming that is close to or exceeds the community aggregated T_{opt} for growth represents such a limit (Bárcenas-Moreno et al., 2009; Donhauser et al., 2020), but the result from the present study is not in accordance with this, since even our highest estimate of the threshold warming represents a soil MAT of approximately 17 °C, compared to a community T_{opt} of about 30 °C in ambient soils (Fig. 1).

An alternative explanation for the observed tipping point are time lag effects; that is, changes take time, especially at low temperatures. It is possible that given enough time all communities reach a compositional and physiological "equilibrium" state relative to soil temperature. Indications of this are that changes in T_{min} have been found in gradient studies at low temperatures, where changes in MAT are well below T_{opt} for bacterial growth (Rinnan et al., 2009; Nottingham et al., 2019). The rate of this process of equilibration appears itself to be driven by temperature, in that warmer soils will reach the new steady state more rapidly than colder soils, even with the same extent of warming (Pettersson and Bååth, 2003; Nottingham et al., 2021). In our case, the threshold temperature is an increase from a low ambient MAT (5.2 °C), suggesting a relatively slow rate of temperature adaptation. Even longer periods of slow adaptation may, however, be expected given that a grassland that had a warming duration of at least 50 years - close by the locations of the present study - still showed threshold dynamics in community composition (Radujković et al., 2018).

There appeared to be an upper limit to the magnitude of temperature-related impacts on community composition, SI or diversity; temperature effects appeared to 'level-off' in all soils >+15 °C above ambient ones (Figs. 2 and 5a and b). We are, however, reluctant to draw any firm conclusion on this. Because of the way it is calculated, it is difficult to study such a wide gradient using SI: measurements at the low temperature (in our case 4 °C) will be close to T_{min} for bacterial growth in samples taken from soils with the highest warming. Measurements taken close to the minimum temperature for growth will lead to large uncertainties when calculating SI. In the case of community composition metrics, the lack of temperature effect at the higher end of the temperature gradient could be due to a real lack of turnover in species composition at higher temperatures. Alternatively, this could be an artefact of the dimension-reduction procedure, combined with relatively few samples at the higher end of the gradient. For example, taxa that distinguish among higher temperature samples but are completely absent from the (more numerous) lower temperature samples would likely not contribute to the first two principle coordinates axes presented here.

4.4. Implications for further research

Regardless of the underlying mechanism responsible for the threshold dynamics, the observation that a major shift in community composition occurred at the same level of warming as a similarly abrupt change in temperature adaptation provides strong, although not conclusive evidence that shifts in the composition were driven by direct temperature effects. Soil and vegetation parameters have also changed along this geothermal gradient (Sigurdsson et al., 2016; Walker et al., 2020; Verbrigghe et al., 2022), implying that at least some of observed changes in bacterial community could be indirect effects. In particular, with our data we cannot definitively rule out a role for changing substrate supply in influencing both community composition and temperature adaptation. However, in our opinion, the close relationship

between community composition and SI would be unlikely if drivers such as substrate, pH, or soil texture were the dominant control, especially since the same adaptation to temperature was found both in forest and grassland, with very different initial communities and quality of soil carbon inputs.

Oliverio et al. (2017) compiled data on bacterial taxa that could be used as indicator species for warming. This idea of a key set of responsive species was partially supported by our data, since both sites had community changes along PCoA1, which could be explained by temperature. However, there were also temperature changes along PCoA2, that were specific for the grassland soil, and thus the overlap in indicator species was relatively small. This suggests that a large part of the warming response will be soil-specific, presumably due to the community filtering effects of other soil physico-chemical characteristics, e.g. pH (Lauber et al., 2009). More comparisons of response patterns across a broader range of soil types are therefore needed. Nevertheless, the use of growth-based trait or function determination, as used here for temperature, may be an efficient tool to disentangle driving factors for the community composition (Hicks et al., 2022), as done earlier for pH (Fernández-Calviño and Bååth, 2010), soil salinity (Rath et al., 2019), heavy metals (Fernández-Calviño et al., 2011), and moisture (de Nijs et al., 2019).

Although this geothermic gradient is a natural event and therefore worth studying in its own right, much of the interest in similar areas is related to predict effects of future global change. However, geothermic warming (and similar effects due to heating cables) is not a perfect simulation of a warming climate, as the warming effects are limited to the soil and, only to a lesser extent, the immediately overlying vegetation (Sigurdsson et al., 2016). This drawback is of minor importance here because of the focus on direct temperature effects on the soil microbes, since the organisms will be directly affected by temperature irrespective of whether the above ground environment is warmed or not. The direct temperature effects on the soil bacterial community observed here, as induced by temperature adaptation of the community, thus likely also applies to climate warming. In both cases, soil warming is the driving force of microbial responses. We therefore recommend incorporation of the observed threshold dynamics in ecosystem models that aim to predict impacts of climate warming/novel environments and conditions on microbial dynamics.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2022.108914.

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