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. A unique area in Iceland, where soil temperatures have increased due to geothermic activity four years prior to sampling, creating a stable gradient of ambient to +40°C, allowed us to investigate the shape of the response to warming of soil bacterial communities, 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)). Samples were taken from ≥9 levels of warming (covering almost up to +40°C above ambient soil temperature), under both grassland (*Agrostis capillaris*) and forest (*Picea sitchensis*) vegetation. The soils had very different community composition, but temperature adaptation was the same. Both diversity and community composition as well SI showed similar threshold dynamics along the soil temperature gradient. There were no significant changes up to soil warming of approx. 6-9 °C, beyond which all indices shifted in parallel. The consistency of these responses gives strong support for a decisive role for direct temperature effects in driving bacterial community shifts along soil temperature gradients.

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). Community responses to temperature and other climate-related variables is a fundamental ecological pattern which must be understood to fully explain the drivers of microbial distribution and abundance (Zhou et al., 2016; Delgado-Baquerizo et al., 2018). 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)

For both fundamental ecological understanding, and for applications to ecosystem modelling, 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 at a higher magnitude than other parts of the globe (Post et al., 2019), and temperature sensitivity of soil microbial activity is higher at low temperatures (Kirschbaum, 1995, 2000). There is thus a large potential for a positive climate feedback via warming-induced increases in net C release, which may be mediated by the activity and temperature sensitivity of soil microbial communities (Cavicchioli et al., 2019).

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), 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

43 studies may introduce additional confounding factors related to soil and vegetation. In
44 experimental warming studies practical considerations compel investigators to choose a small
45 number of (usually small) temperature steps (De Boeck et al., 2015). Studying temperature
46 responses of soil communities along geothermal gradients at local-scales (10 – 100s of
47 metres) overcomes these limitations (O'Gorman et al., 2014). Potential biogeographic and
48 large-scale edaphic confounders are held constant; and a range of temperatures can be studied
49 allowing for the study of dynamics not possible with step-wise experimental set-ups.

50 A near-surface geothermal system that arose after an earthquake in SW Iceland provides a
51 valuable model system for studying soil temperature effects on ecosystem processes
52 (Sigurdsson et al., 2016). This system has been used to study the effects of warming on a
53 wide range of processes in soil ecology and biogeochemistry (Walker et al., 2018, 2020;
54 Marañón-Jiménez et al., 2019; Poeplau et al., 2020; Zhang et al., 2020) Focussing on the
55 microbial community composition, Radujković et al. (2018) found that bacterial and fungal
56 communities changed only at warming levels exceeding +6-8 °C above ambient. However,
57 the authors could not decisively conclude whether this change was a direct effect of
58 temperature or indirect effects due to, for example, effects on vegetation growth and
59 phenology (Leblans et al., 2017), or an observed reduction in soil organic matter
60 concentration, and soil texture (Poeplau et al., 2017; Verbrigghe et al., 2022). It is difficult to
61 distinguish between potential drivers using community data alone, given that the relationship
62 between specific taxonomic groups and ecological functions is largely unknown (Prosser,
63 2012).

64 A possible method for more precisely evaluating the direct effects of temperature on
65 microbial communities is to directly characterize their physiological adaptation to
66 temperature. It has been shown that soil bacterial communities adapt to the thermal
67 environment, shifting measurable aspects of their aggregated temperature response (e.g.

optimal [T_{opt}], and theoretical minimal temperatures [T_{min}] for growth) with changing temperature (Rinnan et al., 2009; Rousk et al., 2012; Bååth, 2018; Nottingham et al., 2019; Li et al., 2021). 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., 2021). A similar approach has been used to understand microbial community responses to soil pH changes (Pettersson and Bååth, 2013), and to link community composition and salt tolerance across a salinity gradient (Rath et al., 2019) .

While community adaptation to temperature has been expressed in different ways (Bradford et al., 2008; Karhu et al., 2014; Dacal et al., 2019) the use of the apparent minimum temperature for growth (T_{min}), estimated using the square root (also called the Ratkowsky) equation (Ratkowsky et al., 1982), provides an easily interpretable index (Pietikäinen et al., 2005; Bååth, 2018). In measurements taken on communities extracted from soils, T_{min} increases as a response to increasing soil temperature (Rinnan et al., 2009; Rousk et al., 2012; Bååth, 2018; Nottingham et al., 2019). This is also seen in pure culture studies, where differences in T_{min} can be used to differentiate between psychrophiles, mesophiles and thermophiles (Ratkowsky et al., 2005; Corkrey et al., 2016). 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). A high value of SI indicates community adapted to relatively higher temperatures, while a low value reflects low-temperature adaptation. SI has earlier shown to correlate well with T_{min} (Rinnan et al., 2009, Nottingham et al., 2019).

In this study, we combined measures of temperature adaptation with taxonomic characterization of bacterial communities in order to test whether the previously observed shifts in bacterial community composition along the FORHOT 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 community composition (a forest and grassland soil). We hypothesized that 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 SI indicating adaptation to higher temperatures. We also hypothesized that the same correlations would be found in the two different soils, despite having different original community composition. Thus, we expected temperature adaptation to be dependent on the temperature increase to the same degree in both soils.

Materials and Methods

Site description and sampling

The study area is the ForHot research site in the Hengil 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. Sampling at the research site is concentrated in two vegetation types: an unmanaged grassland (in the area denoted "GN" in Sigurdsson et al., 2016) dominated by *Agrostis capillaris*, *Ranunculus acris* and *Equisetum pratense*; and a planted *Picea sitchensis* forest ("FN" in Sigurdsson et al., 2016), with no significant understorey vegetation. In May 2008 an earthquake shifted geothermal systems in the area surrounding the research site, resulting in hot groundwater warming the underlying bedrock and thus increasing the soil temperature in previously un-warmed soils. This resulted in transects with a gradient of increasing temperatures, from un-warmed controls to +40°C compared to the ambient temperature in the forest and grassland site. 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 site four replicate transects were established, each consisting of 10 or 11 sampling plots located to cover to produce a set of temperatures on each transect. The target temperatures were: ambient, +1, + 2, +3, +4, +6, + 8, +20, +40 and plots were located at these temperatures using a handheld temperature probe inserted to 10cm depth. Subsequent monitoring of the soil temperature in each plot revealed some divergence from target temperatures, and for all analyses below we use the plot-level averages of temperature offset relative to ambient soils,

134 measured on 4 – 10 different occasions in the period from October 2011 to July 2013 (see
135 Appendix 1 for further discussion of temperature measurements). Note that although in the
136 same area, these plots are not the same as those forming the permanent transects established
137 in 2013 and described in several recent papers (Sigurdsson et al., 2016; Marañón-Jiménez et
138 al., 2018; Radujković et al., 2018; Walker et al., 2018, 2020). For bacterial community
139 analyses, three soil cores were taken at each plot with a 3cm diameter soil corer and the soil
140 from 5 – 10 cm depth were retained for further analyses. Soil samples for community
141 temperature adaptation measurements were taken 7 days later from the same sites using the
142 same sampling method.

143

144 **DNA extraction, amplification and sequencing**

145 Soil samples were stored at 4°C within two hours of sampling, and processed in the following
146 48 hours. Soils were sieved and mixed, and DNA extracted using MoBio PowerSoil DNA kit
147 following the manufacturer's protocol. DNA samples were checked for quality and quantity
148 using a Nanodrop spectrophotometer and prepared for Illumina sequencing of the V3 region
149 of the bacterial 16S rRNA gene using the primers in Bartram et al. (2011) following the
150 procedures described in Weedon et al. (2017). Libraries were sequenced on an Illumina
151 MiSeq using 2x 150 cycle paired-end sequencing (V2 chemistry) at the VUmc Clinical
152 Genetics sequencing facility (Amsterdam, The Netherlands).

153

154 **Bioinformatics**

155 Initial sequence processing and OTU clustering was performed using the USEARCH
156 software (Edgar, 2013). Paired-end sequences were assembled with 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. Operational taxonomic units (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 will be deposited in the NCBI Sequence Read Archive.

Community temperature adaptation measurements

Soil samples were stored at 17°C until analysed (within 2 months). The higher temperature used (not the more commonly used 4°C) was in order to not affect temperature adaptation in the soils with highest temperature. Still, 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 (denoted log (growth at 40ºC/4ºC) 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 (Rinnan et al., 2009; Nottingham et al., 2019). The index correlates to the more commonly used T_{min} (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.

For each replicate, bacteria were extracted from soil (1 g soil in 25 mL water) by shaking, followed by a low speed centrifugation (1000 x g for 10 min). The bacterial suspension was then distributed in microcentrifugation vials (1.5 ml in each) and ^{3}H-Leu 37 MBq mL^{-1} and 5.74 TBq mmol^{-1}, Perkin Elmer, USA) and unlabeled Leu (final concentration of 275 nmol L^{-1}) was added after 30 min at the chosen incubation time. Growth was terminated by adding trichloroacetic acid after incubating for 2.5h at 40°C and 24h 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).

Control 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 original T_{min} and T_{opt} for bacterial growth. 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 the log ratio growth at 35°C/0°C was found in a gradient spanning 20°C in mean annual temperature (Nottingham et al., 2019). This was used together with a conversion factor from

206 the growth ratio 40°C/4°C to 35°C/0°C (unpublished) to calculate corresponding values of
207 T_{min} from growth ratios 40°C/4°C.

208

209 **Statistics**

210 Alpha diversity (Shannon index) was computed for each bacterial community sample.
211 Pairwise weighted Unifrac distances (Lozupone et al., 2006) were computed for all sample
212 combinations and visualized using principle coordinates analysis (PCoA). The first PCoA
213 axis (explaining 25% of the dataset variation) was subsequently used as a univariate proxy of
214 community composition. The significance and relative magnitude of site and temperature
215 elevation effects on total community composition was analysed with a permutational
216 multivariate analysis of variance (Anderson, 2001) on the Unifrac distance matrix.

217 Alpha diversity indices, PCoA scores and SI were each modelled as a function of temperature
218 elevation separately for each site. Initial visualization indicated a step-wise pattern for all
219 responses at both sites. This was further investigated by fitting regression trees to each
220 dataset and comparing the resulting break-point model with normal linear regression using
221 AIC values. Given that there was some uncertainty as to the temperature elevations at each
222 plot (see Appendix 1) we used a parametric bootstrap approach to estimate the uncertainty
223 associated with the breakpoint in the tree regression for each response x site combination.
224 Bootstrap distributions of the breakpoint were generated by generating 1000 sets of
225 temperature points created by adding Gaussian distributed noise to each of the temperature
226 values, and refitting the regression tree model. The standard deviations of the added noise
227 were based on the degree of confidence of each interpolated temperature elevation (ranging
228 from 0.5 to 2.5 °C). To directly test for a relation between community temperature adaptation

and composition, separate linear regressions were performed between PCoA1 scores and SI for each site.

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 Bonferroni method as for false discovery rate correction.

Results

Community adaptation to temperature

Bacterial growth in ambient soils from both sites 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 soils, -6.6°C and -5.4°C in the grassland and forest, respectively. T_{opt} was not specifically determined, but appeared to be 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.

Bacterial community composition

In ambient soils the bacterial communities of both the grassland and forest sites were dominated by OTUs assigned to the phyla Proteobacteria (35% of reads in the forest site, 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. Bacterial community composition was significantly different between the two vegetation types (Weighted Unifrac PERMANOVA $P = 0.004$, $R^2 = 0.27$, $n = 13$).

Soil temperature effects on bacterial community composition

The bacterial community was significantly affected by increasing soil temperatures at both sites. This was visible at the level of OTU 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 a large number of differentially abundant OTUs (Fig. 4; ANCOMBC; $P<0.05$).

Alpha diversity decreased from around 6.5 to 7 in both ambient sites to between 5 and 6 in the warmer soils (Fig. 3). 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 was 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 magnitude as site differences in explaining variation in the bacterial community composition (weighted 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, 849 out of 2926 OTUs for the forest site and 303 out of 3120 OTUs for the grassland site showed differential abundance across the soil warming gradient. Only 104 OTUs were differentially abundant in both sites, of which most OTUs belonged to the phyla Proteobacteria, Acidobacteria, Actinobacteria, and Verrucomicrobia (Fig. 4, Table 1). In the forests soils, the 186 OTUs that increased in abundance went from contributing 0.7 % of the total reads in the ambient soil samples to 49.3 % in the soils warmed above 6 °C. In total 663 OTUs decreased in abundance significantly, reducing the abundance of the total group from 60.2 to 17.3% of the total reads. In grassland soils, 142 OTUs increased in abundance from 3.3 % of the total reads in the ambient soil to 25.5 % in the soils warmed above 6 °C. In total 161 OTUs decreased in abundance significantly, reducing in their contribution to total reads from 13.7 to 3%.

Soil temperature effects on bacterial community adaptation to temperature

Increasing soil temperatures resulted in increased SI in both the forest and the grassland (Fig. 5), 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°C in ambient sites to around -1 °C in the sites with >+15°C above ambient temperatures (MAT increasing from +5°C to >20°C). Thus, T_{min} increased around 5°C with an increase in MAT of >15°C.

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)), with the exception of SI in the grassland site, 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 7.5, 6.1 and 8.6°C for community profile, SI and Shannon Diversity in the forest site, with corresponding values for the grassland being 9.5, 9.0 and 9.0°C (Fig. 6). However, when accounting for uncertainty in soil temperature measurements, the range of estimated threshold

305 temperatures spanned 5 to 12 ° C (end points of bootstrap 95% confidence intervals) with
306 relatively more uncertainty at the grassland site.

307 As noted above, the observed shifts in bacterial community composition and SI with
308 temperature followed very similar dynamics in both sites (Fig. 5). Accordingly, there were
309 significant linear correlations between these two measures at both sites (R^2=0.78 and 0.74,
310 for forest and grassland, respectively (Fig. 7).

Discussion

In this study we characterized bacterial community composition and physiological 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 change significantly relative to ambient conditions when subject to more than approximately 6-9 °C warming (Radujković et al., 2018; Walker et al., 2018). Crucially, we provide the first evidence that this community shift is driven by direct responses to temperature, by showing that community change coincided closely with increases in temperature adaptation of the community. This overlapping of community trait and compositional measures was observed in both grassland and forest soils, even though each environment had distinct bacterial community profiles at both ambient and warmed conditions.

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, which was demonstrated by more OTUs declining in abundance over the warming gradient than OTUs increasing in abundance (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). This suggests different effects of temperature in response to long-term (natural temperature gradients) versus short-term warming (4 years in the present temperature gradient).

333 The warm responders made up a small fraction of the bacterial community (0.6% in forest
334 and 3.3% in grassland) in the ambient soil but increased to 48.7 and 25.5% of the total
335 community above 6°C warming in the forest and grassland, respectively. A similar (partial)
336 community turnover was also observed in an incubation of alpine soils, where increasing
337 temperatures lead to a community dominated by presumably warm-adapted taxa (Donhauser
338 et al., 2020). Community-level responses to warming, either experimentally imposed, or
339 measured along climatic gradients, have been observed for bacteria in a number of soil
340 environments (Yergeau and Kowalchuk, 2008; DeAngelis et al., 2015; Oliverio et al., 2017;
341 Monteux et al., 2018; Radujković et al., 2018), although they are not always present (Weedon
342 et al., 2017). The responding OTUs appeared to be fairly evenly distributed amongst the
343 major phyla, and within each phylum there were OTUs that both increased and decreased
344 with soil warming. This emphasizes that temperature niche seems to be largely decoupled
345 from high-level taxonomic identity (Oliverio et al., 2017; Radujković et al., 2018) supporting
346 the idea that temperature preference of bacteria is a shallowly conserved trait (Martiny et al.,
347 2015).

348 **Bacterial community adaptation to temperature**

349 The bacterial community adaptation to temperature, which we estimated based on the log
350 ratio of bacterial growth rates measured at 40 and 4 °C (SI), was relatively constant along the
351 warming gradient up to between 6 and 9°C before abruptly increasing. Most previous studies
352 of the relationship between soil temperature and temperature adaptation of bacterial
353 community growth have expressed the results in terms of T_{min} (the theoretical minimum
354 temperature for growth) derived from fitting the Ratkowsky square root model to
355 temperature-growth data (Bååth, 2018). Nottingham *et al.* (2019) showed that there was a
356 close correlation between T_{min} and SI, meaning that not only is SI a good indicator of
357 temperature adaptation, but our observed SI shift would be approximately equivalent to an

358 increase in T_{min} from around -6°C to between -1 and -2 °C at temperatures >15ºC above
359 ambient - an increase of 0.27 to 0.33°C per 1°C increase in temperature. A T_{min} of -6°C with
360 a MAT of +5°C is within the expected range for this subpolar oceanic climate (Bååth, 2018).
361 The increase due to the soil warming is also comparable to earlier estimations based on
362 climatic gradients (Rinnan et al., 2009; Nottingham et al., 2019), where an increase of 0.2 to
363 0.5°C per degree increase in MAT is typically found. Higher values (around 0.8 per 1°C
364 warming) have also been reported from incubation studies (Birgander et al., 2013;
365 Donhauser et al., 2020), whereas field warming studies have tended to estimate smaller or
366 even absent effects (Rinnan et al., 2011; Rousk et al., 2012). Still, the increase in T_{min} per
367 degree increase in MAT appears always to be less than one. Bååth (2018) suggested, based
368 on the studies above, a tentative value for a T_{min} increase of 0.3°C/degree increase in MAT,
369 which is well in accordance with our results.

370 An alternative to SI, although less sensitive, is to compare Q_{10} values (the fold change per
371 10°C warming of a biological process) measured over a defined interval. For example, Q_{10} of
372 soil respiration increases at higher temperature conditions and decreases at cooler
373 temperatures (Kirschbaum, 1995). Previous studies of respiration at the FORHOT grassland
374 site have used this approach (Marañón-Jiménez et al., 2018; Walker et al., 2018), reporting
375 the same temperature sensitivity in warmed areas compared to control sites. This implies no
376 community adaptation to increasing temperature in contradiction of our results from growth
377 assays. In the case of the results from Walker et al. (2018) only soils with warming up to 6°C
378 above ambient temperature were included, that is, their results are compatible with the
379 present study, since this is similar or less than our estimated threshold temperature. Marañón-
380 Jiménez et al. (2018) studied respiration of grasslands soils with warming levels up to 15.9°C
381 without any significant changes in the apparent temperature sensitivity of microbial
382 respiration. One reason for this different result may be due to varying sensitivity of

383 temperature adaptation for soil respiration as opposed to bacterial growth. However, Bååth
384 (2018) compiled data on both bacterial growth and respiration and found that these processes
385 appeared to have comparable T_{min} in soils globally, and therefore presumably similar
386 responses to warming. Li et al. (2021) also found that T_{min} for soil respiration correlated to
387 MAT in a large set of soils covering alpine to tropical soils. An alternative explanation for the
388 discrepancy could be the relative large variations in the respiration data, as well as the use of
389 fewer soils sampled from areas with temperatures at >8°C above ambient (only 2 areas in one
390 site compared to our two sites with 3-5 areas each). Indeed, a closer inspection of their
391 results (Table 3 in Marañón-Jiménez et al. (2018)) reveals that sites with Amb+<8°C had Q_{10}
392 values of 2.29 to 2.83, while the two sites with Amb+>8°C both had higher Q_{10} for
393 respiration, 3.09 and 4.77, suggesting a lack of statistical power may have led to the
394 conclusion of no change in temperature sensitivity over the warming gradient. Instead, a
395 threshold in temperature adaptation similar to described in the present study may also be
396 present for soil respiration.

397

398 **Mechanisms behind threshold dynamics**

399 For all community composition measures the change with temperature was abrupt rather than
400 gradual, with the critical warming level triggering the shift estimated to be between
401 approximately 6 and 9 °C above ambient conditions, depending on the vegetation type and
402 community measure. The clear evidence of a threshold dynamic in the response of
403 community composition at both sites to increasing soil temperature confirmed previous
404 observations from this geothermic area (Radujković et al., 2018; De Jonge et al., 2019).
405 However, our study provides the first evidence that this shift is accompanied by a change in
406 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 we estimate a threshold warming of > + 6 °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).

Threshold dynamics in community changes with warming could arise as the results 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). Furthermore, changes in T_{min} has been found in gradient studies at low temperatures, where changes in MAT is well below T_{opt} for bacterial growth (Rinnan et al., 2009; Nottingham et al., 2019) An alternative explanation for the shape of the response 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. However, if the rate of this process of equilibration is itself driven by temperature, then warmer soils will reach the new steady state more rapidly than areas with more modest warming (Pettersson and Bååth, 2003). In our case, the threshold temperature is an increase from a low ambient MAT (5.2°C). This means that even ambient +6°C of warming only has a MAT of around +11ºC. This interpretation is supported by the observation from a reciprocal transplant study over an altitudinal gradient

that showed slower (decade-scale) temperature adaptation to cooler environments compared to adaptation to warmer environments (Nottingham et al., 2021). However, a comparison of grassland sites with different warming durations (50+ years vs 4 years, the same site as the current study) did not produce any evidence to support this time lag interpretation (Radujković et al., 2018).

Implications and further research

Regardless of the underlying mechanism responsible for the observed threshold dynamics, the observation that a major shift in community composition occurred at the same level of warming as a similarly abrupt change in SI provides strong evidence that shifts in the composition of the community was driven by direct temperature effects. A large variety of soil and vegetation parameters have also changed along this geothermal gradient (Sigurdsson et al., 2016; Walker et al., 2020), implying that at least some of observed changes in bacterial community could be indirect effects. However, the close relationship between community composition and SI would be unlikely if drivers such as substrate, pH, or soil texture were the dominant control. Even so, given the inevitable confounding of potential drivers in natural settings, controlled experiments that manipulate temperature, while holding other important driving factors constant, are still needed to confirm the predominance of direct temperature adaptation effects in determining responses of bacterial community composition to warming.

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., 2021), as done earlier for pH (Fernández-Calviño and Bååth, 2010), soil salinity (Rath et al., 2019), heavy metals (Fernández-Calviño et al., 2011), or 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 the need to better understand temperature effects on soil processes in order to predict effects of future global change. It is important to bear in mind that 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, to a lesser extent, the immediately overlying vegetation (Sigurdsson et al., 2016). However, for the present study with focus on direct temperature effects on the soil microbes, this drawback is of minor importance, since the soil organisms will be directly affected by temperature irrespective of whether the above ground environment is warmed to the same extent. Thus, our result on bacterial adaptation to warming, the extent of adaptation and the possibility of threshold temperatures for effects will be of interest for prediction and understanding of future global change effects.

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

760

Phylum	Total # OTUs	Increasing indicators Grassland	Decreasing indicators Grassland	Increasing indicators Forest	Decreasing indicators Forest	Increasing indicators both sites	Decreasing indicators both sites
Acidobacteria	1000	26	33	23	158	6	19
Actinobacteria	743	18	11	10	83	0	6
Bacteroidetes	592	4	13	11	29	2	6
Chloroflexi	461	5	5	12	25	1	1
Firmicutes	73	5	0	1	5	0	0
Gemmatimonadetes	159	2	1	1	8	0	0
Nitrospirae	70	2	3	4	7	0	2
Proteobacteria	1904	31	33	34	160	8	13
Verrucomicrobia	435	8	18	4	57	0	8
WS3	66	0	4	0	3	0	1

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763 **Table 2** Model selection metrics (AIC) comparing linear regression and single breakpoint
764 stepwise function models for each of community profile (PCoA axis 1 scores), temperature
765 sensitivity index (SI, log (growth at 40°C/4 °C)), and bacterial alpha diversity (Shannon) for
766 both sites.

		Linear Model (k = 3)	Breakpoint model (k = 4)
Forest	Community profile (n = 41)	-83	**-101**
	SI (n = 35)	1.8	**-7.6**
	Alpha diversity (n = 41)	63	**47**
Grassland	Community profile (n = 35)	-72	**-81**
	SI (n = 42)	**2.9**	4.1
	Alpha diversity (n = 35)	31	**15**

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Appendix 1 *Interpolation and uncertainty propagation of soil temperature data*

The plots established along the soil temperature gradients in both the grassland and forest sites were based on snapshot soil temperature measurements using a hand-held probe. In subsequent years, after the samples for the current study were taken, permanent soil temperature sensors and loggers were installed to allow more precise and dynamic soil temperature data. Due to the imprecision of the hand-held temperature measurements, and to allow for the possibility of discrepancy between temperature elevation measured on a given day, and longer-term trends in temperature elevation, it was decided to use the temperature logger data to calculate better estimates of average temperature elevation.

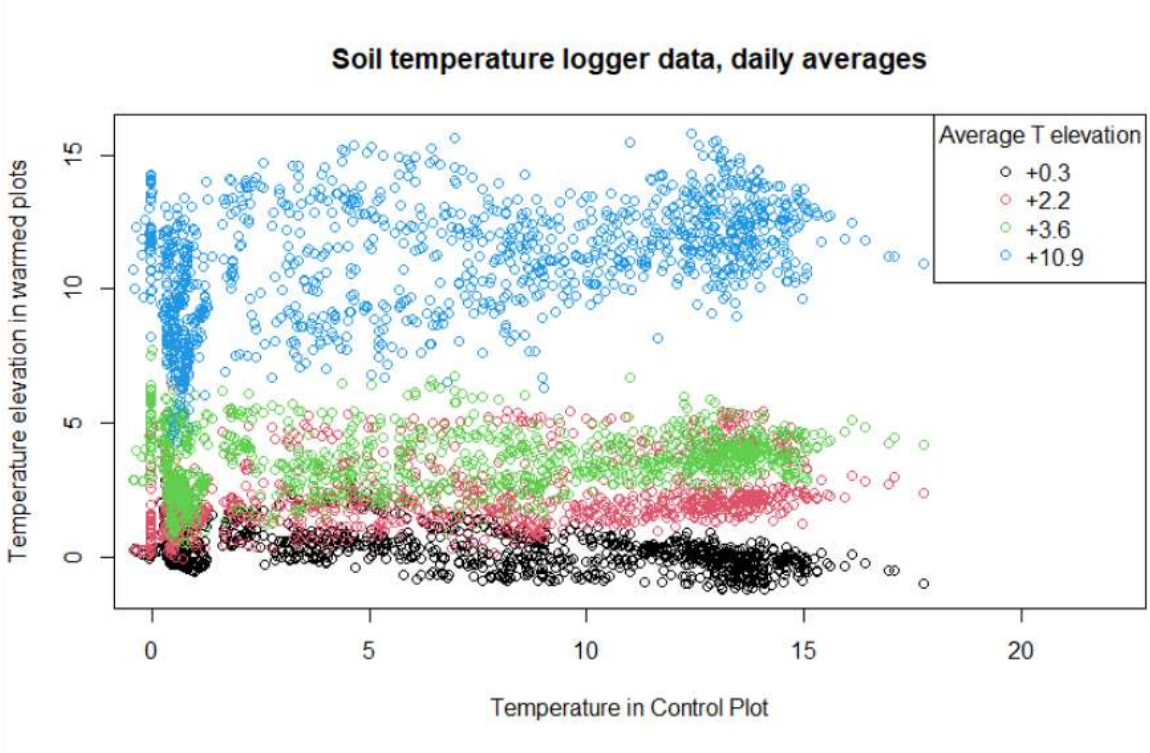
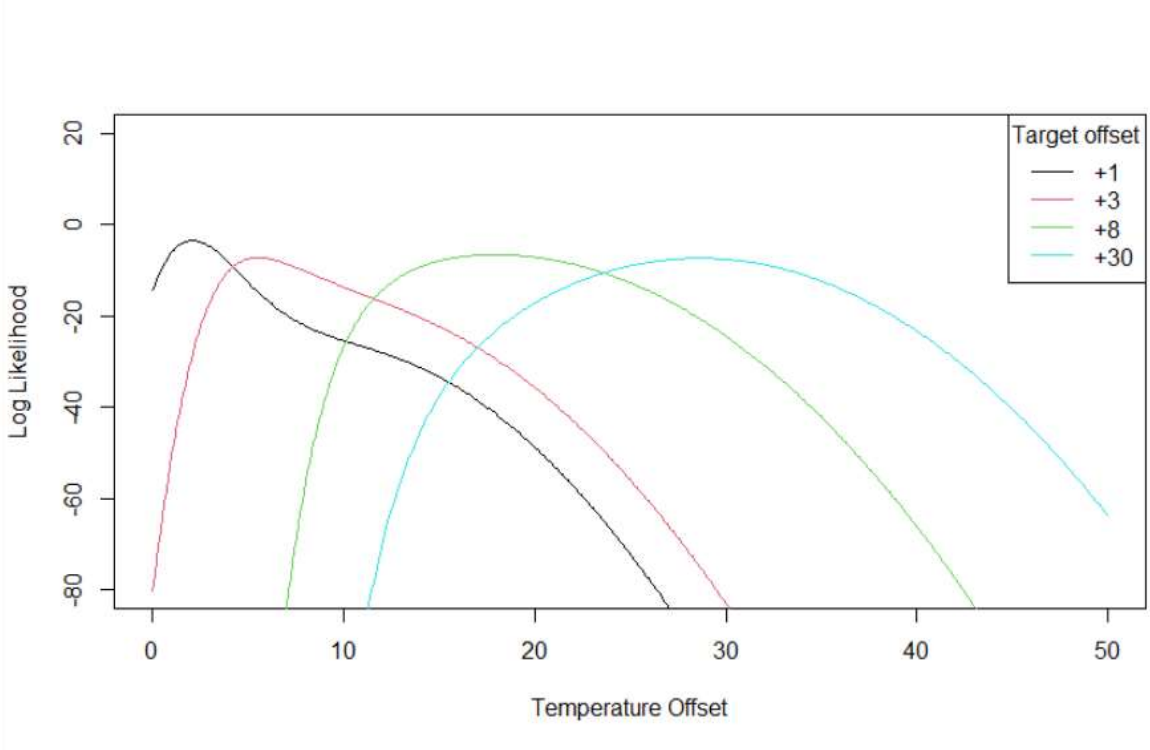


Figure S 1 Relationship between temperature elevation and soil temperature in control (ambient) plots over three years of sampling. Each point represents a daily average. The overall tendency of elevation of each plot is relatively consistent throughout the year (no linear trend) but there is considerable day to day variation. This variation was estimated and incorporated into the estimation procedure relating hand-held soil temperature measurements to long-term average elevations.

Logger data from 5 locations in each site were used to model the relationship between daily temperature averages in control plots and for each of the 4 other plots (spanning temperature elevations between +0.3 and +11.0 °C relative to ambient, Figure S1). The estimates of variance as a function of temperature elevation were then combined with the hand-held soil temperature measures (n = 1 – 3 different measurements depending on plot) to calculate

793 maximum likelihood estimates and associated errors of the long-term average temperature
794 elevation for each site (Figure S2)



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796 **Figure S 2** Example of maximum likelihood estimate of temperature offset based on patchy hand-held temperature data.
797 Curves are likelihood profiles for a parameter representing the real (unmeasured) long-term temperature offset. Likelihood
798 profiles were generated by assuming normal errors with variance estimated from the logger data (see Figure S1). Wider
799 profiles indicate greater uncertainty in the estimate of the underlying temperature offset.

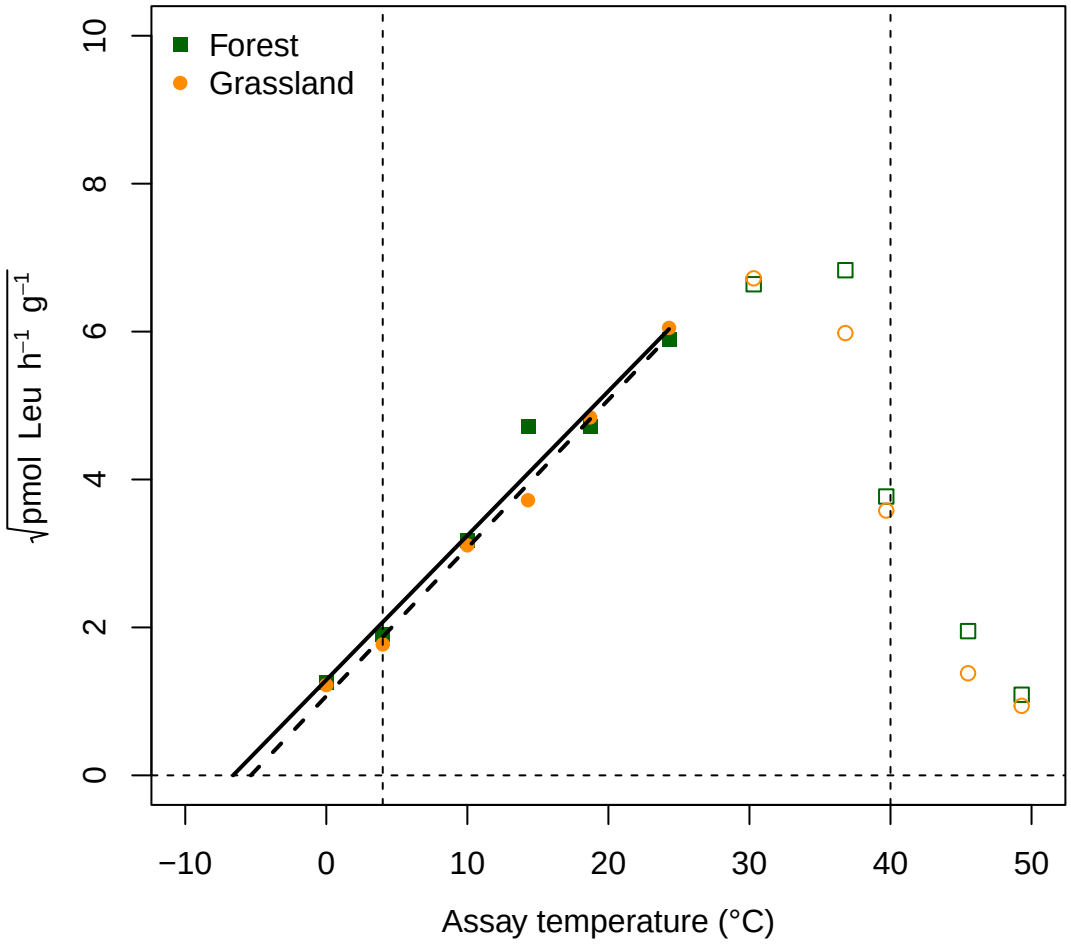

Figure 1: Temperature dependence of growth (Leu uptake) from bacterial communities sampled from soils with ambient temperatures (MAT 5.2°C) in the grassland (GN, squares and solid line) and forest (FN, circles and dashed line) habitats. The data are plotted using square root transformation, with filled 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).

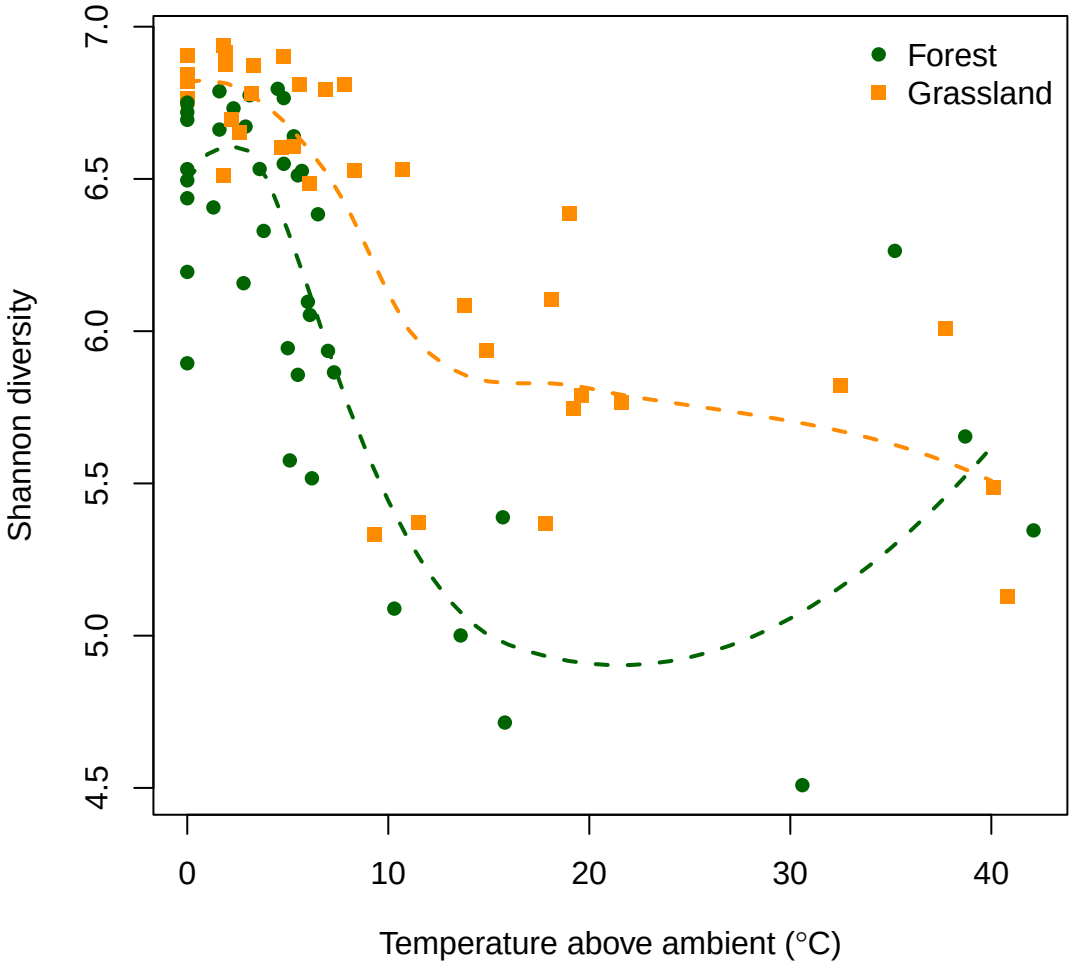

Figure 2: Alpha diversity of the bacterial community (Shannon index) versus soil temperature above ambient (MAT 5.2°C) along two geothermal soil temperature gradients (grassland,GN, yellow squares and forest, FN, green circles). Dashed lines represent a loess smoothing function.

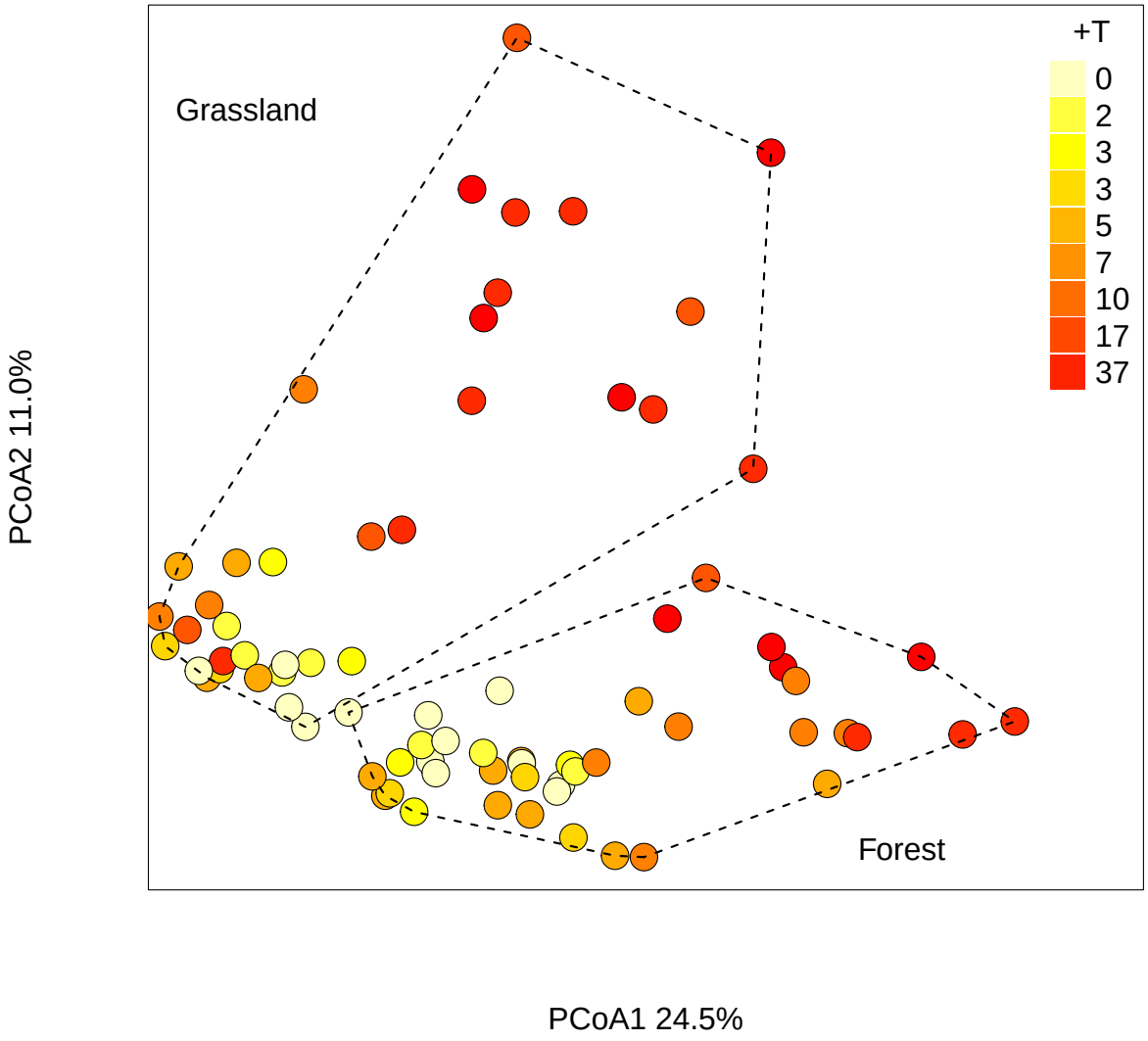

Figure 3: Principal coordinates analysis ordination (PCoA) of bacterial community profiles from two geothermal soil temperature gradients (grassland, GN, and forest, FN). Ordinations are based on weighted-Unifrac distances computed from 16S rRNA gene amplicon data and colour coded according to temperature elevation (in °C) above ambient soils (MAT 5.2°C)

Figure 4: Abundance shiftsof the OTUs that were differentially abundant (P<0.05) across the warming gradient for both Forest and Grassland , expressed in $\log_2$-fold changes and grouped by Order rank on the y-axis. Colors indicate Phylum rank.

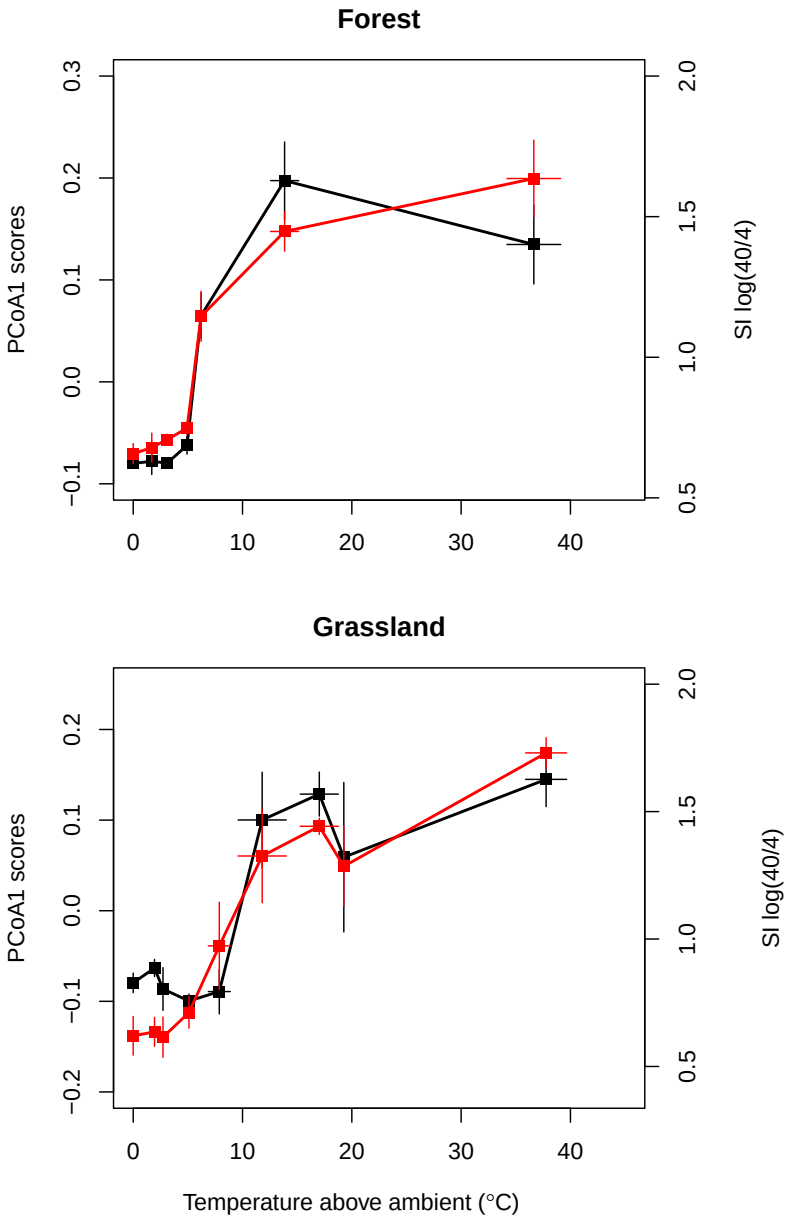

Figure 5: Relationships between bacterial community composition and community adaptation to temperature along soil temperature gradients. Bacterial community expressed as axis scores of PCoA ordinations of UniFrac distances (black points and line) and community growth adaptation as SI, (log growth at 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 temperature elevations; 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, Figure 6) was performed on plot-level observations.

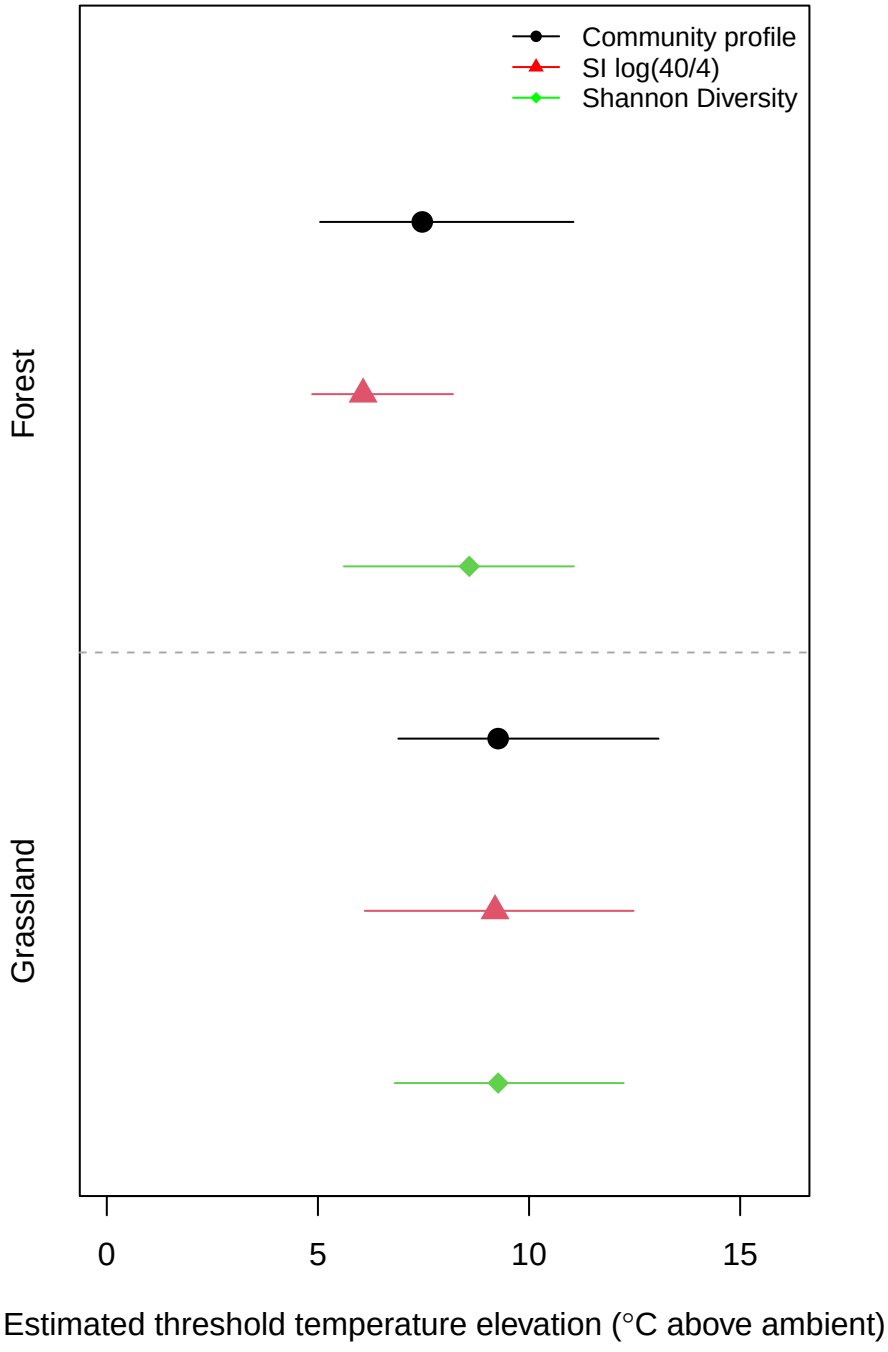

Figure 6: Estimates of temperature thresholds for significant changes and associated uncertainties for bacterial community composition (PCoA1 scores, see Fig. 3), growth adaptation to temperature as a Sensitivity Index (SI, log growth at 40°C/at 4°C), and Shannon diversity at two geothermal soil temperature gradients (grassland, GN, and forest, FN). Distributions were generated using parametric bootstrapping of temperature data, based on computed uncertainties in temperature estimates (for methology see Supplements). Bars and points correspond to the 95% confidence interval and median, respectively, of bootstrap distributions of the breakpoint in tree regression models.

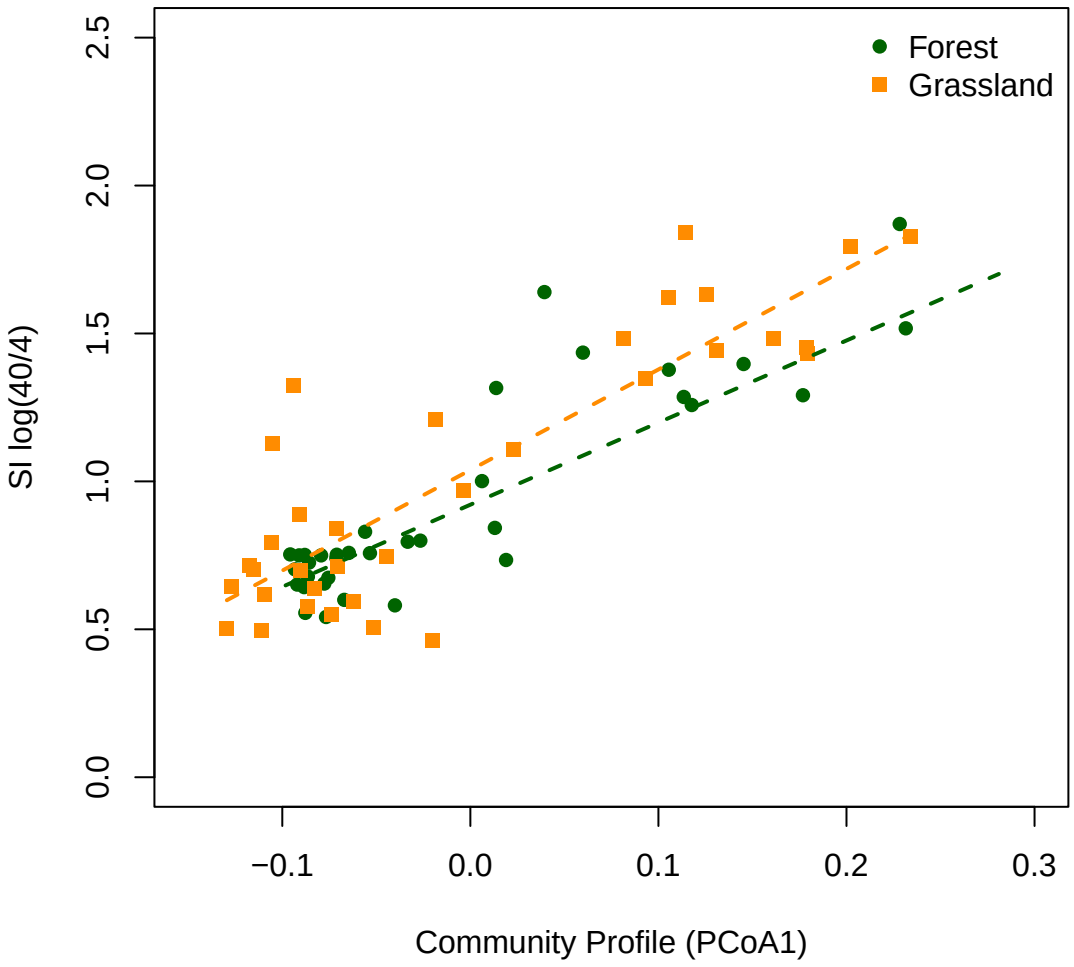

Figure 7: Relationships between bacterial community composition (PCoA1 scores, see Fig. 3) and growth adaptation to temperature as a Sensitivity Index (SI, log growth at 40°C/at 4°C) for samples along two geothermal soil temperature gradients (grassland, GN, orange squares, and forest, FN, green circles). Lines represent linear regressions, in both cases $P < 0.05$, $R^2 = 0.78$ (forest), 0.74 (grassland).