Maximum summer temperatures predict the temperature adaptation of Arctic soil bacterial communities

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Abstract

Rapid warming of the arctic terrestrial region has the potential to increase soil decomposition rates and form a carbon-driven feedback to future climate change. For accurate prediction of the role of soil microbes in these processes it will be important to understand the temperature responses of soil bacterial communities and implement them into biogeochemical models. The temperature adaptation of soil bacterial communities for a large part of the Arctic region is unknown. We evaluated the current temperature adaption of soil bacterial communities from 12 sampling sites in the sub- to High Arctic. Temperature adaptation differed substantially between the soil bacterial communities of these sites, with estimates of optimal growth temperature (T_{opt}) ranging between 23.4 ± 0.5 and 34.1 ± 3.7 C. We evaluated possible statistical models for the prediction of the temperature adaption of soil bacterial communities based on different climate indices derived from soil temperature records, or on bacterial community composition data. We found that highest daily average soil temperature was the best predictor for the T_{opt} of the soil bacterial communities, increasing 0.63 °C per °C. We found no support for the prediction of temperature adaptation by regression tree analysis based on relative abundance data of most common bacterial species. Increasing summer temperatures will likely increase T_{opt} of soil bacterial communities in the Arctic. Incorporating this mechanism into soil biogeochemical models and combining it with projections of soil temperature will help to reduce uncertainty in assessments of the vulnerability of soil carbon stocks in the Arctic.

1. Introduction

The Arctic terrestrial biome has the potential to undergo particularly large losses of soil organic carbon and controls the potential loss or gain of global carbon stocks (Crowther et al., 2016; Wieder et al., 2019). This is because of the large soil organic carbon stock in arctic soils (Tarnocai et al., 2009) and the strong response of soil respiration rates to warming in these cold ecosystems (Carey et al., 2016). Bacterial soil communities in the Arctic terrestrial region are adapted to perform well at

low temperatures (Bååth, 2018). However, these bacterial communities are likely to be exposed to increasing soil temperatures in this century (Post et al 2018) and it remains uncertain whether these soil bacterial communities will adapt their response to temperature when exposed to warmed conditions (Rinnan et al., 2011; Weedon et al., 2023; Rousk et al., 2012). Knowledge of the climate conditions under which such an adaption takes place will help in estimations of the potential vulnerability of arctic soil carbon stocks to warmer climate conditions (Bååth, 2018; Bradford et al., 2019; García-Palacios et al., 2021).

The temperature adaptation of soil bacterial communities is most often characterized in relation to respiration, growth or enzymatic activity. A commonly applied method is to estimate the relationship between whole community growth and temperature with an assay that measures ^{3}H-leucine uptake (Bååth et al., 2001). This relationship between temperature and bacterial growth can be described by the Ratkowsky model, which has three cardinal points: the (theoretical) minimum growth temperature (Tmin), optimal growth temperature (Topt) and maximum growth temperature (Tmax) (Ratkowsky et al., 1983). Previous research has shown that the temperature-growth relationships of soil bacterial communities adapt to their local environment, such that there is a positive correlation between mean annual air temperature (MAAT) and the parameters describing the temperature-growth relationships of soil bacterial communities (cardinal points) (Bååth, 2018). For example, recently it has been found that across an elevation gradient in the Peruvian Andes Tmin increased 0.2 degrees per degree Celsius increase in MAAT (Nottingham et al., 2019) and a similar correlation was found between MAAT and Topt across a natural climate gradient in Europe (Cruz Paredes et al., 2021). This correlation has also been shown in the Antarctic, where the temperature-growth relationships of soil bacterial communities show higher values of Tmin with higher mean annual soil temperature (Rinnan et al., 2009). However, no comparable large-scale study on the temperature-growth relationships of soil bacterial communities in the Arctic has been performed yet. Such a large scale study is needed for arctic soil bacterial communities, as the Arctic differs from lower latitudinal regions in terms of its current climate(Convey, 2013), predicted climate changes (Post et al., 2019) and importance for the global soil carbon stock (Wieder et al., 2019).

Despite strong correlations over large spatial scales, an increase in the mean annual soil temperature does not necessarily lead to a shift in temperature-growth relationships of bacterial communities when soils are experimentally warmed in lab incubation and field studies (Pietikäinen et al., 2005; Birgander et al., 2013, 2018; Rinnan et al., 2011; Weedon et al., 2023). Instead, a common observation is a rapid change in the temperature-growth relationships driven by a community turnover when soils are incubated above the optimal growth temperature of the *in situ* soil bacterial community, (Birgander et al., 2013; Donhauser et al., 2020). This suggests that the *maximum* soil temperature is an important predictor of the temperature-growth relationships of bacterial communities. Supporting evidence for this comes from a study in the Antarctic, where coastal water bacterial communities are adapted to lower temperatures (lower Tmin) than soil bacterial communities in the same region, despite the mean annual temperature of Antarctic water being higher than that of Antarctic soils (van Gestel et al., 2020). The Antarctic soils are exposed to higher summer temperatures than the Antarctic marine environment, leading to the hypothesis that the maximum temperature, rather

60 than the annual average, is a more important driver for the temperature adaptation of bacterial communities across different habitats (Birgander et al., 2013; van Gestel et al., 2020).

Analogous to the maximum temperature, the coldest soil temperature could also influence temperature-growth relationships. In desert soils, the upper layer (0-5 cm) is characterized by relatively large amplitude fluctuations in temperature over both diurnal and annual timescales. Consequently, the bacterial communities of these upper layers tend

65 to have lower Tmin values and higher Topt values than deeper soil layers that are exposed to more moderate and stable soil temperatures (van Gestel et al., 2013). These studies show that while the mean annual temperature might correlate strongly with the cardinal points of the temperature-growth relationships of soil bacterial communities, the temperature adaption might be more directly related to other selective pressures of the thermal regime such as the highest or lowest soil temperature.

70 To predict future temperature-growth relationships of soil bacterial communities in the Arctic, more knowledge is needed on 1) the current temperature adaptation of soil bacterial communities in the Arctic and 2) the specific mechanisms driving temperature adaptation. Bacterial communities from polar ecosystems are hypothesized to be adapted to low temperatures, shown by a low Tmin (Baath, 2018). For example, sub-Arctic bacterial communities exhibit lower cardinal points of their temperature-growth relationships compared to bacterial communities of temperate ecosystems, with a

75 Tmin of –9.6 to -7.0 °C and Topt 25 to 30 °C (Cruz-Paredes et al., 2021; Rinnan et al., 2011). It is likely that soil warming will shift the temperature-growth relationships of sub-Arctic soil bacterial communities (Weedon et al., 2023; Rijkers et al., 2022). However, the *in situ* temperature-growth relationships of soil bacterial communities in the mid- to High Arctic are so far unknown and will need to be evaluated to understand the current temperature adaptation of soil bacterial communities and drivers of temperature adaptation under future climate conditions.

80 It is important to evaluate which soil thermal parameters are the most accurate predictor for soil bacterial communities in the sub- to High Arctic, as this might not be accurately predicted from the mean soil annual temperature alone. In these (sub-) Arctic regions the maximum and minimum daily soil temperatures are only weakly correlated with the mean annual soil temperature, due to the influence of local environmental parameters on the soil climate extremes. For example, winter soil temperatures also vary greatly on the meter-scale in the Arctic, due to the influence of snow cover

85 on winter microclimate (Karjalainen et al., 2018). On the other hand, summer soil temperature is more closely related to the air temperature, which varies less between (sub-) arctic soils (Fig. 1). Implementing knowledge about these possible drivers of the temperature adaptation of soil bacterial communities at these high northern latitudes will support accurate predictions of soil decomposition of the large carbon stock present in the Arctic under future climates.

Due to the possible influence of multiple soil thermal parameters, accurate prediction of temperature adaptation by soil bacterial communities will likely require high-resolution soil temperature data. However, soil temperature logger data are particularly scarce in the Arctic region (Lembrechts et al., 2021), leading to a need for potential alternative predictors of soil microbial temperature adaptation. DNA-based bacterial community composition measures have recently been shown to correlate with shifts in the temperature growth relationships of a soil bacterial communities (Donhauser et al., 2020; Rijkers et al., 2022; Weedon et al., 2023). More generally, temperature traits differ between members of bacterial communities from arctic soils (Wang et al., 2021), and specific bacterial taxa have been associated with warming in forest soils across North America (Oliverio et al., 2017). The aggregated community response, such as the temperature-growth relationship, might therefore be predictable using the abundance of specific species that are associated with a warm or cold adapted community (Hicks et al., 2021). In a pan-arctic survey soil bacterial community showed a large diversity of species, with 15 common taxa shared between all soils (Malard et al., 2019). Therefore, potentially there are bacterial species that could indicate the current temperature adaptation of arctic soil bacterial communities. If so, this provides opportunities to determine the temperature adaptation of soil bacterial communities in the Arctic where long term soil temperature logging is absent.

In this study we tested which soil thermal parameters best predicts the cardinal points of the temperature-growth relationships of bacterial communities from 12 soils collected in the sub- to high Arctic region. We hypothesized that the highest and lowest daily soil temperatures would be the best predictor of the corresponding cardinal points of the temperature-growth relationships. We also compared the DNA-based compositional profiles across soil types and explored whether such compositional data can be used as an alternative predictor for the temperature-growth relationships of the soil bacterial communities in Arctic soils.

2. Methods

2.1 Sample collection

In the summers of 2018 and 2020, soil samples were collected from 12 soil types at 9 sites ranging from sub- to High Arctic (Figure 1). The 2018 sampling at Toolik Lake Field station, Svalbard, Abisko and Iceland has been previously described in (Rijkers et al., 2022). In brief, soil cores of 10 cm depth were collected from Toolik Field Station, USA (68°38' N, 149°36' W) at the LTER Heath site, LTER Moist Acidic Tussock and LTER Non-Acidic Tussock; on Svalbard, from the Bjorndalen site (78°13'N, 15°19'E), dominated by *Carex* sp. vegetation; at the FORHOT site in Iceland (64° 00′N, 21° 11′W), a grassland (*Agrostis capillaris*) and forest site (*Picea sitchensis*) were sampled. Lastly, soil samples were collected from the blanket bog (*Sphagnum* sp.) where the ITEX warming experiment is located, close to the Abisko Research Station in Sweden (68°21'N, 18°49'E).

In 2020 a second sampling campaign collected triplicate soil cores to a depth of 10 cm at sites in Greenland (two sites), Canada, Norway, and Finland. On Disko Island, Greenland soil cores were collected near the AWS-2 logger at Østerlien site of the Greenland Ecosystem monitoring (GEM; 69°15''' N, 53°30'W), which were covered by *Vaccinium* sp. At Kobbefjord, Greenland soil samples with *Empetrum sp.* cover were collected near the SoilEMP logger of GEM (64°08'N 51°22'W). At Inuvik, Canada soil cores were sampled at Inuvik airport bog (68º 18.9342 N, 133º 26.0214 W), which is characterized by low shrubs (Nixon et al 2003). In Finland, samples were collected directly next to the ITEX site in Kilpisjarvi (69.4 N, 20.490E), for which the vegetation cover is dominated by *Vaccinium* and *Empetrum* sp (Ylänne et al 2015). Lastly, soil samples were collected at Petersfjellet in Norway (N69°35.5277' E29°55.1939'), which was covered by *Empetrum nigrum*.

2.2 Soil temperature data

Soil temperature records were collected from the involved research stations (at Abisko (Dorrepaal et al., 2004), Svanhovd (BioForsk Svanhovd; http://lmt.bioforsk.no/agrometbase/getweatherdata_new.php?weatherStationId=36), Inuvik (National Resources Canada), Svalbard (Global Terrestrial Network for Permafrost database; http://gtnpdatabase.org/boreholes/view/166), Toolik Lake (Hobbie and Laundre, 2021), FORHOT research site in Iceland (Sigurdsson et al., 2016), Kilpisjarvi ITEX site (unpublished, personal communication Sari Stark), Greenland sites (Green Ecosystem monitoring database; https://data.g-e-m.dk/)). To overcome differences in the time intervals of data collection between sites, we calculated the mean daily temperature for each day that soil temperature records were available (all records >3 years, except for Kilpisjarvi; Table 1). Based on the daily soil temperature records of each soil, we determined the mean annual temperature (*MAT*), mean warmest day (*STmax*), mean coldest day (*STmax*) based on the annual records for the warmest day, coldest day and mean daily temperature per year (Table 2).

2.3 Soil analysis

After collection, soils were shipped on ice and cooled upon arrival at 4°C. The upper 10 cm was sampled for the following analyses: density of the soils samples was determined by rapid submersion in a water filled cylinder. The water content was calculated based on the weight before and after drying the soil at 70° C for 48 h. The dried samples were ground with a Retsch MM200 ball mill (Retsch, Haan, Germany) for 1 min at 30 rounds per second. A subsample was then ashed at 600ºC for 6 h. The carbon and nitrogen content of ashed and non-ashed subsamples were measured on a Flash EA 1112 (ThermoFisher, Waltham, USA). For the calculation of organic carbon in the soil, the carbon content of the ashed samples was subtracted from the total amount of carbon content. Soil pH was measured by adding 5 g of soil to 25 ml deionized water, after which the slurries were shaken for 1 h at 100 rpm. The soil pH was then measured on a WTW Inolab level2 pH meter (Xylem Analytics, Rye Brook, New York, USA). The slurry was then centrifuged at 200 rpm, for 1 hr and then filtered on 0.45µm nylon filter. The filtered solution was used for the measured of extractable dissolved organic carbon content on a TOC-L CPH/CPN (Shimadzu, Columbia, USA). with NPOC method by manufacturer's protocol. For the soil samples of Svalbard only pH measurements were performed due to limited amounts of soil.

2.4 Temperature-growth relationships of soil bacterial communities

For the assessment of the temperature sensitivity of bacterial growth, 1 gram of soil was subsampled for a leucine incorporation assay using methods adjusted from Bååth et al., (2001). Briefly, 20 ml of sterilized deionized water was added to the soil samples and these slurries were vortexed for 2 min at full speed. After 10 min centrifugation at 1000 G, the 1 ml aliquots of the supernatant were suspended in 2 ml screw-top Eppendorf tubes. A 20 µl mixture ^{3}H-labeled and unlabeled leucine was added, resulting in a final concentration of 401 nM and 72.5 kBq ml^{-1} in the assay tube. The sample aliquots were incubated either 24 h at 0°C, 8 h at 4°C, 4 h at 10 and 15°C and 2 h at 24.5, 28.5, 33 or 40°C.. Trichloroacetic acid was added to the assay tubes to terminate the leucine incorporation. Washing steps for removal of non-incorporated leucine were followed as described in (Bååth et al., 2001). For scintillation 1 ml Optiphase HiSafe 3 (PerkinElmer, Waltham, Massachusetts, USA) was finally added to the biomass pellet after the washing steps. 3H-activity was measured on a Tricarb2800T (Perkin Elmer), Waltham, USA) with 5 minute measurement for ^{3}H. Finally, the leucine incorporation rate, nM leucine 1 h -1 g dry weight soil, was calculated based on ^{3}H activity measured.

2.5 Bacterial community composition

For the characterization of the soil microbial community, 0.2 grams of soil were subsampled for DNA extraction and amplicon sequencing of the 16S rRNA gene. DNA was extracted by the use of Powersoil kit (Qiagen, Hilden, Germany), following the manufacturer's protocol with elution of the purified DNA into 60 sterile µl Millipore water. Amplicons

were generated by a two-step PCR of the 16S V4 rRNA gene with primers designed by Caporaso et al., (2012). An initial PCR consisted of 24 cycles with an initial denaturation step of 1 min at 98°, followed by 25 cycles of denaturation for 10 s at 98 °C, annealing for 30 s at 55°C, elongation for 30 s at 72°C, followed by a final extension of 5 min at 72°C. Amplicons were then 50x diluted in σ-purified water and then indexed by a PCR with unique barcode primers for 8 cycles with the same steps as the initial PCR amplification. Purification of the PCR product was done with Ampure XP beads (Beckman Coulter, Brea, California, USA), following manufacturer's protocol. The indexed PCR products were then sequenced using paired-end Illumina MiSeq runs with V3-2x300 cycle chemistry. In total 1,243,600 sequences were generated for 39 samples (Median sequencing depth; 32,089 sequences per sample). Sequences were truncated at 250 nucleotides on the forward reads and 220 nucleotides on the reverse reads due to deteriorating quality scores for later cycles (average Phred score < 30). Raw sequences are available in the NCBI Sequence Archive, under BioProject Accession number PRJNA857550. Amplicon sequence variants (ASVs) were generated by dereplication and chimera removal of the truncated sequences using DADA2 allowing a maximum expected error of 2 and 'consensus' chimera removal mode. Phylogenetic distances between the ASVs were estimated using MAFFT alignment (Katoh and Standley, 2013) and Fasttree (Price et al., 2009). Taxonomic classification of the ASVs was performed based on the SILVA v138 database (Yilmaz et al., 2014) using a scikit-learn naive Bayes machine-learning classifier (Bokulich et al., 2018) with a confidence threshold for limiting taxonomic depth at 70%. ASVs identified as mitochondria or chloroplasts as well as singletons were discarded prior to further statistical analyses.

2.6 Statistical analyses

All statistical analyses were performed in R (v4.0.2) (R Core Team, 2020). Soil daily temperature records were filtered for datapoints between 2002 and 2021. Leucine incorporation rates were fitted to a Ratkowsky model for bacterial growth (Ratkowsky et al., 1983) by the use of R-package 'nls.multistart' (Padfield and Matheson, 2018). The Ratkowsky model is based on the following equation;

Eq. 1

$$\sqrt{Leu} = a(T - Tmin) \times (1 - e^{b(T-Tmax)})$$

where Leu is the rate of leucine incorporation, a is the coefficient below optimal growth temperature, T is the assay temperature, Tmin is the theoretical minimum growth temperature, b is coefficient above the optimal growth temperature and Tmax is maximum growth temperature (Table 2). The optimal temperature was determined by numerical interpolation. All figures were made with the 'ggplot2' R-package. To test for the effects of soil thermal parameters on the temperature adaptation of soil bacterial communities, we performed linear regression between the cardinal points of the temperature-growth relationships and minimum (*STmin*), mean (MAST), and maximal annual soil temperature

(*STmax*). These linear regression models tested the relationship between Tmin and minimum soil temperature, Tmax and the maximum soil temperature and Topt with minimum, mean and maximum soil temperature as independent variable. We fitted a linear regression for the relationship between the temperature range (Tmin – Tmax) of the temperature-growth relationships of the soil bacterial communities and amplitude of thermal soil regime (minimum *STmin* to maximum soil temperature *STmax*) with a linear regression model.

Processing the microbial community data was done using the R-package 'phyloseq' (McMurdie and Holmes, 2013). Samples were rarified to depth of 23687 reads. Permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was performed on the weighted UniFrac distances (Lozupone and Knight, 2005) of the sample of the 11 sites, excluding the Svalbard due to lack of data, (Suppl. Table 1.) using the mean annual soil temperature, pH, organic carbon content, organic nitrogen content, community Topt and one of the three soil temperature variables (mean, maximum or minimum daily soil temperature) as independent variables in the 'vegan' R-package. We determined the common ASVs by filtering for mean relative abundance above 0.001 % in at least 2, 3, or 4 soil types. The relative abundance of the common ASVs in three datasets varying in cut off filter was used to predict the Topt of the soil bacterial communities. The relative abundance of these common ASVS was then used to perform a 3 types of regression tree analysis on the Topt of soil bacterial communities using the R-package 'caret' (Kuhn, 2008). Data were randomly split into training (9 soils) and validation (3 soils) dataset, after which a regression tree analysis was performed with 'rpart1SE' function using the control settings (maxdepth=4, minsplit=4, minbucket =2). We also build a regression tree with cross validation (10 folds, 10 repeats) using the 'rpart' function using the same control settings. Additionally, we used 'Rborist' function with the default setting to calculate a random forest regression tree to predict Tmin based on the relative abundance of common ASVs in the training soils. For direct comparison with regression models, we performed an additional linear regression using Topt as independent variable and *STmax* as dependent variable using the 9 soils of the training dataset and 3 soils in the validation dataset. Due to the small datasets that these models were based on, the random division into training and validation dataset had a strong influence on the computed RMSE (root mean square error) value. Therefore, we trained each of the 4 models on all 220 possible combinations of soils in the training and validation dataset of the dataset with the cut-off filter for common ASVs found in 4 soil types (with a 9:3 split between soil for train and testing, respectively). We then compared the performance of the 4 different models based on median RMSE over the 220 simulations.

3. Results

3.1 Temperature adaptation of soil bacterial communities

From sub- to High Arctic, mean annual soil temperatures at 10 cm depth varied between -3.5 and 6.1 °C (Table 1, Figure 1). The sampled bacterial communities varied in Tmin between -11.1 ± 4 (s.d) in Østerlien and -5.5 ± 2.1 in the Icelandic grassland. Topt varied between 23.4± 0.5 in Toolik Lake MAT and 34.1 ± 3.7 in Kilpisjarvi (Figure 2). Tmax varied between 42.2 ± 1.0 in Svalbard and 57.8 + 9.3 at Toolik Lake Heath. Temperature range of growth, (Tmin - Tmax) varied between 48.7 and 65.2.

The MAST of soils was not significantly related with Topt (P= 0.5) nor was Tmin (P= 0.78, Adj. R^2 = -0.1). However, Topt did relate significantly with *STmax*, increasing 0.63 °C per °C (Figure 2; P < 0.01, Adj. R^2 = 0.63) and also showed a significant but weaker relationship to the mean summer temperature (P<0.05, Adj. R^2=0.34). In contrast, Topt was not significantly correlated to the number of days above 0°C (P=0.9). Tmin showed no significant correlation to mean winter soil temperature and coldest daily soil temperature (P>0.05). The temperature range of growth was significantly related to the amplitude of the temperature soil temperature (Linear regression; Adj. R^2= 0.3, P= <0.05). We computed the optimal growth temperature of soil bacterial communities across the Arctic based on combining the Soil Temp database (Lembrechts et al., 2021) with our estimates of the relationship between soil temperature (*STmax*) and Topt (Figure 4).

3.2 Bacterial community composition

After filtering for singletons, we retrieved a total of 967,146 reads across the samples, belonging to 12692 ASVs. PERMANOVA analyses showed bacterial community composition to be significantly influenced by pH and MAST of the sampling sites (Table 3, Figure 5), but showed no significant correlation with maximum nor minimum daily soil temperature (P >0.05; Supplementary Table 2 and 3) . The bacterial community composition was not significantly related with the Topt of the bacterial communities (P= 0.124). Proteobacteria (25.9%), Acidobacteriota (21.9%), Actinobacteriota (18.4%), Verrucomicrobiota (7%), Bacteroidota (6.7%), Chloroflexi (5.2%), Planctomycetota (5.1%), and Myxococcota (2.1%) were the most abundant phyla across all samples (Figure 3).

We found 118 ASVs and 32 ASVs that were observed in 2 or 3 soils with a relative abundance greater than 0.001 %. Additionally, we observed only 12 ASVs that occurred at relative abundance greater than 0.001 % in four or more sites (Table 4). The common ASV datasets differing in cut-offs showed similar RMSE-values for predicting Topt of the bacterial community (Supplementary Table 4). We therefore focussed on the dataset ASVs that occurred in at least 4 soils for further analysis, as these ASVs are most likely useful in soil types outside this study. Both regression tree and random forest analyses based on the relative abundance of these common ASVs in at least 4 soils showed the relative abundance of ASV11 was the best predictor of the corresponding community Topt (Suppl. Figure 1), in which it

differentiated of ASV11 absence from the community and relative abundance > 0.055%. The pruned regression tree showed RMSE lower than the full tree on the validation dataset (Suppl. Figure 2; Suppl. Table 4). The linear regression model based on the *STmax* as dependent variable showed larger predictive power of Topt than the pruned regression tree

260 and random forest, since summarized across the 220 possible training sets, the median RMSE of the linear model was lower than that median RMSE of the pruned tree and random forest for common ASVs found in at least 4 soil types (2.17, 4.14 and 3.51, respectively; Suppl. Figure 3).

4. Discussion

4.1 Temperature adaptation across the Arctic

In this study we explored the role of soil thermal parameters on the temperature adaptation of soil bacterial communities in the Arctic. The cardinal points estimated from bacterial communities sampled at 12 Arctic locations were comparable to other bacterial communities from polar soils and showed a large variety between sites and soil types. We found Tmin to vary between -11.1 and -5.5 °C, which is comparable to soils sampled from sub arctic soils (Cruz-Paredes et al., 2021; Rinnan et al., 2011; Weedon et al., 2023). Tmin was lowest at the low arctic site Østerlien, which is lower than any the Tmin of previously described for Arctic soil bacterial community, but fits within the range of Tmin of bacterial communities previously described in Antarctic soils (Rinnan et al., 2009). In contrast to Tmin, Topt is hypothesized to vary less between thermal environments (Rinnan et al., 2009). At the Toolik Lake Moist Acidic Tundra site, estimated Topt was 23.5 °C, which is so far the lowest Topt described for a soil bacterial community in the Arctic (Rinnan et al., 2011; Weedon et al., 2023; Cruz-Paredes et al., 2021) and is also comparable to soil bacterial communities from Antarctica (Donhauser et al., 2020; Rinnan et al., 2009, 2011; van Gestel et al., 2020). This site was characterized by relative low summer temperatures and moderate annual mean temperatures, compared to the other sites (Table 1, Figure 1).

4.2 Temperature adaptation is influenced by mean daily maximum soil temperature

Of the soil thermal parameters we tested, only *STmax* had a significant correlation with temperature-growth relationships of Arctic bacterial communities (Figure 2). Temperatures above the optimum growth temperature can induce heat-related death of bacterial cells, which results in a strong selective pressure by the maximum soil temperature on the bacterial community (Bárcenas-Moreno et al., 2009; Birgander et al., 2013; Donhauser et al., 2020). Consequently, the optimal growth temperature of soil bacterial communities is always observed to greatly exceed the maximum soil temperatures at a given location (Bárcenas-Moreno et al., 2009; Birgander et al., 2018; van Gestel et al., 2013; Rinnan et al., 2009). Our results show that even in cold biome environments the maximum soil temperature is an important determinant of the temperature physiology of soil bacterial communities. For two of our sites (Inuvik and Svalbard), soil temperatures were recorded at depths that differed from the depth where the soil samples were taken (Table 1). Excluding these sites from our analysis, we still found the same relationship between Topt and the maximum soil temperature with a slope coefficient and intercept differing by less than 2% (Supplementary methods). While samples in this study were collected in summer, temperature-growth relationships are not affected by seasonal dynamics (van Gestel et al., 2013; Birgander et al., 2018), making it likely that the *STmax* is the most important predictor of thermal adaptation amongst those we measured. All in all, the evidence collected in this study provides further support for the idea that temperature adaptation of soil microbial communities is best explained by the optimum-driven hypothesis (Alster et al., 2020). According to this hypothesis temperature-growth relationships are driven by the maximum soil temperatures, and this was previously proposed as temperature adaptation could only be induced after exposure of communities to conditions above a certain threshold temperature (Bárcenas-Moreno et al., 2009; Birgander et al., 2013, 2018).

4.3 No evidence for influence of soil thermal parameters on Tmin or Tmax

In contrast to the clear relationship between *STmax* and Topt, we found no evidence for a relationship between soil thermal parameters and the minimum and maximum cardinal points, nor with the thermal breadth of the bacterial temperature-growth relationships. This non significance could in principle be a result of statistical artefacts, since for the estimation of Tmin and Tmax, both cardinal points are extrapolated beyond the assay temperatures, which could cause a large standard deviation of the mean and increase the chance of type II errors. Indeed, the mean of site-level standard deviations across sites was relatively high for both Tmin and Tmax (respectively mean s.d. of 1.94 and 2.8). However, this variation was on the same order as that observed for Topt estimates amongst the sampled soil bacterial communities (mean s.d. of 2.06), implying that the lack of significance is most likely not due to limited power of the statistical analysis.

Given the importance of Tmin for determining activity at low temperatures, we expected that Tmin of communities would be related to site *STmin*. However, we did not detect a significant influence of MinT on the Tmin of soil bacterial communities. There is a general consensus that constantly frozen subsoils (permafrost) are an unlikely environment for proliferation of soil microbial life (Abramov et al., 2021). Due to this limited growth, cold-adapted (low Tmin) species might not necessarily thrive at subzero temperature but are likely to be better equipped to survive the winter conditions. Consequently, winter temperatures might not pose an environmental filter for the community assembly. Soil temperatures above freezing might have a larger influence on the temperature adaption of soil bacterial communities, when soil bacteria are most metabolically active (van Gestel et al., 2020). Therefore, the high soil temperatures in summer might induce a large environmental influence on the assembly of the bacterial communities. Additionally, strategies to survive subzero temperatures might not necessarily be indicative of the optimal growth temperature, as many microbial species that can cope with subzero temperature still grow best at relatively high temperature and are best described as psychro-tolerant rather than as true psychrophiles (Cavicchioli, 2015). These factors might therefore be the reason why we are unable to make predictions of Tmin based on the temperature parameters measured in this study.

Since *STmax* influenced the Topt of the soil bacterial communities, we expected that this parameter would also correlate with the Tmax value of the soil bacterial community. Tmax has been hypothesized to increase with higher soil temperatures (Rinnan et al., 2009; Birgander et al., 2013), but to date this has not been directly tested. In our results, Tmax was not influenced by any of the measured soil thermal parameters. As noted above, Topt was far above maximum soil temperatures, which suggests that the measured growth rates of bacterial communities above Topt are rarely relevant in the soil environment. Therefore, it is likely that Tmax is less relevant for the performance of soil bacterial species and consequently, not subject to selection *in sensu* Vellend (2010).

4.4 What will happen in response to warming?

Since *STmax* was found to be most important predictor, it follows that changes to summer temperatures are likely to be the most important factor determining temperature-growth relationships of bacterial communities in Arctic soils under a changing

climate. Arctic summer air temperatures are predicted to increase less than the mean annual and winter temperature (Karjalainen et al., 2018). While it has been estimated that mean annual soil temperature will rise ~ 2 - 4 °C around the Arctic by 2100 under RCP 4.5 (Aalto et al., 2018), accurate predictions of summer soil temperature in the Arctic are complicated by a variety of environmental factors that influence soil temperatures. At the local scale, soil temperatures are largely influenced by air temperature, solar radiation and precipitation (Karjalainen et al., 2018), leading to > 5 °C variation on the microscale (Aalto et al., 2013; Karjalainen et al., 2018). Increasing air temperatures in the Arctic can also lead to changes in vegetation height and shrub expansion (Mekonnen et al., 2021), which moderate increasing soil temperature by shading during the summer season (Paradis et al., 2016; Blok et al., 2011). Furthermore, it is likely that the Arctic terrestrial region will experience more frequent and extreme heatwaves, which could induce rapid change in the temperature-growth relationships if soil temperatures exceed historical maximum soil temperatures and/or the Topt of the soil bacterial communities (Bárcenas-Moreno et al., 2009; Birgander et al., 2013; Donhauser et al., 2020). These complicated local scale effects imply that more microclimatic data will be needed for more accurate assessments of temperature adaptation of soil bacterial communities in the Arctic.

Our study covered a large portion of the range of maximum soil temperature within the Arctic region, as these temperatures currently vary between -0.4 and 20.6 °C (Lembrechts et al., 2021). Figure 4 shows that the Topt of Arctic soil bacterial communities likely varies between 22 and 35 °C. A combination of this pan-arctic projection, predicted future summer (soil) temperatures, and other spatial databases such as soil C maps, could be useful to identify locations where soil bacterial communities will be sensitive to future warming, where potential shifts in the temperature-growth relations can occur, and where these may have disproportionate impacts on regional biogeochemistry. For example, by identifying regions where local soil temperatures are expected to rise rapidly and soil organic stocks are large.

4.5 Can we use microbial community data for predicting temperature adaption?

Predicting temperature adaptation of soil bacterial communities across the Arctic might be limited by lack of long term soil temperature data across the Arctic as most Arctic research has focused on only few research sites (Metcalfe et al., 2018). To explore the potential use of microbial 'bio-indicators' for predicting the temperature-growth relationships of *in situ* soil bacterial communities (Hicks et al., 2021), we evaluated whether microbial community data can reveal the temperature adaptation of microbial communities. We found that regression tree analysis using bacterial ASVs as potential predictors (Suppl. Fig. 4) produced larger estimation errors on prediction of the Topt of soil bacterial communities when compared with the linear regression against *STmax* (Supplementary Table 4). This can be partially attributed to the low effective sample sizes resulting from the use of cross-validation methods to prune the regression trees, but likely also reflects a lack of consistent signal in the bacterial composition data. Although this does not refute the potential for using compositional data to predict community-broad temperature growth relationships (Hicks et al., 2021), it implies that such methods would need a larger training dataset with more sample sites for proper validation, and more accurate predictions. The full regression tree used a

low number of ASVs (Supplementary Figure 2, Supplementary Table 5), which were not observed in all soil types, which might indicate limited use for other datasets. This suggests that indicator species, if they exist, might be indicative of the temperature adaptation of bacterial communities for only certain particular soil types or climatic regions. Despite these caveats, it is notable that the pruned regression tree and random forest model both identified the abundance of ASV11 as effective in predicting the Topt (Supplementary Figure 1). ASV11 matches 100% to ASV that is the most commonly observed bacterial in arctic soils (Malard et al., 2019). The genus of Candidatus Udaeobacter, to which ASV11 matches, is commonly found in soil environments globally (Brewer et al., 2016). It has been proposed to be a small oligotrophic and resilient soil bacteria characterized by aerobic heterotrophic metabolism with small genome size (2.8-3.2 Mbp), large diversity of antibiotic resistance genes and a preference for acidic soils (Brewer et al., 2016; Willms et al., 2020, 2021). However, so far no study has successfully cultivated the any lineage of the genus 'Candidatus Udaeobacter' and traits related to temperature preferences have not been recorded. In the pruned tree (Supplementary Figure 4) the presence or absence of ASV11 was indicative a Topt of 26.3 or 31.4, respectively. As this taxon was absent in 7 out of 12 soils, the utility as an indicator of temperature adaption is quite limited. In summary, although there is some potential utility in using community data to estimate and predict aspects of soil bacterial temperature physiology, our results suggest that more accurate predictions can be made from soil temperature records.

5. Conclusions

In this study, we showed a large variety in the temperature adaptation of soil bacterial communities from the sub to High Arctic region. Due to the large influence of maximum soil temperatures, we predict that summer warming, to the extent that leads to higher maximum soil temperatures, will lead to increasing community-level increase the Topt of these bacterial communities under future climate conditions in the Arctic. The influence of shifting optimal growth temperature for soil bacterial communities on soil carbon cycling will need further investigation to evaluate the contribution to the vulnerability of soil carbon stock in the Artic under future climate conditions.

Data availability

The data used for statistical analysis and visualization is available on Figshare (doi; 10.6084/m9.figshare.20977588, doi: 10.6084/m9.figshare.20977582, doi: 10.6084/m9.figshare.20977585). The raw sequences of the 16S rRNA gene amplicon sequences have been deposited on NCBI's Sequence Read Archive under BioProject PRJNA856638.

Authors Contributions

RR and JTW designed the study. MD and RR performed soil analysis and bacterial community analysis. RR performed leucine assays and statistical analysis. All authors contributed to writing of the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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References

Aalto, J., Le Roux, P. C., and Luoto, M.: Vegetation Mediates Soil Temperature and Moisture in Arctic-Alpine environtments, Arctic, Antarct. Alp. Res., 45, 429–439, 2013.

Aalto, J., Karjalainen, O., Hjort, J., and Luoto, M.: Statistical Forecasting of Current and Future Circum-Arctic Ground Temperatures and Active Layer Thickness, Geophys. Res. Lett., 45, 4889–4898, https://doi.org/10.1029/2018GL078007, 2018.

Abramov, A., Vishnivetskaya, T., and Rivkina, E.: Are permafrost microorganisms as old as permafrost?, FEMS Microbiol. Ecol., 97, 1–12, https://doi.org/10.1093/femsec/fiaa260, 2021.

Alster, C. J., von Fischer, J. C., Allison, S. D., and Treseder, K. K.: Embracing a new paradigm for temperature sensitivity of soil microbes, Glob. Chang. Biol., gcb.15053, https://doi.org/10.1111/gcb.15053, 2020.

Anderson, M. J.: A new method for non-parametric multivariate analysis of variance, Austral Ecol., 26, 32–46, https://doi.org/10.1046/j.1442-9993.2001.01070.x, 2001.

Bååth, E.: Temperature sensitivity of soil microbial activity modeled by the square root equation as a unifying model to differentiate between direct temperature effects and microbial community adaptation., Glob. Chang. Biol., https://doi.org/10.1111/gcb.14285, 2018.

Bååth, E., Pettersson, M., and Söderberg, K. H.: Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria, Soil Biol. Biochem., 33, 1571–1574, https://doi.org/10.1016/S0038-0717(01)00073-6, 2001.

Bárcenas-Moreno, G., Brandón, M. G., Rousk, J., and Bååth, E.: Adaptation of soil microbial communities to temperature: Comparison of fungi and bacteria in a laboratory experiment, Glob. Chang. Biol., 15, 2950–2957, https://doi.org/10.1111/j.1365-2486.2009.01882.x, 2009.

Birgander, J., Reischke, S., Jones, D. L., and Rousk, J.: Temperature adaptation of bacterial growth and 14C-glucose mineralisation in a laboratory study, Soil Biol. Biochem., 65, 294–303, https://doi.org/10.1016/j.soilbio.2013.06.006, 2013.

Birgander, J., Olsson, P. A., and Rousk, J.: The responses of microbial temperature relationships to seasonal change and winter warming in a temperate grassland, Glob. Chang. Biol., https://doi.org/10.1111/gcb.14060, 2018.

Blok, D., Schaepman-Strub, G., Bartholomeus, H., Heijmans, M. M. P. D., Maximov, T. C., and Berendse, F.: The Response of Arctic Vegetation to the Summer Climate: relation between shrub cover, NVDI, surface albedo and temperature, Environ. Res. Lett., 2011.

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., and Gregory Caporaso, J.: Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin, Microbiome, 6, 1–17, https://doi.org/10.1186/s40168-018-0470-z, 2018.

Bradford, M. A., McCulley, R. L., Crowther, T. W., Oldfield, E. E., Wood, S. A., and Fierer, N.: Cross-biome patterns in soil microbial respiration predictable from evolutionary theory on thermal adaptation, Nat. Ecol. Evol., 3, 223–231, https://doi.org/10.1038/s41559-018-0771-4, 2019.

Brewer, T. E., Handley, K. M., Carini, P., Gilbert, J. A., and Fierer, N.: Genome reduction in an abundant and ubiquitous soil bacterium "Candidatus Udaeobacter copiosus," Nat. Microbiol., 2, https://doi.org/10.1038/nmicrobiol.2016.198, 2016.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., and Knight, R.: Ultra-high-throughput microbial community analysis on the

Illumina HiSeq and MiSeq platforms, ISME J., 6, 1621–1624, https://doi.org/10.1038/ismej.2012.8, 2012.

Carey, J. C., Tang, J., Templer, P. H., Kroeger, K. D., Crowther, T. W., Burton, A. J., Dukes, J. S., Emmett, B., Frey, S. D., Heskel, M. A., Jiang, L., Machmuller, M. B., Mohan, J., Panetta, A. M., Reich, P. B., Reinsch, S., Wang, X., Allison, S. D., Bamminger, C., Bridgham, S., Collins, S. L., de Dato, G., Eddy, W. C., Enquist, B. J., Estiarte, M., Harte, J., Henderson, A.,
460 Johnson, B. R., Larsen, K. S., Luo, Y., Marhan, S., Melillo, J. M., Peñuelas, J., Pfeifer-Meister, L., Poll, C., Rastetter, E., Reinmann, A. B., Reynolds, L. L., Schmidt, I. K., Shaver, G. R., Strong, A. L., Suseela, V., and Tietema, A.: Temperature response of soil respiration largely unaltered with experimental warming, Proc. Natl. Acad. Sci., 113, 13797–13802, https://doi.org/10.1073/pnas.1605365113, 2016.

Cavicchioli, R.: On the concept of a psychrophile, ISME J., 1–3, https://doi.org/10.1038/ismej.2015.160, 2015.
465 Convey, P.: Antarctic Ecosystems, Encycl. Biodivers. Second Ed., 1, 179–188, https://doi.org/10.1016/B978-0-12-384719-5.00264-1, 2013.

Crowther, T., Todd-Brown, K. E. O., Rowe, C., Wieder, W., Carey, J., Machmuller, M., Snoek, L., Fang, S., Zhou, G., Allison, S., Blair, J., Bridgham, S., Burton, A., Carrillo, Y., Reich, P., Clark, J., Classen, A., Dijkstra, F., Elberling, B., Emmett, B., Estiarte, M., Frey, S., Guo, J., Harte, J., Jiang, L., Johnson, B., Kröel-Dulay, G., Larsen, K., Laudon, H., Lavallee, J., Luo, Y.,
470 Lupascu, M., Ma, L., Marhan, S., Michelsen, A., Mohan, J., Niu, S., Pendall, E., Penuelas, J., Pfeifer-Meister, L., Poll, C., Reinsch, S., Reynolds, L., Schmidth, I., Sistla, S., Sokol, N., Templer, P., Treseder, K., Welker, J., and Bradford, M.: Quantifying global soil C losses in response to warming, Nature, 540, 104–108, https://doi.org/10.1038/nature20150, 2016.

Cruz-Paredes, C., Tájmel, D., and Rousk, J.: Can moisture affect temperature dependences of microbial growth and respiration?, Soil Biol. Biochem., 156, https://doi.org/10.1016/j.soilbio.2021.108223, 2021.
475 Cruz Paredes, C., Tajmel, D., and Rousk, J.: Microbial temperature adaptation across a European gradient, in: EGU General Assembly Conference Abstracts, EGU21-4867, https://doi.org/10.5194/egusphere-egu21-4867, 2021.

Donhauser, J., Niklaus, P. A., Rousk, J., Larose, C., and Frey, B.: Temperatures beyond the community optimum promote the dominance of heat-adapted, fast growing and stress resistant bacteria in alpine soils, Soil Biol. Biochem., 107873, https://doi.org/10.1016/j.soilbio.2020.107873, 2020.
480 Dorrepaal, E., Aerts, R., Cornelissen, J. H. C., Callaghan, T. V., and Van Logtestijn, R. S. P.: Summer warming and increased winter snow cover affect Sphagnum fuscum growth, structure and production in a sub-arctic bog, Glob. Chang. Biol., 10, 93–104, https://doi.org/10.1111/j.1365-2486.2003.00718.x, 2004.

García-Palacios, P., Crowther, T. W., Dacal, M., Hartley, I. P., Ye, S., and Bradford, M. A.: Evidence for large microbial-mediated losses of soil carbon under anthropogenic warming, Nat. Rev. Earth Environ., https://doi.org/10.1038/s43017-021-
485 00178-4, 2021.

van Gestel, N. C., Reischke, S., and Bååth, E.: Temperature sensitivity of bacterial growth in a hot desert soil with large temperature fluctuations, Soil Biol. Biochem., 65, 180–185, https://doi.org/10.1016/j.soilbio.2013.05.016, 2013.

van Gestel, N. C., Ducklow, H. W., and Bååth, E.: Comparing temperature sensitivity of bacterial growth in Antarctic marine water and soil, Glob. Chang. Biol., 26, 2280–2291, https://doi.org/10.1111/gcb.15020, 2020.
490 Hicks, L. C., Frey, B., Kj, R., Lukac, M., Moora, M., Weedon, J. T., and Rousk, J.: Towards a function-first framework to make soil microbial ecology predictive, Ecology, https://doi.org/10.1002/ecy.3594, 2021.

Hobbie, S. and Laundre, J.: Hourly temperature and humidity data from the LTER Moist Non-acidic Tussock Experimental plots (MNT), Environmental Data Initiative, https://doi.org/10.6073/pasta/a48892da5bc9eab27b18d2364dea6998, 2021.

Karjalainen, O., Luoto, M., Aalto, J., and Hjort, J.: New insights into the environmental drivers of the circumpolar ground
495 thermal regime, Cryosph., 1–17, https://doi.org/10.5194/tc-2018-144, 2018.

Katoh, K. and Standley, D. M.: MAFFT multiple sequence alignment software version 7: Improvements in performance and usability, Mol. Biol. Evol., 30, 772–780, https://doi.org/10.1093/molbev/mst010, 2013.

Kuhn, M.: Building predictive models in R using the caret package, J. Stat. Softw., 28, 1–26, https://doi.org/10.18637/jss.v028.i05, 2008.
500 Lembrechts, J. J., van den Hoogen, J., Aalto, J., Ashcroft, M. B., De Frenne, P., Kemppinen, J., Kopecký, M., Luoto, M., Maclean, I. M. D., Crowther, T. W., Bailey, J. J., Haesen, S., Klinges, D. H., Niittynen, P., Scheffers, B. R., Van Meerbeek, K., Aartsma, P., Abdalaze, O., Abedi, M., Aerts, R., Ahmadian, N., Ahrends, A., Alatalo, J. M., Alexander, J. M., Nina Allonsius, C., Altman, J., Ammann, C., Andres, C., Andrews, C., Ardö, J., Arriga, N., Arzac, A., Aschero, V., Assis, R. L., Johann Assmann, J., Bader, M. Y., Bahalkeh, K., Barančok, P., Barrio, I. C., Barros, A., Barthel, M., Basham, E. W., Bauters,
505 M., Bazzichetto, M., Belelli Marchesini, L., Bell, M. C., Benavides, J. C., Luis Benito Alonso, J., Berauer, B. J., Bjerke, J. W.,

Björk, R. G., Björkman, M. P., Björnsdóttir, K., Blonder, B., Boeckx, P., Boike, J., Bokhorst, S., Brum, B. N. S., Brůna, J., Buchmann, N., Buysse, P., Luís Camargo, J., Campoe, O. C., Candan, O., Canessa, R., Cannone, N., Carbognani, M., Carnicer, J., Casanova-Katny, A., Cesarz, S., Chojnicki, B., Choler, P., Chown, S. L., Cifuentes, E. F., Čiliak, M., Contador, T., Convey, P., Cooper, E. J., Cremonese, E., Curasi, S. R., Curtis, R., Cutini, M., Johan Dahlberg, C., Daskalova, G. N., Angel de Pablo, M., Della Chiesa, S., Dengler, J., Deronde, B., Descombes, P., Di Cecco, V., Di Musciano, M., Dick, J., Dimarco, R. D., Dolezal, J., Dorrepaal, E., Dušek, J., Eisenhauer, N., Eklundh, L., Erickson, T. E., et al.: Global maps of soil temperature, Glob. Chang. Biol., https://doi.org/10.1111/gcb.16060, 2021.

Lozupone, C. and Knight, R.: UniFrac: A new phylogenetic method for comparing microbial communities, Appl. Environ. Microbiol., 71, 8228–8235, https://doi.org/10.1128/AEM.71.12.8228-8235.2005, 2005.

Malard, L. A., Anwar, M. Z., Jacobsen, C. S., and Pearce, D. A.: Biogeographical patterns in soil bacterial communities across the Arctic region, FEMS Microbiol. Ecol., 95, 1–13, https://doi.org/10.1093/femsec/fiz128, 2019.

McMurdie, P. J. and Holmes, S.: Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data, PLoS One, 8, https://doi.org/10.1371/journal.pone.0061217, 2013.

Mekonnen, Z. A., Riley, W. J., Berner, L. T., Bouskill, N. J., Torn, M. S., Iwahana, G., Breen, A. L., Myers-Smith, I. H., Criado, M. G., Liu, Y., Euskirchen, E. S., Goetz, S. J., Mack, M. C., and Grant, R. F.: Arctic tundra shrubification: a review of mechanisms and impacts on ecosystem carbon balance, Environ. Res. Lett., 16, https://doi.org/10.1088/1748-9326/abf28b, 2021.

Metcalfe, D. B., Hermans, T. D. G., Ahlstrand, J., Becker, M., Berggren, M., Björk, R. G., Björkman, M. P., Blok, D., Chaudhary, N., Chisholm, C., Classen, A. T., Hasselquist, N. J., Jonsson, M., Kristensen, J. A., Kumordzi, B. B., Lee, H., Mayor, J. R., Prevéy, J., Pantazatou, K., Rousk, J., Sponseller, R. A., Sundqvist, M. K., Tang, J., Uddling, J., Wallin, G., Zhang, W., Ahlström, A., Tenenbaum, D. E., and Abdi, A. M.: Patchy field sampling biases understanding of climate change impacts across the Arctic, Nat. Ecol. Evol., 1–6, https://doi.org/10.1038/s41559-018-0612-5, 2018.

Oliverio, A. M., Bradford, M. A., and Fierer, N.: Identifying the microbial taxa that consistently respond to soil warming across time and space, Glob. Chang. Biol., 23, 2117–2129, https://doi.org/10.1111/gcb.13557, 2017.

Padfield, D. and Matheson, G.: nls. multstart: robust non-linear regression using AIC scores, R Packag. version, 1, 1–5, 2018.

Paradis, M., Lévesque, E., and Boudreau, S.: Greater effect of increasing shrub height on winter versus summer soil temperature, Environ. Res. Lett., 11, https://doi.org/10.1088/1748-9326/11/8/085005, 2016.

Pietikäinen, J., Pettersson, M., and Bååth, E.: Comparison of temperature effects on soil respiration and bacterial and fungal growth rates, FEMS Microbiol. Ecol., 52, 49–58, https://doi.org/10.1016/j.femsec.2004.10.002, 2005.

Post, E., Alley, R. B., Christensen, T. R., Macias-Fauria, M., Forbes, B. C., Gooseff, M. N., Iler, A., Kerby, J. T., Laidre, K. L., Mann, M. E., Olofsson, J., Stroeve, J. C., Ulmer, F., Virginia, R. A., and Wang, M.: The polar regions in a 2°C warmer world, Sci. Adv., 5, https://doi.org/10.1126/sciadv.aaw9883, 2019.

Price, M. N., Dehal, P. S., and Arkin, A. P.: Fasttree: Computing large minimum evolution trees with profiles instead of a distance matrix, Mol. Biol. Evol., 26, 1641–1650, https://doi.org/10.1093/molbev/msp077, 2009.

R Core Team: R: A Language and Environment for Statistical Computing, https://www.r-project.org/, 2020.

Ratkowsky, D. A., Lowry, R. K., McMeekin, T. A., Stokes, A. N., and Chandler, R. E.: Model for bacterial culture growth rate throughout the entire biokinetic temperature range, J. Bacteriol., 154, 1222–1226, https://doi.org/10.1002/14651858.CD002209.pub2, 1983.

Rijkers, R., Rousk, J., Aerts, R., and Weedon, J. T.: Optimal growth temperature of Arctic soil bacterial communities increases under experimental warming, Glob. Chang. Biol., https://doi.org/doi:10.1111/gcb.16342, 2022.

Rinnan, R., Rousk, J., Yergeau, E., Kowalchuk, G. A., and Bååth, E.: Temperature adaptation of soil bacterial communities along an Antarctic climate gradient: Predicting responses to climate warming, Glob. Chang. Biol., 15, 2615–2625, https://doi.org/10.1111/j.1365-2486.2009.01959.x, 2009.

Rinnan, R., Michelsen, A., and Bååth, E.: Long-term warming of a subarctic heath decreases soil bacterial community growth but has no effects on its temperature adaptation, Appl. Soil Ecol., 47, 217–220, https://doi.org/10.1016/j.apsoil.2010.12.011, 2011.

Rousk, J., Frey, S. D., and Bååth, E.: Temperature adaptation of bacterial communities in experimentally warmed forest soils, Glob. Chang. Biol., 18, 3252–3258, https://doi.org/10.1111/j.1365-2486.2012.02764.x, 2012.

Sigurdsson, B. D., Leblans, N. I. W., Dauwe, S., Gudmundsdóttir, E., Gundersen, P., Gunnarsdóttir, G. E., Holmstrup, M., Ilieva-Makulec, K., Kätterer, T., Marteinsdóttir, B., Maljanen, M., Oddsdóttir, E. S., Ostonen, I., Peñuelas, J., Poeplau, C.,

Richter, A., Sigurdsson, P., Van Bodegom, P., Wallander, H., Weedon, J., and Janssens, I.: Geothermal ecosystems as natural climate change experiments: The ForHot research site in Iceland as a case study, Icelandic Agric. Sci., 29, 53–71, https://doi.org/10.16886/IAS.2016.05, 2016.

560 Tarnocai, C., Canadell, J. G., Schuur, E. a G., Kuhry, P., Mazhitova, G., and Zimov, S.: Soil organic carbon pools in the northern circumpolar permafrost region, Global Biogeochem. Cycles, 23, 1–11, https://doi.org/10.1029/2008GB003327, 2009.

Vellend, M.: Conceptual synthesis in community ecology, Q. Rev. Biol., 85, 183–206, https://doi.org/10.1086/652373, 2010.

Wang, C., Morrissey, E. M., Mau, R. L., Hayer, M., Piñeiro, J., Mack, M. C., Marks, J. C., Bell, S. L., Miller, S. N., Schwartz, E., Dijkstra, P., Koch, B. J., Stone, B. W., Purcell, A. M., Blazewicz, S. J., Hofmockel, K. S., Pett-Ridge, J., and Hungate, B. A.: The temperature sensitivity of soil: microbial biodiversity, growth, and carbon mineralization, ISME J.,

565 https://doi.org/10.1038/s41396-021-00959-1, 2021.

Weedon, J. T., Bååth, E., Rijkers, R., Reischke, S., Sigurdsson, B. D., Oddsdóttir, E. S., Van Hal, J., Aerts, R., Janssens, I. A., and Van Bodegom, P. M.: Community adaptation to temperature explains abrupt soil bacterial community shift along a geothermal gradient on Iceland, Soil Biol. Biochem., 177, 108914, https://doi.org/https://doi.org/10.1101/486966, 2023.

Wieder, W. R., Sulman, B. N., Hartman, M. D., Koven, C. D., and Bradford, M. A.: Arctic Soil Governs Whether Climate

570 Change Drives Global Losses or Gains in Soil Carbon, Geophys. Res. Lett., https://doi.org/10.1029/2019GL085543, 2019.

Willms, I. M., Rudolph, A. Y., Göschel, I., Bolz, S. H., Schneider, D., Penone, C., Poehlein, A., Schöning, I., and Nacke, H.: Globally Abundant " Candidatus Udaeobacter" Benefits from Release of Antibiotics in Soil and Potentially Performs Trace Gas Scavenging , mSphere, 5, https://doi.org/10.1128/msphere.00186-20, 2020.

Willms, I. M., Bolz, S. H., Yuan, J., Krafft, L., Schneider, D., Schöning, I., Schrumpf, M., and Nacke, H.: The ubiquitous soil

575 verrucomicrobial clade 'Candidatus Udaeobacter' shows preferences for acidic pH, Environ. Microbiol. Rep., 13, 878–883, https://doi.org/10.1111/1758-2229.13006, 2021.

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., and Glöckner, F. O.: The SILVA and "all-species Living Tree Project (LTP)" taxonomic frameworks, Nucleic Acids Res., 42, 643–648, https://doi.org/10.1093/nar/gkt1209, 2014.

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Figure Captions

Figure 1. A) Map with polar projection showing the 12 sampling sites across the Arctic and B) the average values of soil thermal regimes for each site including the maximum (red), mean (grey) and minimum (blue) soil temperature. Error bars indicate the standard deviation across the years.

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Figure 2. A) Estimated growth curves for each soil type depicted by the normalized leucine incorporation over incubation temperature. Colors indicate the maximum soil temperature of each sampling site. B) Linear relationships between the optimal growth temperature and maximum soil temperature, error bars indicate the standard error.

590 **Figure 3**. Bar plot showing the relative abundance (%) of top 10 most abundance phyla across all soil samples. Color shades indicate the two most abundant Order for each of these phyla.

Figure 4. Map of the predicted Topt of soil bacterial communities across the Arctic based on the linear relationship between maximum soil temperature (from SoilTemp database) and Topt.

Figure 5. Principal coordinate analysis of bacterial community composition (16s rRNA amplicon sequencing) based on
595 weighted Unifrac distances. Lines show the correlation of measured environmental factors with the axes of the ordination space

Table 1. Thermal regimes of the 12 soil types

600 **Table 2.** Description of abbreviations used to described temperature-growth relationships and soil thermal regimes

Table 3. Results of PERMANOVA showing the influence of soil parameters on the bacterial community composition

Table 4. Taxonomy of commonly observed bacterial ASVS. N indicates number of soils the ASV was observed in.
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Table 1. Thermal regimes of the 12 sampling sites in ° C. *STmax* depicts the warmest day of the year, MAST the mean annual temperature and *STmin* the coldest day of the year. ± indicate standard deviation of the mean value recorded of the temperature record from the first year (Start) till the last year (End). Depth indicated the soil temperature logger depth in centimeters.

Site	Start	End	Depth	*STmax*	MAST	*STmin*
Abisko	2015	2019	10	13.2 ± 3.7	0.8 ± 0.1	-9.8 ± 5.3
Blaesedalen	2013	2020	10	10.5 ± 1.3	-0.7 ± 1.3	-14.5 ± 4.8
Iceland Forest	2013	2019	10	11.8 ± 0.3	5.1 ± 0.3	0 ± 0.3
Iceland Grassland	2013	2018	10	14.2 ± 0.7	6.1 ± 0.6	0.1 ± 0.2
Inuvik	2002	2018	5	13.5 ± 2.2	1.3 ± 0.7	-6.1 ± 3
Kobbefjord	2008	2019	10	12.3 ± 0.8	1.9 ± 0.5	-5.3 ± 2.5
Svalbard	2008	2016	25	9 ± 0.8	-3.5 ± 0.5	-18.5 ± 2.3
Svanhovd	2014	2021	10	15 ± 0.7	3.6 ± 0.6	-3.4 ± 0.1
Toolik Lake Heath	2002	2019	10	13.5 ± 1	-2.2 ± 1.2	-13.8 ± 3.8
Toolik Lake MAT	2008	2021	10	2.7 ± 0.4	-2.2 ± 0.6	-8.1 ± 2.2
Toolik Lake MNAT	2012	2021	10	4.7 ± 0.8	-1.7 ± 0.8	-8.2 ± 2.9
Kilpisjarvi	2019	2019	10	16.3		

Table 2. Description of abbreviations used to describe temperature-growth relationships and soil thermal regimes

	ABBREVIATION	DESCRIPTION
CARDINAL POINTS OF TEMPERATURE-GROWTH RELATIONSHIPS	Tmin	Theoretical minimum temperature of growth
	Topt	Optimal growth temperature
	Tmax	Theoretical maximum growth temperature
SOIL TEMPERATURES	*STmin*	Mean minimum daily soil temperature
	MAST	Mean annual soil temperature
	STmax	Mean maximum daily soil temperature

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Table 3. PERMANOVA showing the association between mean annual soil temperature (MAST) and other environmental (pH, water content, organic carbon and nitrogen) and community physiological measures (Topt) and bacterial community composition calculated using the weighted UniFrac metric based on 16 rRNA gene sequences across the 11 sampled sites (n = 1 –3 per site) Bold numbers indicate significance (P<0.05)

	Df	SumOfSqs	R2	F	Pr(> F)
MAST	1	0.013	0.061	3.136	**0.037**
pH	1	0.085	0.412	21.115	**0.001**
Water Content	1	0.008	0.037	1.882	0.115
Topt	1	0.008	0.037	1.919	0.124
Organic C	1	0.004	0.021	1.084	0.290
Organic N	1	0.008	0.040	2.068	0.095
Residual	20	0.081	0.391		
Total	26	0.207	1		

Table 4. Taxonomy of commonly observed (present in samples from 4 or more sites) bacterial ASVs. N indicates number of soils the ASV was observed in.

SV	PHYLUM	CLASS	ORDER	FAMILY	GENUS	SPECIES	N
1	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae (Subgroup 1)	Granulicella	Uncultured soil	4
2	Acidobacteriota	Acidobacteriae	Subgroup 2	Subgroup 2	Subgroup 2	Uncultured forest	4
3	Acidobacteriota	Acidobacteriae	Subgroup 2	Subgroup 2	Subgroup 2	Uncultured eubacterium	6
4	Actinobacteriota	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Conexibacter		4
5	Actinobacteriota	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium		4
6	Actinobacteriota	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus		4
7	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae			1
8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium		4
9	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae			5
10	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae			7
11	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus Udaeobacter	Uncultured soil	5
12	Proteobacteria	Gammaproteobacteria	WD260	WD260	WD260	Uncultured eubacterium	4
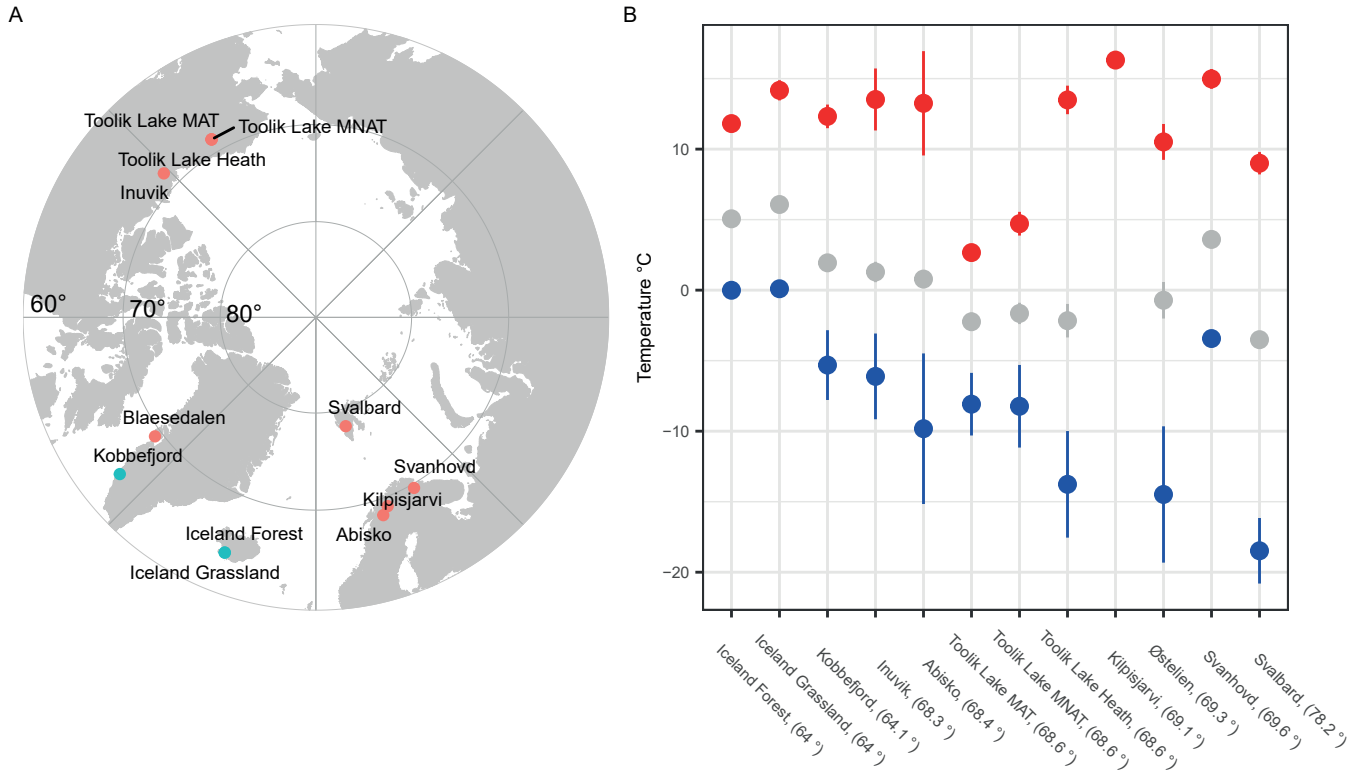
Figure 1. A) Map with polar projection showing the 12 sampling sites across the Arctic and B) the average values of soil thermal regimes for each site including the maximum (red), mean (grey) and minimum (blue) soil temperature. Error bars indicate the standard deviation across the years.
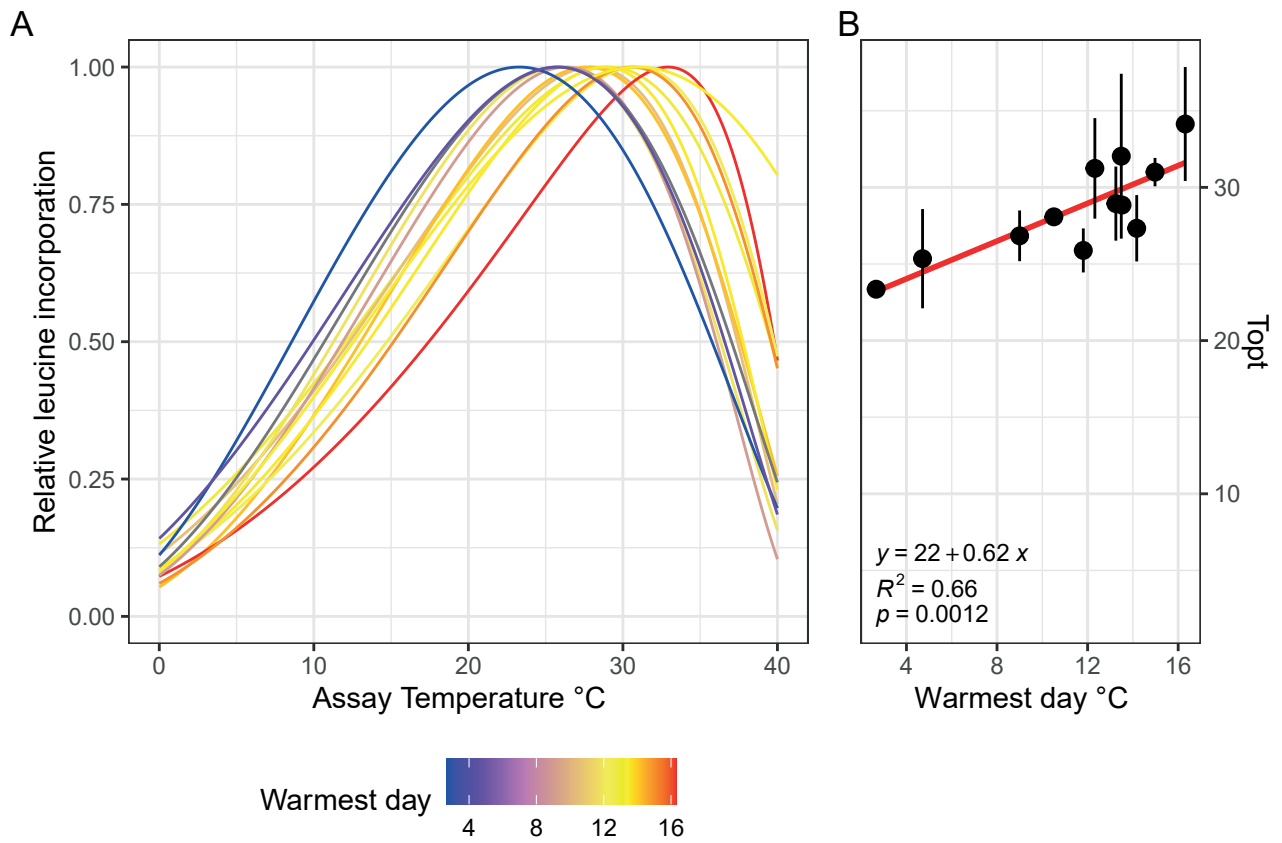
Figure 2. A) Estimated growth curves for each soil type depicted by the normalized leucine incorporation over incubation temperature. Colors indicate the maximum soil temperature of each sampling site. B) Linear relationships between the optimal growth temperature and maximum soil temperature, error bars indicate the standard error.

Figure 3. Bar plot showing the relative abundance (%) of top 10 most abundance phyla across all soil samples. Color shades indicate the two most abundant Order for each of these phyla.
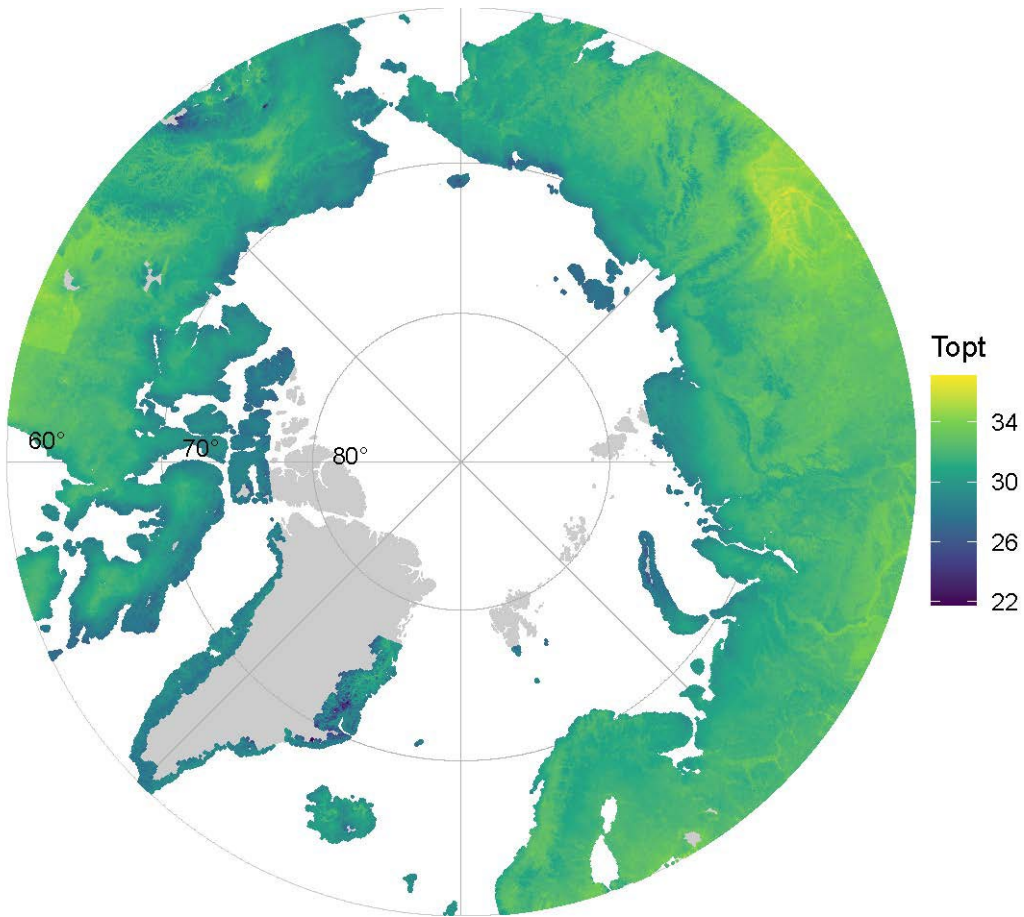
Figure 4. Map of the predicted Topt of soil bacterial communities across the Arctic based on the linear relationship between maximum soil temperature (from SoilTemp database) and Topt.
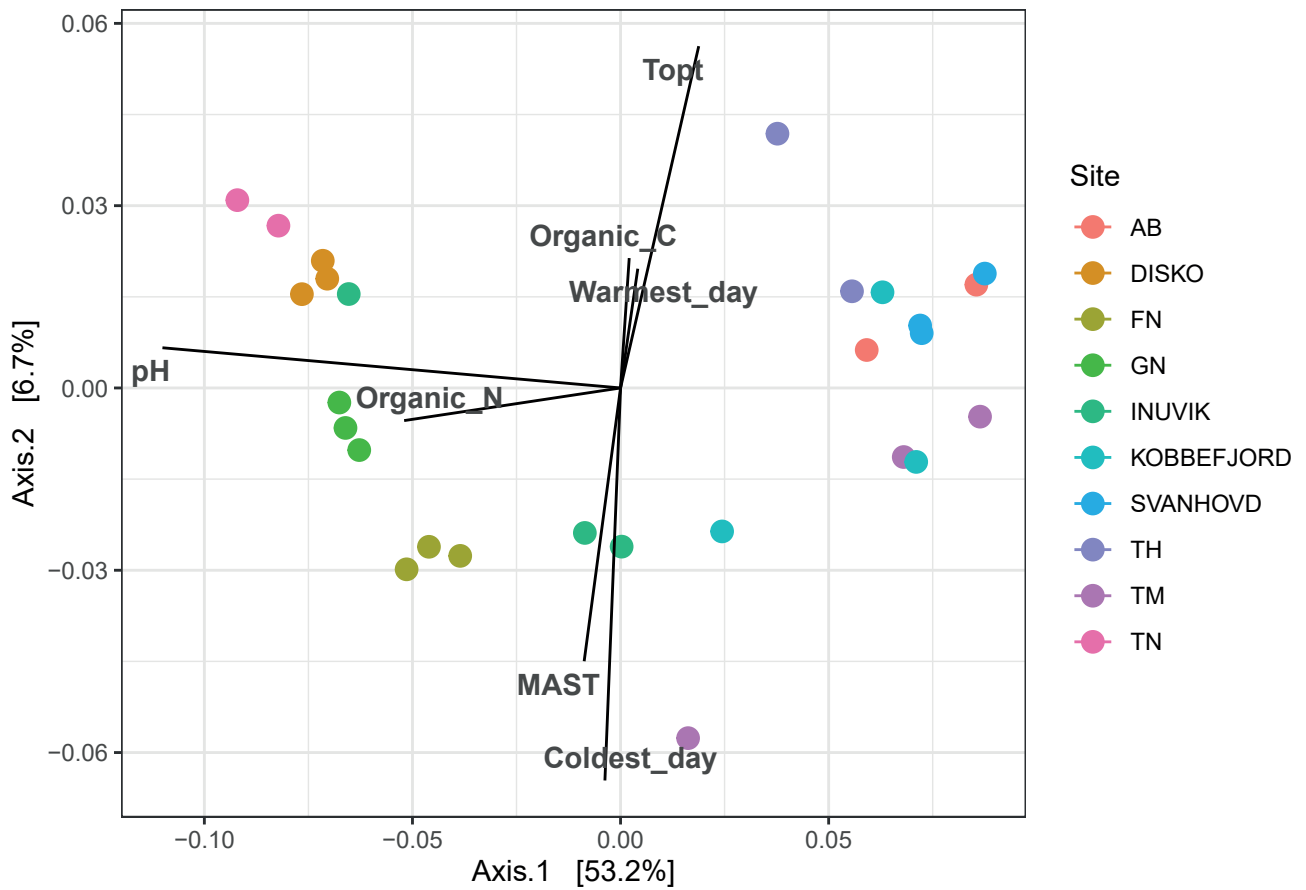
Figure 5. Principal coordinate analysis of bacterial community composition (16s rRNA amplicon sequencing) based on weighted Unifrac distances. Lines show the correlation of measured environmental factors with the axes of the ordination space.