Temperature adaptation of soil bacterial communities across the Arctic

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Temperature adaptation of soil bacterial communities across the Arctic

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door
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geboren te Rijswijk

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“Seconds from annihilation

But no one stopped to think about the people

Or how they would survive”

We Almost Lost Detroit

Gil Scott-Heron

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Chapter 1

General Introduction

Soil carbon cycling

The microorganisms that inhabit soil play a crucial role in the mineralization of carbon belowground, by breaking down plant litter and root exudates (Schimel and Schaeffer, 2012). During this decomposition process, plant-derived carbon is largely respired back into the atmosphere as CO_2. A large part of the plant-derived carbon that remains in soils is transformed into microbial biomass (Kallenbach et al., 2016). Consequently, the remains of dead microorganisms, microbial necromass, can comprise as much as 50% of the organic carbon in soil (Ludwig et al., 2015; Liang et al., 2019; B. Wang et al., 2021). Microorganisms also further decompose soil organic matter to support their catabolic and anabolic needs (Liang et al., 2017). By their transformation of plant litter and exudates into remnant pools of soil organic matter or through the return of carbon to the atmosphere in the form of CO_2 or CH_4, microorganisms are therefore a key driver for the storage capacity of carbon and soil carbon cycling (Figure 1.1).

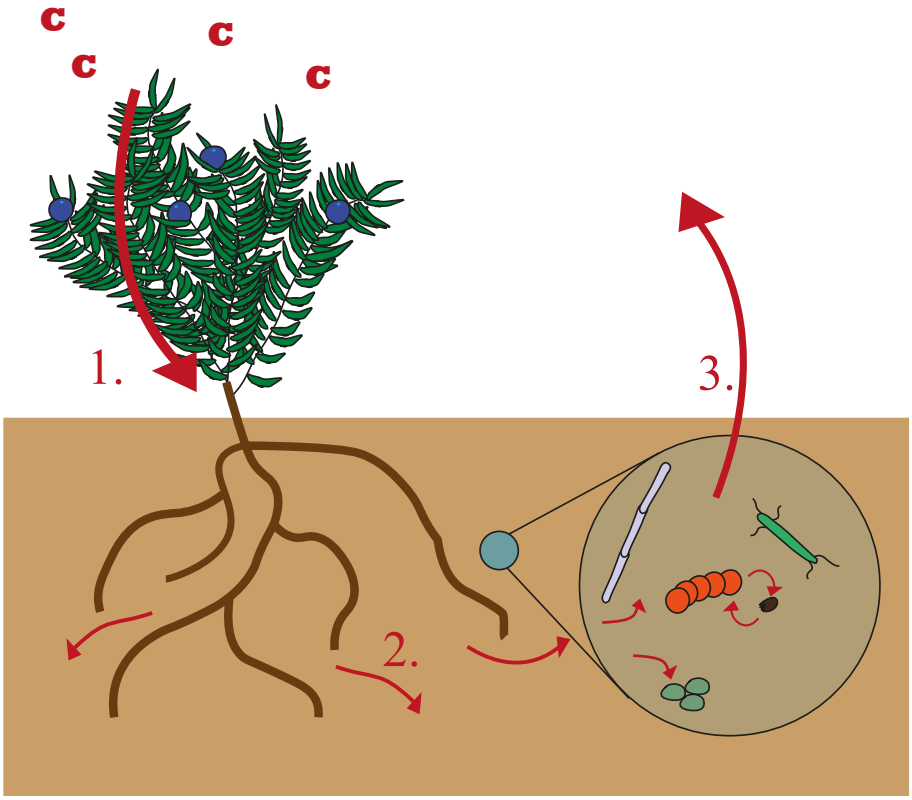

Figure 1.1 Soil carbon cycle (red lines) showing the 1. input of plant litter and 2. root exudates as carbon into the soil, which is 3. largely mineralized back to the atmosphere by microbial organisms, or stabilized as soil organic matter. It is expected that the rate of all these processes will increase at higher temperatures, especially in cold biomes.

Soils play an important role in global carbon cycling and in the feedbacks to climate change. Carbon flows in different forms through the atmosphere, vegetation and soils, of which roughly two thirds of the carbon in this cycle resides belowground (Lal, 2008). Loss of soil carbon can therefore have a considerable impact on atmospheric CO_2 concentrations. Since the processes involved in the formation, stabilization and mineralization of soil organic matter can all respond differently to climate change, it is still uncertain whether soils will gain or lose organic carbon under future climate conditions (Bradford et al., 2016). Notably in cold biomes, plant productivity could be stimulated by increasing temperatures (Ainsworth and Long, 2005). This could lead to increased production of plant litter and root exudates, therefore increasing the allocation of carbon to belowground and stimulating the formation of soil organic matter. However, some portion of the additional CO_2 that is captured by photosynthesis of plants will likely be transferred back to the atmosphere, due to increased decomposition by soil bacterial communities (Heath et al., 2005; van Groenigen et al., 2017). Higher air temperatures also induce an increase in the activity of microbial enzymes that breakdown soil organic matter (Davidson and Janssens, 2006; Conant et al., 2011). Increased microbial activity under warmed conditions has the potential to mineralize both fresh carbon and remnant organic matter in soils. Thus, there is the potential for terrestrial ecosystems to turn from net carbon sinks to carbon sources under future climatic conditions (Peñuelas et al., 2017), especially in the northern latitudes, with the released carbon contributing to further warming (Crowther et al., 2016). This phenomenon is known as the 'soil carbon feedback' to climate change. To understand the fate of soil organic matter under climate change both the input and output of soil organic matter need to be understood (Figure 1.1). Moreover, given the importance of microbially driven decomposition for estimating the fate of soil organic carbon globally, there is an urgent need to understand what environmental and biological factors influence the functioning of soil microbial communities.

Effects of warming on soil organic matter and microbial communities

Current predictions estimate that by 2100, Earth's surface air will warm between 1.6 °C and 2.8 °C compared to pre-industrial times (Collins et al., 2013). This has the potential to induce substantial global losses of organic carbon from soils into the atmosphere due to increased microbial activity (Naylor et al., 2020). However, it is still uncertain whether soil carbon stocks will increase or decrease, with predicted changes in soil organic carbon stocks varying between a gain of 248 Pg or a loss of 95 Pg of carbon from soils globally by 2100 (Carey et al., 2016; Crowther et al., 2016; Todd-Brown et al., 2018; van Gestel et al., 2018). The uncertainty of the projected fate of soil organic matter is to a large extent caused by the unknown magnitude of responses of soil microbial processes to climate change (Wieder et al., 2015, 2019; Bradford et al., 2021). Due to the large potential soil carbon feedback to climate change, many efforts have been made to investigate the effects of temperature on soil carbon efflux in various contexts. The magnitude by which soil decomposition rates increase under higher temperatures is influenced by many factors related to the biochemistry of the soil organic matter itself, the environment, and the microbial communities present (Davidson and Janssens, 2006; Bradford et al., 2016).

From a biochemical perspective, the substrates that microbial enzymes break down into smaller organic compounds have a certain activation energy, which is the minimum energy required to transform the first molecule into the latter. The activation energy is higher for more complex and larger molecules, which leads to a stronger response of the enzymatic rate to temperature (Arrhenius, 1889). Since the temperature sensitivity of decomposition differs between small and large molecules, warming will therefore most strongly affect the decomposition rate of more chemically complex "recalcitrant" soil organic matter (Conant et al., 2011). Aspects of the soil environment can also influence temperature sensitivity of decomposition. For example, mineral interactions and soil aggregation can both affect the availability of soil organic matter for the degradation by microbial enzymes (Cotrufo et al., 2019; Hartley et al., 2021). With shifts in temperature, the absorption and desorption of carbon to minerals change, which implies that soil mineralogy influences the apparent temperature sensitivity of the decomposition of soil organic matter (Lugato et al., 2021). Additionally, soil moisture and oxygen concentrations impact the temperature sensitivity (Davidson and Janssens, 2006). Finally, the response of soil decomposition rates to warming is dependent on the

temperature itself. The temperature sensitivity of decomposition rates is particularly high at low temperatures and decreases with increasing temperatures (Kirschbaum, 1995; Tuomi et al., 2008; Hamdi et al., 2013). This can be explained by thermodynamic constraints (Hobbs et al., 2013; Schipper et al., 2014; Arcus et al., 2016). Taking all these chemical and physical factors together, the temperature sensitivity of decomposition rates of soil organic matter can vary greatly along gradients of soil depth (Karhu et al., 2010; Wang et al., 2022), vegetation type (Gutiérrez-Girón et al., 2015; Ding et al., 2016) and environmental gradients (Wieder et al., 2019).

The temperature response of decomposition rates is not solely explained by geochemical and environmental variables (Alster et al., 2016; Liu et al., 2018; Li et al., 2020). Soil microbial communities from colder regions show a larger response in terms of respiration to temperature than communities from warmer regions, even after accounting for other influences, such as differences in substrate availability and microbial biomass (Dacal et al., 2019). This difference in temperature response of microbial communities has been linked to a difference in microbial community composition between soils from different climatic regions (Balser and Wixon, 2009; Matulich and Martiny, 2015; Strickland et al., 2015; Glassman et al., 2018; Johnston and Sibly, 2018; Tong et al., 2021). It is therefore hypothesized that microbial communities can adapt to their local climate regime via potential phenotypic, genotypic or compositional community changes (Balser and Wixon, 2009; Bárcenas-Moreno et al., 2009; Wei et al., 2014; Chase et al., 2021), which induce different temperature responses for microbial communities obtained from different climatic regions (Karhu et al., 2014; Strickland et al., 2015; Dacal et al., 2019). So far, these differences are not implemented in large scale earth system models (ESMs), which assume the same temperature response across all soils globally (Todd-Brown et al., 2012, 2013; Wieder et al., 2015). Implementing microbial processes into ESMs is however crucial for accurate predictions of the fate of soil organic matter (Allison et al., 2010; Wieder et al., 2013; Bradford et al., 2021) and the feedback to the global climate. To better understand the fate of soil organic matter in the 21st century, it is therefore important to be able to predict the relationship between the climate and the temperature response of soil microbial communities (García-Palacios et al., 2021).

The current frameworks for temperature adaptation of bacterial communities

The relationship between the temperature experienced by a microbial community and a measure of the inherent physiological response of that community to temperature is known as the temperature adaptation of microbial communities. Within soil microbial communities bacteria and fungi are the most important groups with respect to carbon cycling. Since fungal and bacterial soil communities show large similarities in terms of temperature adaptation (Bárcenas-Moreno et al., 2009), this dissertation focusses on the temperature adaptation of soil bacterial communities for reasons of feasibility. The adaptation of soil bacterial communities can be determined relative to many physiological processes, but is most commonly studied in terms of changes in CO_2 production, growth or substrate uptake. A common method for measuring the temperature adaption of bacterial communities is an assay for the incorporation of leucine as a proxy for growth (Bååth et al., 2001). By measuring the growth rate over a controlled gradient of temperatures, the temperature adaptation of individual species or whole communities can be described by the use of the Ratkowsky model (Ratkowsky et al., 1983). In this model, the growth of bacteria is assumed to increase quadratically up to the optimal growth temperature T_{opt}, after which it declines exponentially. The temperature adaptation of soil bacterial communities can be described by the (extrapolated) cardinal points of the fitted curve. These are the minimum growth temperature T_{min}, optimal growth temperature T_{opt} and maximal growth temperature T_{max} (Figure 1.2). Moreover, the temperature range in which soil bacterial communities grow can be described as the interval between T_{max} and T_{min} or, more commonly, since T_{max} is difficult to estimate: T_{opt} and T_{min}. A range of studies have shown that the temperature-growth relationship of soil bacterial communities is tightly linked to the temperature dependence of heterotrophic soil respiration (Bååth, 2018; Birgander et al., 2018; Cruz-Paredes et al., 2021). In contrast to soil respiration, temperature-growth relationships are less affected by other environmental factors (Cruz-Paredes et al., 2021), and are therefore more reliable for estimating the inherent temperature adaptation of bacterial communities (Bååth, 2018). This implies that it should be possible to incorporate knowledge of the temperature adaptation for soil bacterial communities into ESMs in order to estimate the influence of shifts in temperature adaptation on soil carbon cycling and correspondingly accurately predict the

temperature response of decomposition rates of soil organic matter. By comparing the cardinal points of the temperature-growth relationship, researchers can assess the difference in temperature adaptation of soil bacterial communities across large geographical gradients, in changing environments through time, and in response to experimental treatments.

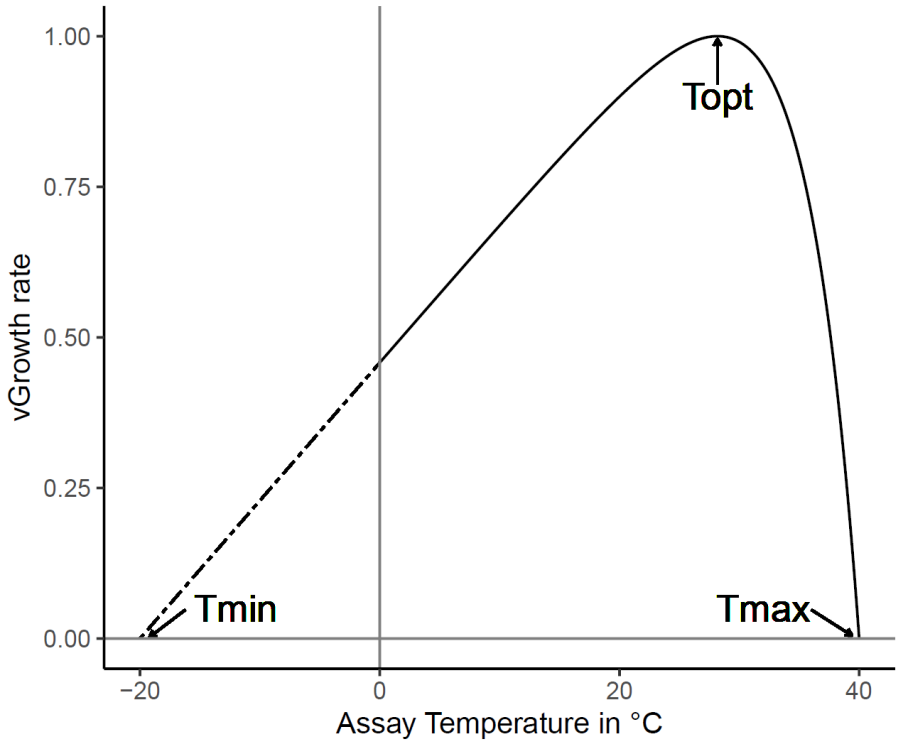

Figure 1.2 Model for the relationship between temperature and bacterial growth as proposed by the Ratkowsky equation, including the cardinal points. Growth in replicate aliquots from a single extract is estimated at several temperature levels between (typically 5 – 7 temperatures between 0 °C and 40 °C). The model is fit to the resulting growth measurements, and the cardinal points computed by extrapolation (for T_{min} and T_{max}) and numerical solution (for T_{opt}). These cardinal points can be used as indices of temperature adaptation.

It is important to identify the drivers of temperature adaptation for an accurate prediction of the temperature response of soil bacterial communities under current and future climate conditions. Across large temperature gradients, soil bacterial communities have been shown to adapt to their local climate. For example, soil bacterial communities from the polar regions typically have an optimal growth temperature of 25 °C (Rinnan et al., 2011; van Gestel et al., 2020), compared to an optimal growth temperature > 40 °C for soil bacterial communities from hot desert communities (van Gestel et al., 2013). Due to the differences in this temperature adaptation of soil bacterial communities between climatic zones, it is hypothesized that warming of soils will induce changes in the temperature adaptation of soil bacterial communities *in situ* (Rinnan et al., 2009; Nottingham et al., 2019a), which would affect soil decomposition rates under future climatic conditions. However, contrasting observations have been made about how shifts in the temperature adaptation of soil bacterial communities are induced and also the mechanisms driving temperature adaptation are so far not fully understood. This limits the current ability to predict when and how shifts in the temperature adaptation of soil bacterial communities will occur with changing climatic conditions.

Based on observations, two types of adaptation can be distinguished. In the first group of observations, laboratory studies have shown that the temperature adaption of soil bacterial communities will only change when they are exposed to temperatures above the optimal growth temperature of the community during short-term incubation experiments (Bárcenas-Moreno et al., 2009; Birgander et al., 2013; Donhauser et al., 2020). Therefore, the current theoretical framework (Birgander et al., 2013) hypothesizes that shifts in temperature adaptation of soil bacterial communities under warmed conditions occur due to heat-induced death of cold-adapted bacterial taxa, which implies that changes in the temperature adaptation of soil bacterial communities will only occur when soil temperatures reach above the T_{opt} of the initial community.

Recently, it has been shown by soil warming experiments that changes in the temperature adaptation of soil bacterial communities also occur under field conditions (Rousk et al., 2012; Nottingham et al., 2019a, 2022; Weedon et al., 2022). This further corroborates the evidence that climate change will indeed affect the temperature adaptation of soil microbial communities. However, in contrast to the

results from lab incubations, these field observations show that changes in the temperature adaption can even occur at temperatures below the optimal growth temperature of the community (Nottingham et al., 2021; Weedon et al., 2022), and that shifts take a longer time to occur under cooler soil climates (Nottingham et al., 2021). Thus, this second group of observations shows that the time needed for the shift in temperature adaptation to occur might differ depending on the magnitude of warming and the climate region (Nottingham et al., 2019a; Weedon et al., 2022).

It is currently hypothesized that the temperature adaptation of soil bacterial communities emerges from environmental filtering for bacterial taxa that perform best in the temperature range that the soils are exposed to (Bárcenas-Moreno et al., 2009; Birgander et al., 2013; Donhauser et al., 2020). This is supported by recent observations that the change in temperature adaptation of soil bacterial communities co-occurs with a shift in the composition of the bacterial communities (Donhauser et al., 2020; Weedon et al., 2022). Additional evidence for this species-sorting hypothesis comes from the fact that soil bacterial communities from colder regions are comprised of more species that are adapted to low temperatures than soil bacterial communities from warmer regions (C. Wang et al., 2021). In case the temperature adaptation of a soil bacterial community directly arises from the temperature preferences of its community members, bacterial taxa with specific temperature traits might thus be indicative of the temperature adaptation of soil bacterial communities. Moreover, the temperature adaption of soil bacterial communities might than be predicted by forecasting the relative fitness of bacteria varying in temperature traits.

The Arctic

High latitude ecosystems are some of the key regions to study the impact of warming on the temperature adaptation and decomposition rates of soil bacterial communities. The Arctic region is currently warming at 2 – 4 times faster than the global average (Collins et al., 2013; Post et al., 2019; Rantanen et al., 2022), which is mostly driven by temperature feedbacks and changing albedo effect, among other factors (Pithan and Mauritsen, 2014; Previdi et al., 2021). While the large response of Arctic microbial communities and their soil decomposition rates to warming is generally recognized (Hamdi et al., 2013; Karhu et al., 2014), we still lack a comprehensive understanding of whether the temperature adaptation of

soil bacterial communities will shift under climate change (Rinnan et al., 2011). It is therefore particularly important to understand what mechanisms drive the temperature adaptation of arctic soil bacterial communities and to use that information to determine if predicted levels of warming will induce shifts in the temperature adaptation of arctic soil bacterial communities (Rinnan et al., 2011; Weedon et al., 2022).

The Arctic terrestrial region contains 50% of the soil organic carbon globally, of which 340 - 1530 Pg of carbon is estimated to be vulnerable to decomposition (Tarnocai et al., 2009, 2017; Hamdi et al., 2013; Schädel et al., 2014). Currently, earth system models differ in their predictions regarding the vulnerability of soil organic carbon stocks to decomposition in the Arctic, while these stocks determine to a large extent whether there is a loss or gain in global soil carbon stocks under climate change (Wieder et al., 2019). Due to the lack of data on the temperature adaptation of soil bacterial communities, it still unknown whether the large temperature response of decomposition rates in the Arctic will alter under warmed conditions.

Soil bacteria in the Arctic experience long winter periods with below-zero temperatures and relatively short time periods above thawing point (Priemé et al., 2017; Poppeliers et al., 2022). Arctic soil bacterial communities are therefore hypothesized to be adapted to low temperatures (Bååth, 2018). This is supported by the observation that bacterial communities in the sub-Arctic grow between -10 and 45 °C , with an optimal growth temperature of 25-30 °C , which is lower than that of bacterial communities from temperate and warm regions (section 1.3; Rinnan et al., 2011; van Gestel et al., 2013; Bååth, 2018). Despite these important observations, a study covering many Arctic sites is still needed to determine the variation in the temperature adaptation of soil bacterial communities across the entire Arctic region. Such a study may clarify whether increasing temperatures will indeed induce shifts in the temperature adaptation within the Arctic region (Rinnan et al., 2011; Weedon et al., 2022).

To improve the predictions for the temperature adaptation of arctic soil bacterial communities, more data is needed from bacterial communities of the Arctic region to understand the impact of soil warming for these communities. For example, data over a large-scale spatial gradient in the Arctic would allow the identification of the main drivers of temperature adaptation in arctic soil bacterial communities.

In addition, soil incubation studies can indicate whether currently projected arctic soil temperatures over the 21st century are likely to induce shifts in the current temperature adaptation of soil bacterial communities in the Arctic. If community dynamics indeed control the temperature adaptation of a soil bacterial community, as discussed in section 1.3, the temperature adaptation of soil bacterial communities might be predictable from the abundance of taxa associated with a specific temperature adaptation of soil bacterial communities, or through modelling the potential growth of species with specified temperature preferences. Improved assessment of the possible temperature adaptation of soil bacterial communities under current and future arctic soil climatic conditions can thereby contribute to better constraints on estimates of soil carbon cycling processes in the future Arctic.

Aims and research questions

The overall aim of this dissertation is to determine the current temperature adaptation of soil bacterial communities and whether potential shifts in the temperature adaptation of bacterial soil communities can influence the fate of soil organic matter under future climate change. This leads to the following research questions:

a. What are the current cardinal points (T_{min}, T_{opt}) of temperature adaptation for soil bacterial communities across the Arctic? How are these related to soil climatic variables?
b. Will warming induce shifts in the cardinal points of the temperature adaptation of soil bacterial communities in the Arctic?
c. Can information about the abundance of indicator taxa in bacterial communities of arctic soils inform us about the temperature adaptation of the community?
d. Are we able to predict the temperature adaptation of soil bacterial communities based on theory about both the temperature traits of soil bacteria and the mechanisms that drive temperature adaptation of soil bacterial communities?

The approach

In this dissertation, a range of approaches is used to evaluate the current temperature adaptation of soil bacterial communities in the Arctic (Figure 1.3). I

assessed the temperature adaptation with ^{3}H-leucine assays as a proxy for bacterial growth. The use of this method allows for comparison to previous work on the temperature adaptation of soil bacterial communities in sub-Arctic and temperate environments. I used a set of sites spanning a large-scale climatic gradient to measure the current temperature adaptation of arctic soil bacterial communities and investigate the key climatic drivers for their temperature adaptation (Chapter 2). To test whether the temperature adaptation of bacterial communities in arctic soils is shifted under warmed conditions, I conducted an incubation study, in which arctic soils were exposed to temperatures of more than 15 °C above the current maximum soil temperature (Chapter 3). I used 16S rRNA gene amplicon sequencing to profile the bacterial communities and elucidate whether changes in community composition can explain the possible shifts in temperature adaptation. To test the use of bacterial taxa as indicators of soil warming and the temperature adaptation of soil bacterial communities, I identified the bacterial taxa that are ubiquitously abundant across the spatial gradient and that consistently respond to increased soil temperature along a well-studied soil warming gradient in Iceland (Chapter 4). Last, a new modelling approach was used to integrate current knowledge about the mechanisms that drive the temperature adaptation of soil bacterial communities into a simple trait-based model (Chapter 5). By evaluating the accuracy of the model, I aimed to improve the conceptual framework for the temperature adaptation of soil bacterial communities (section 1.3).

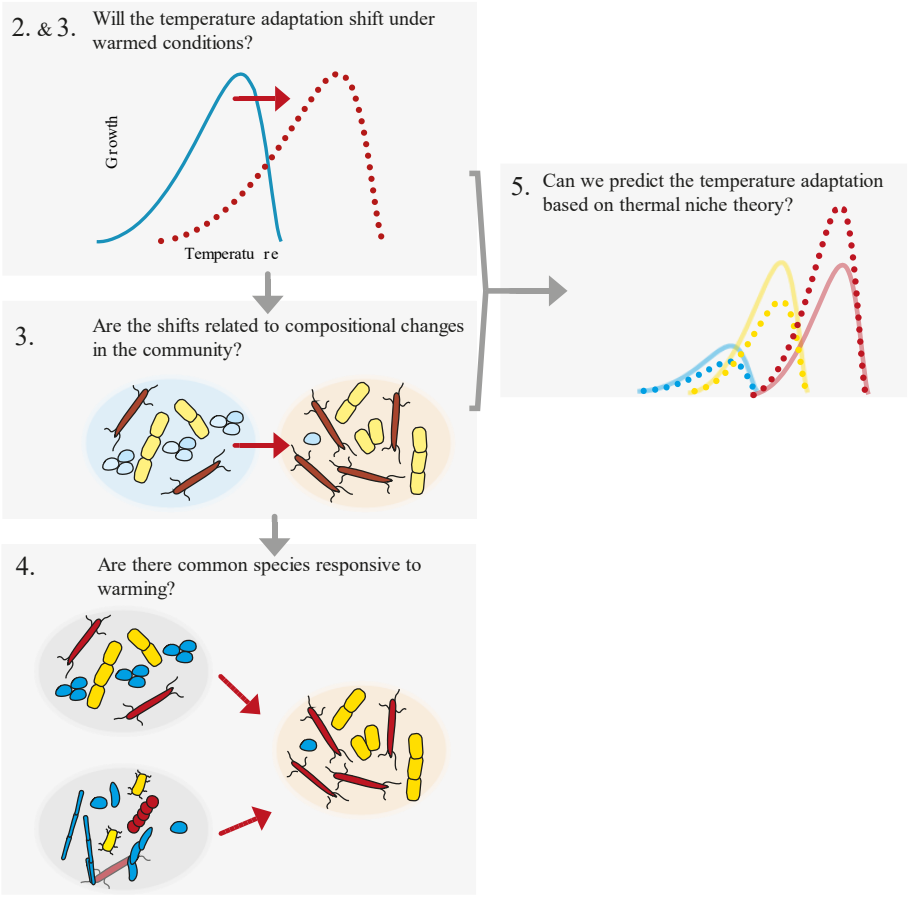

Figure 1.3. Conceptual diagram of the approach where I evaluate (**Chapter 2**); the current temperature adaptation of soil bacterial communities in the Arctic (**Chapter 3**); whether shifts in the temperature adaptation of arctic soil bacterial communities can be induced by experimental warming, (**Chapter 4**); if changes of the abundance of bacterial species within the community are robust indicators of warming; and (**Chapter 5**) whether we can predict the temperature adaptation of soil bacterial communities based on current understanding of thermal niches for soil bacteria (blue, yellow and red lines).

Outline of thesis

In this dissertation, the temperature adaptation of soil bacterial communities in the Arctic region is evaluated and approaches for predicting the distribution of bacterial temperature adaptation under current and future climate condition are constructed and evaluated.

In **Chapter 2**, the current temperature adaptation of soil bacterial communities is determined across multiple sites in the Arctic region and possible drivers of variation in temperature adaptation are evaluated. **Chapter 3** tests whether the temperature adaptation of arctic soil bacterial communities alters in response to experimentally elevated temperatures and whether these shifts are related to compositional changes within the bacterial communities.

The succeeding chapters describe and test potential methodology for predicting current and future responses of soil bacterial communities to warming. In **Chapter 4**, I evaluate the use of bacterial taxa as indicators of the response of bacterial communities to soil warming by using a natural warming gradient in Icelandic grasslands. A multi-year dataset on the bacterial community composition at 6 °C warming was used to test whether bacterial taxa responsive to warming ('bioindicators') show a consistent response both in time and across studies.

In an alternative approach to 'bioindicators', **Chapter 5** compiles the key findings of this dissertation and previous literature to build a model for prediction of the temperature adaptation of soil bacterial communities based on the temperature regime they are exposed to and based on current theory on the distribution and correlation of temperature traits in soil bacteria.

In **Chapter 6**, a synthesis is presented on the key findings of this thesis, resulting in the postulation of a framework for how soil bacterial communities respond to increasing soil temperatures. Furthermore, this chapter identifies ecological questions that need to be addressed for further advancement in accurate predictive modeling of the impacts and feedbacks of climate change to soil carbon cycling.

Chapter 2

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

Ruud Rijkers, Mark Dekker, Rien Aerts and James T. Weedon

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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 (Topt) 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 Topt 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 Topt 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.

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; Rousk et al., 2012; Weedon et al., 2022). 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; Rinnan et al., 2011; Birgander et al., 2013, 2018; Weedon et al., 2022). 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 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 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.

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 Tmin of –9.6 to -7.0 °C and Topt 25 to 30 °C (Rinnan et al., 2011; Cruz-Paredes et al., 2021). It is likely that soil warming will shift the temperature-growth relationships of sub-Arctic soil bacterial communities (Weedon et al., 2022; Chapter 3). 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.

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 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 (Figure 2.1). 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 (Chapter 3; Donhauser et al., 2020; Weedon et al., 2022). More generally, temperature traits differ between members of bacterial communities from arctic soils (C. Wang et al., 2021), and specific bacterial OTUs 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 OTUs 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.

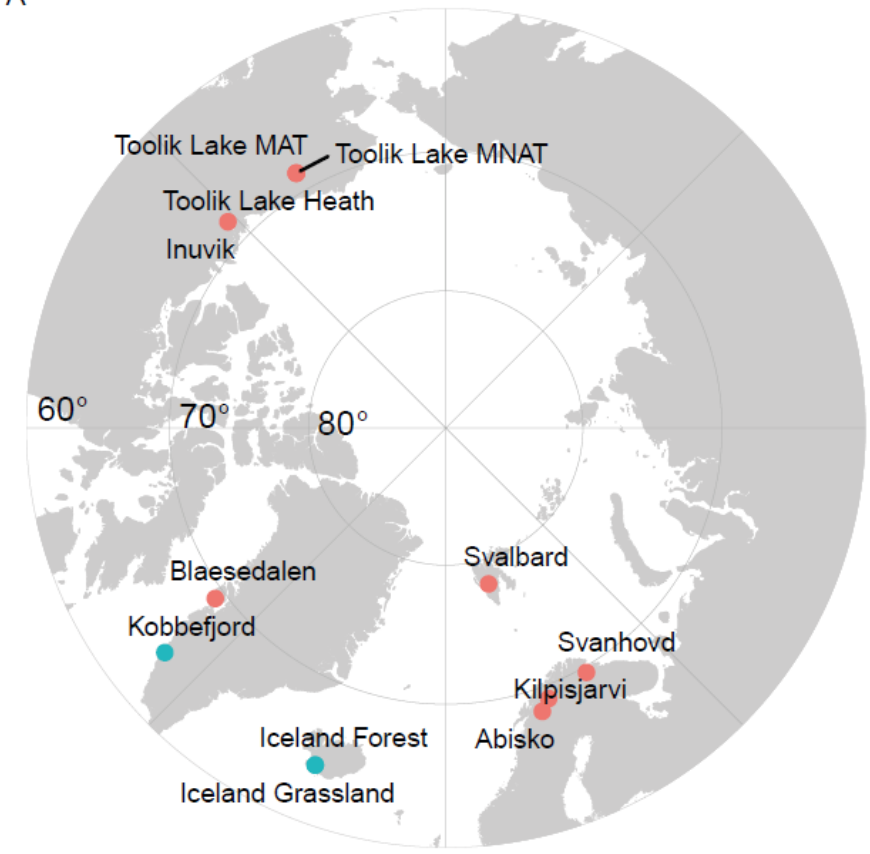
In this study we tested which soil thermal parameters best predicts the cardinal points of the temperature-growth relationships of bacterial communities from 12 soils collected in the sub- to high Arctic region. We hypothesized that the highest and lowest daily soil temperatures would be the best predictor 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.

Methods

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 2.1). The 2018 sampling at Toolik Lake Field station, Svalbard, Abisko and Iceland has been further described in Chapter 3. 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).

A


Toolik Lake MAT
Toolik Lake MNAT
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Inuvik
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Svalbard
Svanhovd
Kilpisjarvi
Abisko
Iceland Forest
Iceland Grassland

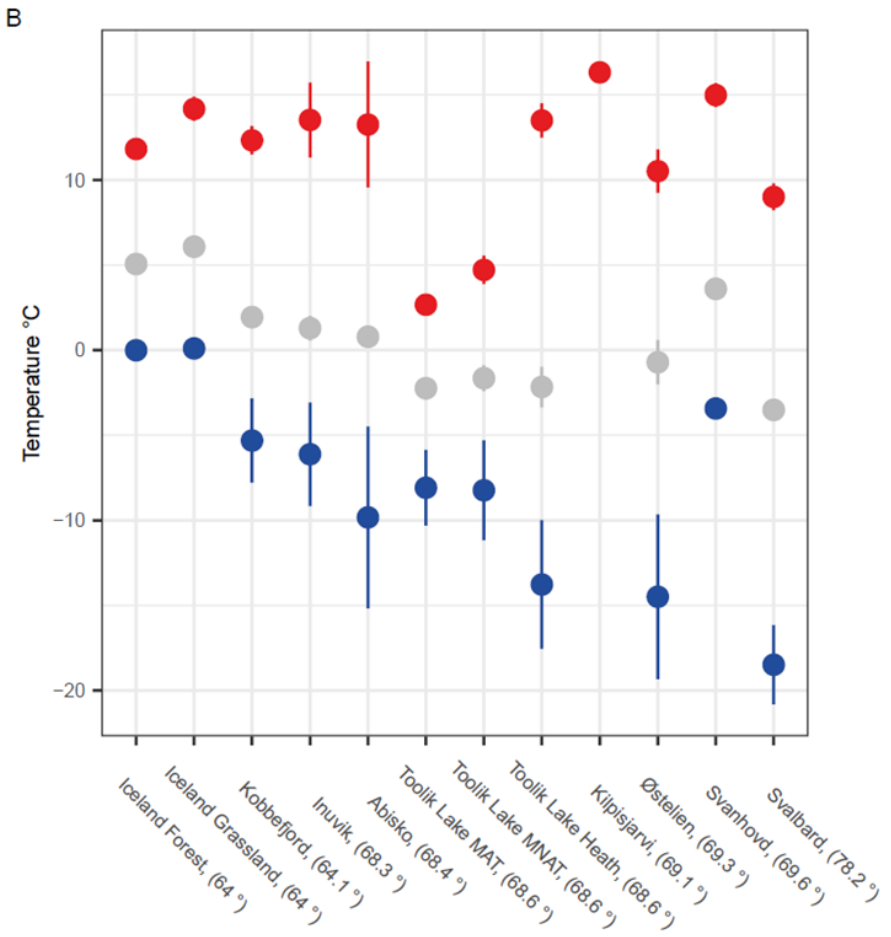


Figure 2.1 A) Map with polar projection showing the 12 sampling sites across the Arctic and B) the average values of soil thermal regimes for each site including the maximum (red), mean (grey) and minimum (blue) soil temperature. Error bars indicate the standard deviation across the years.

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 (68° 18.9342 N, 133° 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*.

Soil temperature data

Soil temperature records were collected from the involved research stations (at Abisko (Dorrepaal et al., 2004a), Svanhovd (BioForsk Svanhovd; 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 (MaxT), mean coldest day (MinT) based on the annual records for the warmest day, coldest day and mean daily temperature per year.

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 600^{0}C 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.

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 at 0, 4, 10, 15, 24.5, 28.5, 33.5 and 40 °C for 24 – 2 hours. 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.

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.

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 equation 2.1;

$$\sqrt{Leu} = a(T - Tmin) \times (1 - e^{b(T-Tmax)}) \qquad (2.1)$$

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. 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 (MinT), mean (MAST), and maximal annual soil temperature (MaxT). 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 MinT to maximum soil temperature MaxT) 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 and community Topt 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 4 soil types. The relative abundance of the common ASVs 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 MaxT 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 (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.

Results

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 2.1, Figure 2.1). 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 2.2). 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 MaxT, increasing 0.63 °C per °C (Figure 2.1; P < 0.01, Adj. R^2 = 0.63). 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).

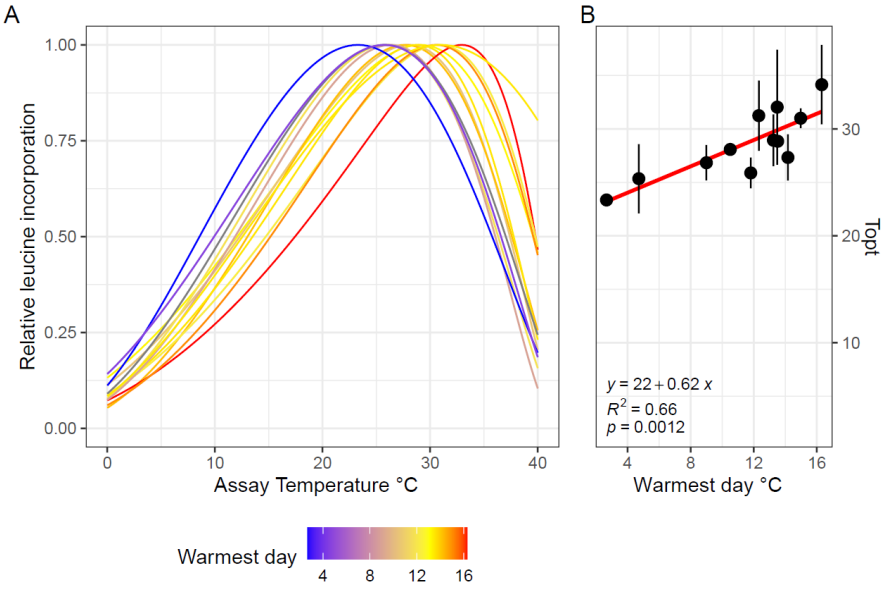


Figure 2.2 Estimated growth curves for each soil type depicted by the normalized leucine incorporation over incubation temperature. Colors indicate the maximum soil temperature of each sampling site. B) Linear relationships between the optimal growth temperature

and maximum soil temperature, error bars indicate the standard error.

Table 2.1. Thermal regimes of the 12 sampling sites in ° C. MaxT depicts the warmest day of the year, MAST the mean annual temperature and Min 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	MaxT	MAST	MinT
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		

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 2.2). 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 2.3).

Table 2.2 Results of PERMANOVA showing the influence of soil parameters on the bacterial community composition.

	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		

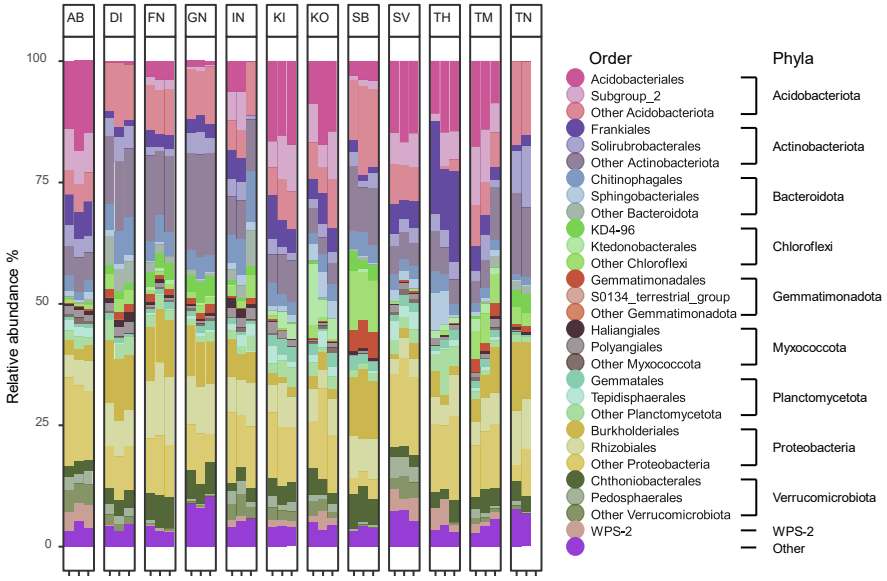


Figure 2.3 Bar plot showing the relative abundance (%) of top 10 most abundance phyla across all soil samples. Color shades indicate the two most abundant Order for each of these phyla.

We observed only 12 ASVs that occurred at relative abundance greater than 0.001 in four or more sites (Table 2.3). Both regression tree and random forest analyses based on the relative abundance of these common ASVs showed the relative

abundance of ASV11 was the best predictor of the corresponding community Topt (Suppl. Figure 2.1), 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 2.2). The linear regression model based on the MaxT as dependent variable showed larger predictive power of Topt than the pruned regression tree and random forest, since summarized across the 220 possible training sets, the median RMSE of for the linear model was lower than that median RMSE of the pruned tree and random forest (2.17, 4.14 and 3.51, respectively; Suppl. Figure 2.3).

Table 2.3 Taxonomy of commonly observed bacterial ASVS. N indicates number of soils the ASV was observed in.

OTU	PHYLUM	CLASS	ORDER	FAMILY	GENUS	SPECIES	N
1	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae (Subgroup1)	Granulicella	Uncultured soil	4
2	Acidobacteriota	Acidobacteriae	Subgroup2	Subgroup2	Subgroup2	Uncultured forest	4
3	Acidobacteriota	Acidobacteriae	Subgroup2	Subgroup2	Subgroup2	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			10
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

Discussion

Temperature adaptation across the Arctic

In this study we have 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 (Rinnan et al., 2011; Cruz-Paredes et al., 2021; Weedon et al., 2022). 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; Cruz-Paredes et al., 2021; Weedon et al., 2022) 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 2.1).

Temperature adaptation is influenced by mean daily maximum soil temperature

Of the soil thermal parameters we tested, only MaxT had a significant correlation with temperature-growth relationships of Arctic bacterial communities. 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; Rinnan et al., 2009; van Gestel et al., 2013; Birgander et al., 2018). 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. 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 MaxT 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).

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

In contrast to the clear relationship between MaxT 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 MinT. 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 MaxT 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.

What will happen in response to warming?

Since MaxT 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 (Blok et al., 2011; Paradis et al., 2016). 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.

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 (MaxT) and Topt (Figure 2.4). 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 2.4 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.

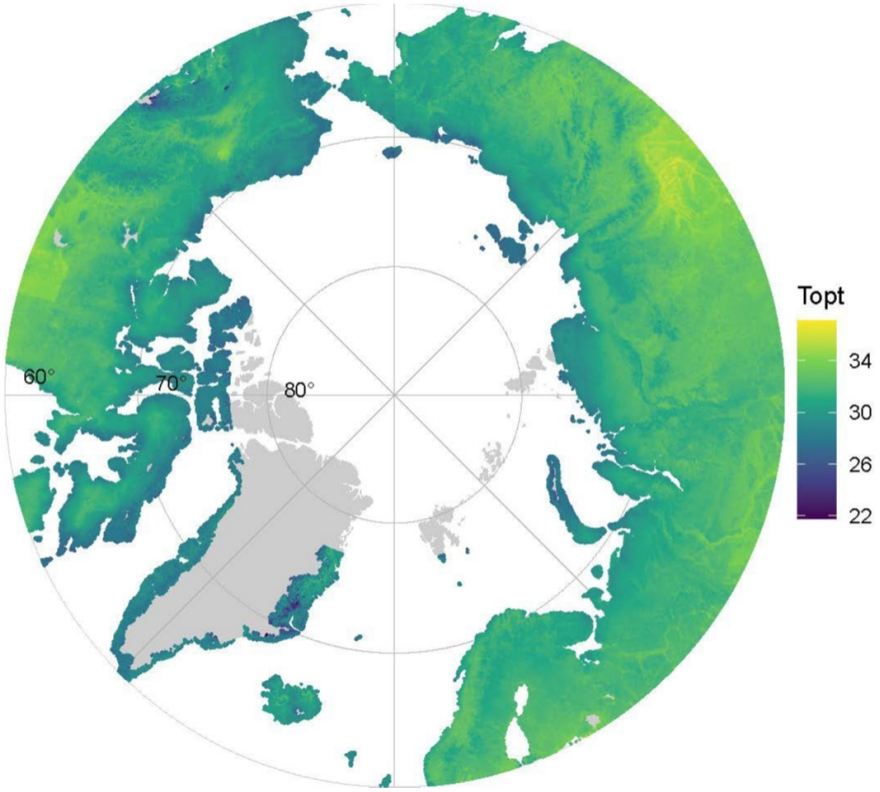


Figure 2.4 Map of the predicted Topt of soil bacterial communities across the Arctic based on the linear relationship between maximum soil temperature (from SoilTemp database) and Topt.

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. 2.4) produced larger estimation errors on prediction of the Topt of soil bacterial communities

when compared with the linear regression against MaxT. 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 doesn't 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 2.2, Supplementary Table 2.2), 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 2.1). 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 2.4) 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.

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.

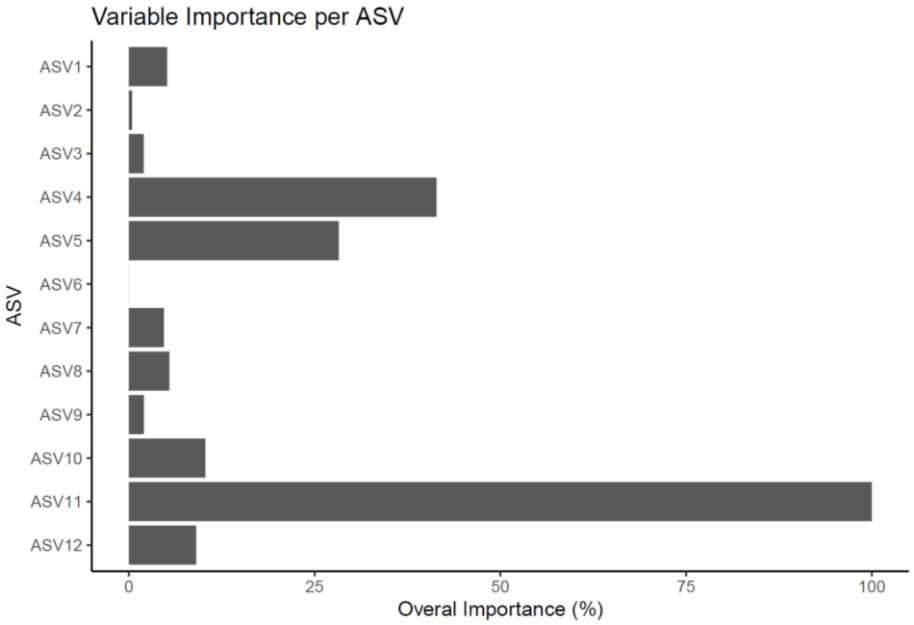
Supplement

Supplementary table 2.1 Soil characteristics of the sampled soils

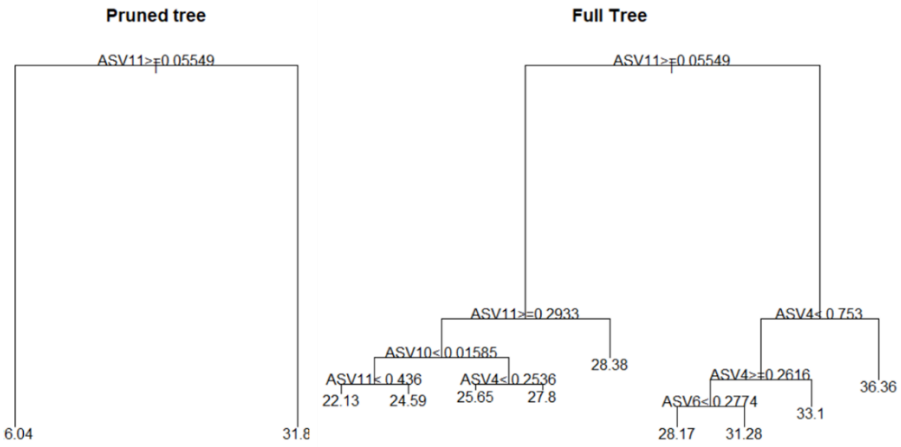
NAME	PH	WATER CONTENT (%)	TOTAL C (%)	TOTAL N (%)	C:N RATIO	ORG. C (%)	ORG. N (%)	DOC (MG/KG)	DENSITY (GR/ML)
ABISKO	4.7	48.0	47.3	0.7	65.3	47.3	0.7	122.0	0.8
ICELAND FOREST	5.9	36.3	9.9	0.7	15.2	9.9	0.7	44.0	1.5
ICELAND GRASSLAND	6.8	37.6	34.2	2.2	15.4	33.7	2.2	17.3	1.4
INUVIK	5.2	74.9	43.1	1.2	36.2	11.5	0.8	103.2	NA
KILPISJARVI	4.2	40.4	12.3	0.6	21.5	15.5	0.5	117.3	NA
KOBBEFJORD	4.6	38.0	12.5	0.5	23.4	16.6	0.6	67.8	NA
ØSTERLIEN	6.3	58.7	22.9	1.2	19.0	9.1	0.7	47.4	NA
SVALBARD	NA	NA	NA	NA	NA	NA	NA	NA	NA
SVANHOVD	3.9	73.1	36.3	1.0	36.5	37.3	1.0	193.3	NA
TOOLIK LAKE HEATH	4.9	36.8	11.4	0.5	23.6	11.4	0.5	82.7	1.2
TOOLIK LAKE MAT	5.3	67.8	7.6	0.3	27.1	7.6	0.3	77.0	0.7
TOOLIK LAKE MNAT	7.1	63.9	13.5	0.5	27.5	13.0	0.5	36.1	1.1

Supplementary Table 2.2. Taxonomy of ASVs commonly found in samples in study and previous work. N= indicates the number of soil types the ASV was present in this study.

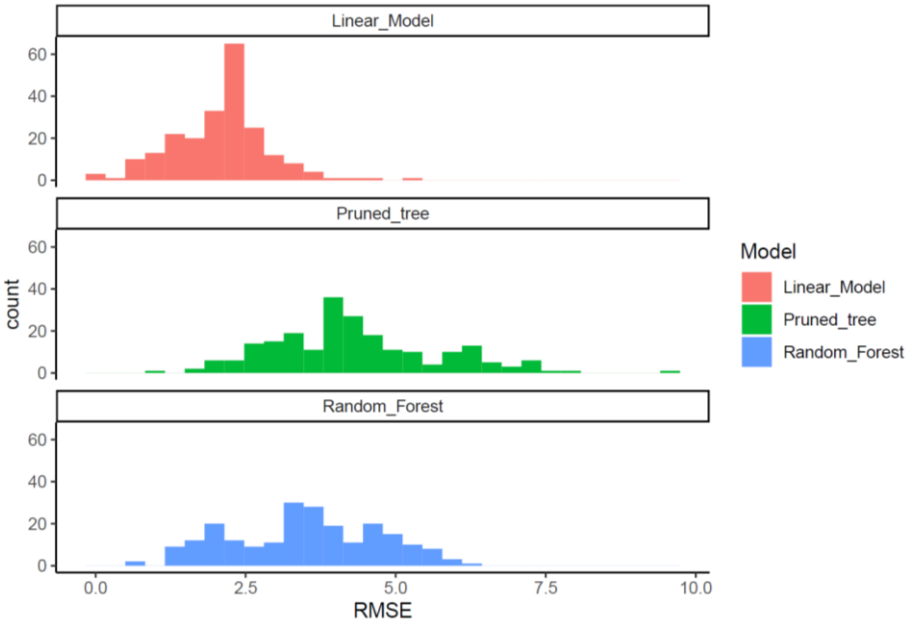
ASV	*Phylum*	*Class*	*Order*	*Family*	*Genus*	*This study (n=)*
1	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae (Subgroup_1)	Granulicella	4
2	Acidobacteriota	Acidobacteriae	Subgroup 2	Subgroup 2	Subgroup 2	4
3	Acidobacteriota	Acidobacteriae	Subgroup 2	Subgroup 2	Subgroup 2	6
4	Actinobacteriota	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Conexibacter	4
5	Actinobacteriota	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	4
6	Actinobacteriota	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus	4
7	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	NA	10
8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium	4
9	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	NA	5
10	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	NA	7
11	**Verrucomicrobiota**	**Verrucomicrobiae**	**Chthoniobacterales**	**Chthoniobacteraceae**	**Candidatus Udaeobacter**	**5**
12	Proteobacteria	Gammaproteobacteria	WD260	WD260	WD260	4



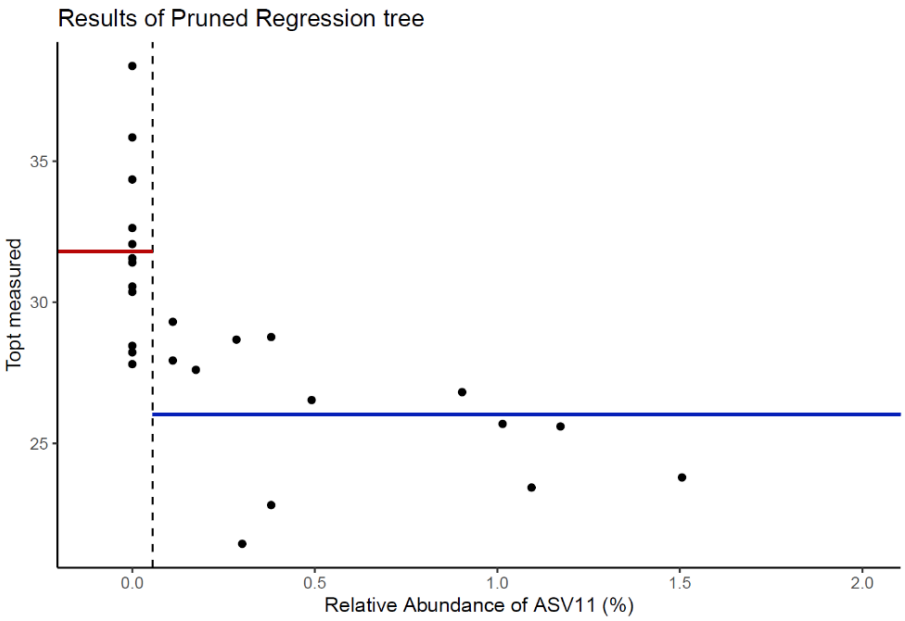
Supplementary Figure 2.1 Importance of variables for Random Forest Tree Regression Analysis



Supplementary Figure 2.2 Regression tree analysis for estimation of Topt of bacterial community based on the relative abundance of the 12 common ASVS across all soil samples.



Supplementary Figure 2.3 Histograms of the performance of each model type by RMSE over on all 220 possible combinations of soils in the training and validation dataset (with a 9:3 split between soil for train and testing, respectively.



Supplementary Figure 2.4 Scatterplot the relationship between Topt of the soil bacterial community and relative abundance of ASV 11 in the community composition. Lines indicate the predicted Topt values by the pruned regression tree indicate.

Chapter 3

Optimal growth temperature of Arctic soil bacterial communities increases under experimental warming

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Abstract

Future climate warming in the Arctic will likely increase the vulnerability of soil carbon stocks to microbial decomposition. However, it remains uncertain to what extent decomposition rates will change in a warmer Arctic, because extended soil warming could induce temperature adaptation of bacterial communities. Here we show that experimental warming induces shifts in the temperature-growth relationships of bacterial communities, which is driven by community turnover and is common across a diverse set of 8 (sub) arctic soils. The optimal growth temperature (T_{opt}) of the soil bacterial communities increased 0.27 ± 0.039 (s.e.) and 0.07 ± 0.028 °C per °C of warming over a 0-30 °C gradient, depending on the sampling moment. We identify a potential role for substrate depletion and time-lag effects as drivers of temperature adaption in soil bacterial communities, which possibly explain discrepancies between earlier incubation and field studies. The changes in T_{opt} were accompanied by species-level shifts in bacterial community composition, which were mostly soil-specific. Despite the clear physiological responses to warming, there was no evidence for a common set of temperature-responsive bacterial amplicon sequence variants (ASVs). This implies that community composition data without accompanying physiological measurements may have limited utility for the identification of (potential) temperature adaption of soil bacterial communities in the Arctic. Since bacterial communities in arctic soils are likely to adapt to increasing soil temperature under future climate change, this adaptation to higher temperature should be implemented in soil organic carbon modeling for accurate predictions of the dynamics of arctic soil carbon stocks.

Introduction

The functional relationships between temperature and the rate of soil microbial processes, such as growth and respiration, differ markedly between geographic regions in a manner that is largely predictable from climatic averages (Bradford et al., 2019; Dacal et al., 2020; C. Wang et al., 2021). This correlation between temperature responses and *in situ* temperature is known as temperature adaptation and can influence the rates of associated biogeochemical rates (Bradford, 2013). Therefore, temperature adaptation of soil microbial processes can influence the vulnerability of soil organic carbon stocks to warming and needs assessment for the prediction of carbon feedbacks to climate change (Allison et al., 2010). For example, microbial respiration in soils from colder regions is generally more responsive to temperature (i.e. higher intrinsic Q_{10}) than soils from warmer regions (Balser and Wixon, 2009; Karhu et al., 2014; Dacal et al., 2019). Importantly, it is largely unknown whether knowledge about temperature adaptation observed over large scale climatic gradients can be used to make predictions about changes to the temperature response of soil bacterial communities at a given locality after exposure to altered temperature regimes. It is particularly important to understand whether extended soil warming can induce temperature adaptation of microbial communities in high latitude northern soils, because besides being particularly temperature sensitive, these soils also contain ~50% of the global soil carbon stock (Tarnocai et al., 2009). For this reason predictions of the fate of global carbon stocks under climate change require mechanistic understanding of the controls of microbial biogeochemistry in Arctic soils (Wieder et al., 2019).

The response of soil microbial activity to temperature can be measured in relation to a variety of biochemical pathways and bio(geo)chemical fluxes (Nottingham et al., 2019b), such as extracellular enzyme activities (Weedon et al., 2014), carbon use efficiencies (Walker et al., 2018; Pold et al., 2020), and respiration (Bradford et al., 2008; Karhu et al., 2014). Many of these measures are potentially confounded by edaphic conditions such as moisture and substrate availability (Kirschbaum, 2004; Davidson and Janssens, 2006; Manzoni et al., 2012). There are therefore advantages in focusing on the temperature response of microbial growth, which can be measured in a way that minimizes the influence of confounding variables (Bååth, 2018; Cruz-Paredes et al., 2021). The physiological adaptation of bacterial growth to their thermal environment can be described in terms of changes in the parameters of the Ratkowsky model (Bååth, 2018; Ratkowsky et al., 1983), an

empirical model for relating microbial growth rates to temperature. By fitting this model to growth rate data, parameters representing the (theoretical) minimal, maximal and optimal temperatures for growth (respectively T_{min}, T_{max} and T_{opt}) can be estimated and used to compare the temperature response of single strains or, more commonly, communities from different environments (Corkrey et al., 2016). Temperature-growth relationships of bacterial communities estimated in this way have been shown to change along natural gradients of increasing soil temperature following elevation in the Andes (Nottingham, Bååth, et al., 2019), and along a continent-scale climate gradient in Antarctica (Rinnan et al., 2009). Likewise, 5 °C of experimental warming increased the parameters of the microbial temperature-growth relationships in a temperate forest, (Rousk et al., 2012). It is therefore likely that long-term warming causes changes in the temperature-growth relationships of soil bacterial communities, and that as a consequence these communities perform less well at low temperatures (Rinnan et al., 2009). In this study, we define temperature adaptation of soil bacterial communities as a change in aggregate temperature-growth relationship of soil bacterial communities.

Numerous incubation studies have identified the optimal growth temperature (T_{opt}) as a key parameter for determining how much experimental soil warming is needed to induce temperature adaptation in soil bacterial communities. Specifically, shifts in the overall temperature growth relationship of a community have been proposed to occur due to selective mortality of microbial taxa when soils are exposed to temperature exceeding the aggregate T_{opt} of the microbial community (Bárcenas-Moreno et al., 2009; Birgander et al., 2013; Donhauser et al., 2020). In the sub-Arctic, soil bacterial communities typically show a T_{opt} between 25 to 30^{o}C and a T_{min} of -8 to -6 °C (Rinnan et al., 2011; Bååth, 2018; Cruz-Paredes et al., 2021). Currently, the maximum soil temperatures in the Arctic do not generally exceed 15 °C for the majority of the soil habitat (Lembrechts et al., 2021, Table 1.) and thus it is unlikely that such a heat-induced-death mechanism will play an important role in the (sub-) Arctic.

In contrast to the evidence from incubations, a small number of field studies have shown that temperature-growth relationships can also shift with soil temperatures well below the T_{opt} of ambient conditions. Shifts in temperature-growth-relationships of bacterial communities even occurred when transplanting soil cores to a cooler climate across an ±20 °C elevation gradient in the Andes (Nottingham et al., 2021). Moreover, soil bacterial communities altered their

temperature-growth relationships at 6-8° degrees of soil warming along a geothermal gradient in Iceland, while the soil temperature of 25°C did not exceed the T_{opt} of the bacterial soil communities (30 °C) in ambient conditions (Weedon et al., 2022). This implies that, at least in some contexts, long-term temperature changes can alter temperature growth relationships of soil bacteria via mechanisms other than heat-induced mortality under warming scenarios that are realistic for future climate change. However, the empirical basis for this remains limited, and in particular it remains unknown whether the effects observed in Iceland generalize to other cold-climate soils.

Temperature-growth relationships of soil bacteria might themselves be predictable from information about the taxonomic composition of the bacterial community (Hicks et al., 2021). This is because warming-induced shifts in temperature-growth relationships have been linked to turnover in community composition (Bárcenas-Moreno et al., 2009; Donhauser et al., 2020; Weedon et al., 2022). For example, a study of North American forest soils identified 189 bacterial taxa that responded to soil warming, during both heating in a field experiment and in lab incubations (Oliverio et al., 2017). To date, a comparable attempt to identify temperature-responsive species specific to Arctic soils has not been attempted. If found to occur, such a relation between specific (groups of) taxa and physiological responses to warming could provide a tool to predict the response of arctic soils to changing climate. Specifically, combining community profiling with measures of temperature adaptation could allow for the identification of potential 'bioindicator' species specifically related to the temperature adaptation of soil bacterial communities (Bååth, 2018), as has been done previously for salt tolerance (Rath et al., 2018).

Studying warming effects on community composition and physiology is complicated by the fact that soils incubated at a range of temperatures will also differ in substrate availability when sampled after a fixed time interval. As such, direct effects of warming are usually confounded with substrate limitation effects, which can potentially bias the identification of temperature-responsive bacterial species (Oliverio et al., 2017) and bias estimates of warming effects on physiological processes (Hartley et al., 2008; Karhu et al., 2014; Walker et al., 2018). A common solution is to add excess substrate to temperature incubations in order to remove indirect effects due to substrate limitation (Dacal et al., 2019), which often alters the soil organic matter quality. As an alternative, the sampling

moments can be standardized such that different temperature treatments are compared after a set amount of substrate use or carbon loss (Whittington, 2019).

In this study, we aimed to evaluate whether temperature adaptation by soil bacterial communities occurs when arctic soils are exposed to experimental warming. Additionally, we asked whether there were general patterns in the temperature-growth relationships and community composition associated with temperature adaptation. To do this we conducted an incubation experiment with 8 soils from 4 different study sites reaching from the sub-Arctic to the high Arctic, varying in mean annual soil temperature, vegetation cover and soil type. We tested whether incubation temperatures between 0 – 30 °C, reaching >15 °C above the observed maximum in situ soil temperatures of all sites, altered temperature-growth relationships and whether the size of the response to incubation temperature is influenced by the mean annual temperature of the soils (Donhauser et al., 2020). Secondly, to separate direct warming effects from indirect effects via substrate depletion, we sampled both after 100 days (T100 samples) as well as at a variable moment (C15 samples) with timing linked to a set amount of CO_2 production (as a proxy for substrate use). Lastly, we tested whether eventual shifts in the temperature-growth relationships in response to incubation temperature are related to changes in the bacterial community composition and if we could identify ubiquitous bacterial taxa that respond to soil warming. We hypothesized that; 1) the growth parameters of the temperature-growth relationships would increase with higher incubation temperature or when incubation temperature exceeded the T_{opt} of the initial bacterial community, 2) and these changes are similar across measurements taken after a fixed incubation period, or fixed amount of cumulative respiration (proxy for substrate availability) ; 3) changes in temperature-growth relationships are accompanied with a change in the community composition; 4) there is a set of 'bioindicator' taxa common to all soils whose changes in abundance correlate with altered temperature-growth relationships.

Methods

Sample collection

In order to test the generality of our hypotheses across diverse Arctic soil and vegetation types and to identify potential ubiquitous temperature responsive bacterial amplicon sequence variants (ASVs), we sampled 8 different soils from 4 locations in the (sub-) Arctic region in the summer of 2018. The sampling locations

varied in mean annual soil temperature at 10 cm depth (from -3.5°C to +6.1°C), vegetation cover and soil type (Table 3.1). We collected two Silandic Andosols from the FORHOT research site in Iceland (64° 00′N, 21° 11′W), with a dominant cover of *Agrostis capillaris* for the grassland site (GN) and *Picea sitchensis* for the forest site (FN) (Sigurdsson et al., 2016). We collected a Histosol from a *Sphagnum* covered bog at the long-term climate manipulation experiment (Dorrepaal et al., 2004b) close to the Abisko Research Station in northern Sweden (AB; 68°21'N, 18°49'E). From the LTER-sites at the Toolik Field Station in Alaska, USA (Shaver et al., 2013; 68°38' N, 149°36' W), we collected three soils classified as Typic Aquiturbels. The vegetation cover of LTER Heath (TH) dominated by *Arctostaphylos alpina*, while LTER Moist Acidic Tussock (TM, pH=3.7) and LTER Non-Acidic Tussock (TN, pH=5.9) have *Eriophorum* and *Carex* species as dominant vegetation (Ping et al., 1998; Gough et al., 2000; Street et al., 2007). At Svalbard, we collected two Cryosols from Bjorndalen (78°13'N, 15°19'E). Both sites were characterized by the presence of *Carex* sp., *Salix* sp. and mosses, where the first, SA, showed a dominance of *Carex* and < 1 cm organic horizon, and the second site, SB, was mainly covered by mosses and showed a thicker organic horizon, 3-6 cm.

Table 3.1 Characteristics of sampling sites, including coordinates, mean annual soil temperature (MAST), maximum and minimum hourly temperature in °C. Organic nitrogen and carbon content are expressed are depicted the percentage of dry soil weight.

	Site	Total N	Total C	Org. N	Org. C_	C:N ratio
1	TH	0.483	11.398	0.483	11.398	23.582
2	TM	0.279	7.564	0.276	7.564	27.106
3	TN	0.492	13.512	0.470	13.017	27.477
4	SA	0.146	2.115	0.138	2.115	14.454
5	SA					
6	FN	0.652	9.934	0.650	9.934	15.242
7	GN	2.218	34.157	2.197	33.743	15.397
8	AB	0.723	47.253	0.723	47.253	65.334

Soil cores were collected with an approximate diameter of 10 cm and depth of 20 cm, and vegetation was removed from the top during sampling. After collection, samples were frozen and shipped cooled to the Vrije Universiteit Amsterdam and stored at -20 °C. For further processing, all samples were thawed at 7^{0}C for seven days, except the Alaskan samples that were thawed at 4^{0}C, to maintain the thawing temperature below the summer soil temperatures. After thawing the soils were homogenized and passed through a 2mm sieve. For each soil type, 21 jars were prepared for incubation by adding 20-40 g of fresh weight soil into autoclaved 300 ml mason jars with rubber septa in the lid. The jars and soil were pre-incubated for 7 days at 7^{0}C. CO_2 was measured in the headspace after 0 and 7 days using an EGM-5 infra-red gas analyzer (PP-systems, Amesbury MA, United States of America) to assess whether soil respiration stabilized over the course of the pre-incubation. The headspace was then flushed with 0.45 µm filtered air. After the pre-incubation, 3 replicates of each soil type were placed into incubators set at 0, 5, 10, 15, 20, 25 or 30^{0}C. The CO_2 concentration in the headspace of the 168 jars was measured at variable intervals between 1-7 days depending on the respiration

rate and incubation temperature, where the 30°C incubated jars were sampled every day and 0 ° every 7 days. Once the CO_2 concentration passed 30.000 ppm the headspace was flushed with 0.45 µm filtered air. After 15 days, 5 g samples were taken from the jars at 30^{0}C for soil analysis, DNA microbial community profiling and aggregate temperature relationship measurements. The cumulative amount of CO_2 produced after 15 days at 30^{0}C was used as reference sampling point, at which all the jars at the other temperatures were sampled between 15 and 285 days (hereafter C15). Additionally, all jars were also sampled after 100 days (hereafter T100). The samples from both time points were used for DNA microbial community profiling and aggregate temperature-growth relationship measurements as described in the following sections.

Characterization of bacterial community composition

Subsamples of 200 mg soil were taken for analysis of soil bacterial community composition. DNA was extracted using a Powersoil Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol, and eluting the final DNA into 60 µl sigma-sterilized Millipore water. Amplicons were generated by a two-step PCR of the 16S V4 rRNA gene with primers designed by Caporaso et al., (2011), 515 forward primer (5'-GTG YCA GCM GCC GCG GTA A-3') and 806 reverse primer (5'-GGA CTA CNV GGG TWT CTA AT -3'). An initial PCR consisted of an initial denaturation step of 1 min at 98°, followed by 24 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 sigma-sterilized water and subsequently indexed by an 8 cycle PCR with unique barcode primers for each sample using the same steps as the initial PCR amplification. Purification of the PCR product was done by with Ampure XP beads (Beckman Coulter, Brea, California, USA), following manufacturer's protocol. The amplicons were sequenced over two paired-end MiSeq Illumina Sequencing runs with V3-600 cycle chemistry, generating 19,079,107 sequences in total. QIIME 2 was used for processing the resulting sequences (Bolyen et al., 2019). Raw sequences were deposited in the NCBI Sequence Read Archive (BioProject accession number: PRJNA856638) Demultiplexed sequences were truncated at 250 bp for forward and reverse reads. DADA2 (Callahan et al., 2016) was used for dereplication, allowing a maximum expected error of 2. Chimera removal was done internally by DADA2, using the 'consensus' mode. Amplicon sequence variants (ASV) were then aligned using MAFFT (Katoh and Standley, 2013) and phylogenetic distances were calculated Fasttree (Price et al., 2009). QIIME 2's scikit-learn naive Bayes machine-learning

classifier (Bokulich et al., 2018) was used for the taxonomic classification based on the SILVA v138 database (Yilmaz et al., 2014). Finally, ASVs matching to mitochondrial or chloroplast sequences were discarded. Lastly, 23 samples were removed from the dataset due to low sequencing depth (<3000 sequences).

Measuring temperature-growth relationships

We used a ^{3}H-leucine growth assay adjusted from Bååth et al., (2001) to estimate the growth rates of soil bacterial communities over a temperature gradient from 0-40 ºC. Each frozen sample was first thawed for two days for the recovery of microbial activity (Koponen and Bååth, 2016). Next, 20 ml of sterile deionized water was added to 1 g of soil and vortexed at maximum speed for 2 min. After centrifugation at 1000 g for 10 min, 9 aliquots of 1 ml were suspended in 2ml screw top tubes. For the measurement of leucine incorporation, 20 µl of a mixture of unlabeled leucine and 3H-leucine (Perkin Elmer), was added to the tubes, resulting in a final concentration of 401 nM and 72.5 kBq ml^{-1}. For every sample, one tube was incubated for 20 h at 0 ºC, 8 h at 4ºC, 4 h at 10 &15 ºC and 2 h at 24.5, 28.5, 33 and 40 ºC. As a negative control, for the last tube 100% TCA was added directly after addition of the leucine mixture to eliminate bacterial growth. To terminate the incubation 75 µl 100% TCA was added, after which samples were stored for no more than 4 days at 4 ºC before further processing. The bacterial cells were then washed by centrifugation for 8 min at 13,000 RPM, removal of the supernatant and addition of 1.5 ml 5% TCA. The TCA washed samples were then washed in the same manner with 80% ethanol. The final pellets were made by one last step of centrifugation and supernatant disposal, after which 1 M NaOH was added to the pellets, followed by incubation at 90 ºC for 30-60 min. At room temperature, 1 ml Optiphase Hisafe 3 was added and tubes were vortexed briefly. Scintillation was measured on a Tricarb 2800T (Perkin Elmer). Leucine incorporation rates were based on the ^{3}H-activity measured and transformed to nM $^{-1}$ h $^{-1}$ g dry weight soil using equation 3.1:

$$k = \frac{dpm * c}{t * w} \qquad (3.1)$$

here k is the final leucine incorporation rate (nM $^{-1}$ h $^{-1}$ g), dpm are the measured disintegrations per minutes, c the conversion factor from Bq to nM (5.31 * 10^{-3} nM/Bq), t is the incubation period in hours and w is the added soil dry weight.

Statistical analysis

For the calculation of total CO_2 produced in each jar, we calculated the cumulative respiration after each time interval and linearly interpolated the cumulative respirations between the measurements. Temperature-growth relationships were estimated for each soil sample (i.e. for each of the two sampling points per replicate incubation) by fitting a square root model for bacterial growth (Ratkowsky et al., 1983, equation 3.2, Chapter 1 Figure 1.2), to the measured leucine incorporation rates using the *nls.multstart* R-package (Padfield and Matheson, 2018):

$$\sqrt{Leu} = a(T - Tmin) \times (1 - e^{b(T - Tmax)}) \quad (3.2)$$

where, Leu is the incorporation rate of leucine, a is the slope parameter for growth below the optimum, T the temperature of the leucine incorporation assay in °C, T_{min} corresponds to the theoretical minimum temperature for growth, b is a slope parameter for growth above the optimum and T_{max} the theoretical maximum temperature for growth (Chapter 1 Figure 1.2). The temperature of optimal growth, T_{opt}, was derived numerically, based on the best-fit parameters.

In previous work, T_{min} and the a-slope parameter were often first estimated by equation 3.3

$$\sqrt{Leu} = a(T - Tmin) \quad (3.3)$$

after which these parameters were used as constants in equation 3.2 for the second step. In general, only 4 assay temperatures fitted to the linear section of the Ratkowsky model, limiting the accuracy for fitting equation 3.2 in a model. We evaluated both approaches and found negligible differences in estimated parameters from the two approaches, but lower AIC values when the model in equation 1 was directly fitted. We discarded 41 samples from the leucine incorporation data set due to improper storage and/or limited leucine incorporation.

To test the influence of the incubation temperature on the growth parameters we fitted a mixed effect models for the growth parameters T_{min}, T_{opt} and maximum growth rate with incubation temperature and sampling moment (C15 and T100) as fixed effects and soil type and the jar number as random effects. We calculated the marginal R^2 for the fixed effects of each model using the R-package *MuMin* (Barton, 2015). To test for potential threshold changes in T_{min}, we compared linear

models and piecewise regression models with T_{min} as response variable and the incubation temperature as predictor for each soil type and timepoint combination using the *segmented* R-package (Muggeo, 2003). We performed a p.score.test on each linear model and evaluated the AIC for both linear and piecewise regression models. To test whether incubation temperature effects on growth parameters were related to the mean annual temperature (MAT) of the sampling sites, we performed an ANCOVA on the relationships between MAT and the estimated coefficient for each site x timepoint combination estimated for the correlation between T_{opt} and the incubation temperature.

For the analyses of bacterial community data, the R package *phyloseq* was used, unless stated otherwise. ASVs with proportional abundance lower than 0.001 % over the whole dataset were excluded, after which we rarefied our bacterial community composition data to the minimum read count of 3283. We estimated the alpha-diversity of the soil bacterial communities using the Shannon index and used mixed effects model to test influence of incubation temperature and sampling moment with soil type and jar as random effects on alpha-diversity. Then, we computed the pair-wise distance matrix using weighted Unifrac distances (Lozupone and Knight, 2005). To study the drivers of community composition we performed a series permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) tests on the calculated dissimilarity distances to determine the influence of incubation temperature, sampling moment (C15 or T100) and soil type on bacterial community composition. We also performed a PERMANOVA test on the calculated dissimilarity distances at C15, with incubation temperature and cumulative respiration amount as predictor variables. Additionally, we tested for a correlation between *in situ* T_{opt} and the overall soil bacterial community composition with PERMANOVA. Last, we performed a PERMOVA test on the Unifrac distances of each soil separately at C15 to test whether the bacterial community composition responded to incubation temperature.

To detect possible abundance differences of ASVs along the incubation temperature gradient, we performed a differential abundance analysis for each soil type and sampling moment individually at phylum, family and ASV level using the ANCOMBC 1.1.4 package (Lin and Peddada, 2020a). In this analysis we tested whether the differential abundance of each taxon was correlated to the incubation temperature, using Bonferroni method for p-value adjustment for multiple

comparisons and default settings of the package. ASVs were considered 'true' temperature responders when the ASVs responded positively/negatively to the incubation temperature at C15 for at least 2 soils. The program R (v4.0.2) was used for all statistical analyses (R Core Team, 2020). Data for CO_2 measurements and parameters of the temperature-growth relationships is available via Figshare (doi: 10.6084/m9.figshare.19516777 and doi: 10.6084/m9.figshare.19516780).

Results

Incubation temperature and soil type influence soil respiration rates

Respiration rates increased with incubation temperature and differed between the 8 soil types, ranging between 0.25 and 2 μg^{-1} day at 10 °C (mixed effects model; marginal R^2 = 0.87, P<0.001). The cumulative amount of CO_2 produced after 100 days varied by a factor of 14 – 69 between soils incubated at 0 and 30 °C depending on the soil type (mixed effects model; marginal R^2= 0.86, P < 0.001, Figure 3.1). At C15, differences between minimum and maximum cumulative respiration were reduced to 1.44 – 2.99-fold across the soil types. There was a weak correlation between incubation temperature and CO_2 produced at C15 (mixed effects model; marginal R^2= 0.01, P < 0.01, Figure 3.1), but when the soils at 0°C were excluded, it was no longer significant (P=0.30).

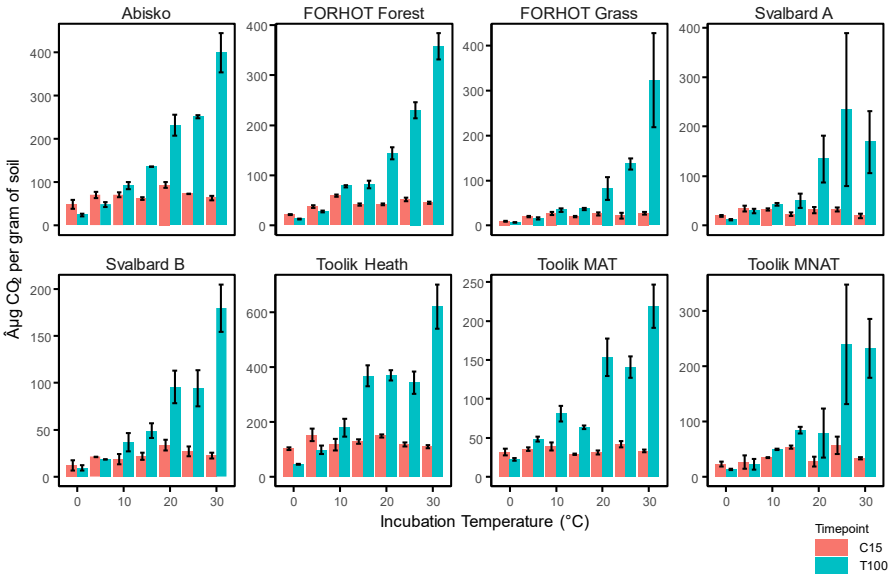


Figure 3.1 Mean cumulative respiration (µg CO2 g-1 soil) measured in soils samples from eight arctic and sub-arctic sites incubated at between 0 and 30°C (*n* = 3 per site x temperature combination). Cumulative respiration determined at two time points per replicate incubation jar are presented. The blue bars show cumulative respiration after 100 days of incubation (T100). Red bars show cumulative respiration determined at a time point chosen such that an amount of respiration approximately equal to the cumulative respiration after 15 days for samples incubated at 30^{0}C (C15, between 15 and 285 days, depending on soil and temperature). Error bars show standard error of the mean. Note: y-axis scale varies between panels.

Linear change in temperature-growth relationships with incubation temperature

The temperature-growth relationships of the bacterial communities were influenced by the incubation temperature. T_{opt} showed a linear increase with the incubation temperature (mixed effects models; $P < 0.05$, Figure 3.2), as piece-wise regression showed higher AIC scores than the mixed-effect linear models. The increase in T_{opt} was significantly higher in T100 samples compared to C15 samples ($P<0.001$), increasing by 0.27 °C ± 0.039 (s.e.) and 0.07 ± 0.028 °C per °C of incubation temperature, respectively. The magnitude of the incubation temperature effect on T_{opt}, was not significantly related to the mean annual temperature of the sampling site (ANCOVA of T_{opt} slopes per site, $P > 0.05$). Alongside the T_{opt} effects, there was a weak but significant positive relationship between T_{max} and the incubation temperature (mixed effects models; $R^2= 0.08$, $P < 0.05$), with no significant difference in T_{max} between the two sampling moments ($P= 0.12$). Lastly, T_{min} of the incubated soil bacterial communities was not significantly influenced by the incubation temperature in either a linear mixed model ($P= 0.47$, Figure 3.2) nor in piecewise regression models ($P>0.05$ for all models). In total, the temperature range of growth ($T_{max} – T_{min}$) increased 0.14 °C per °C of incubation temperature ($P<0.01$) at T100, but at C15 the temperature range of growth was not significantly different across the incubation gradient. The growth rates of the bacterial communities, here defined by the maximum leucine incorporation rates at T_{opt}, showed no significant correlation with the incubation temperature at C15. However, microbial growth rates at T100 significantly decreased by 36.2% from 0 to 30 °C (mixed effect models; marginal $R^2= 0.07$, $P < 0.001$). Overall, the temperature and sampling moment influenced the T_{opt} and T_{max}

parameter regardless of the sampling site MAT, while T_{min} was not significantly altered, resulting in a broader temperature range of growth.

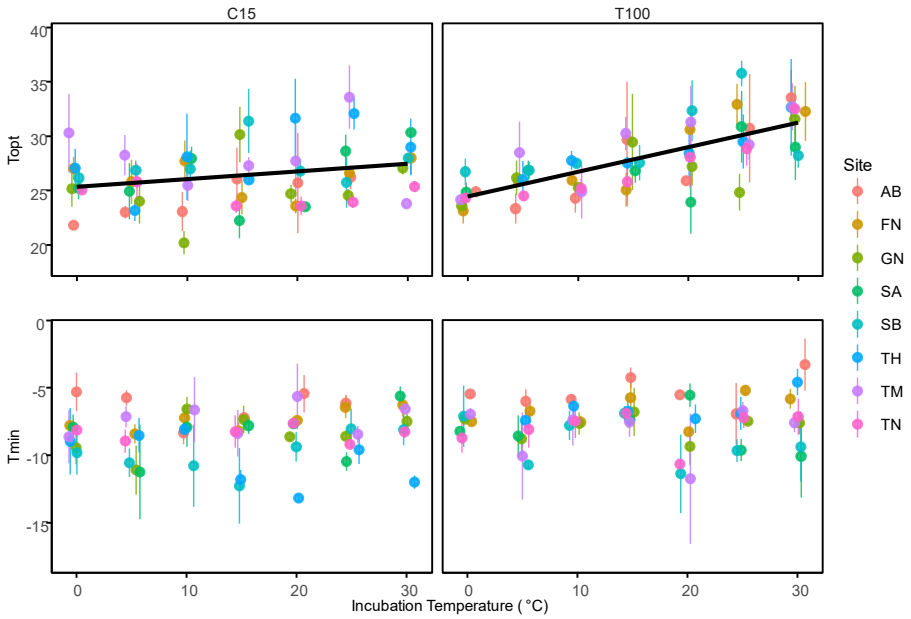


Figure 3.2 Relationships between estimated parameters of the temperature-growth function (upper panels: theoretical minimum temperature for growth, T_{min}; lower panels: estimated optimal growth temperature, T_{opt}) and soil incubation temperature. Parameters were estimated with Leucine incorporation assay using 9 temperatures between 0.5 and 40^{0}C. Assays were performed using soil samples taken at both C15 (left panels) and T100 (right panels, see Methods). Points and error bars show means +/- standard error per site x timepoint combination (n = 3). Points are jittered horizontally for legibility. Lines represent significant linear regressions ($P < 0.05$).

Incubation temperature influences bacterial community composition at both sampling moments

Overall, 5836 ASVs were observed with a mean abundance above 0.001%, of which none were present in all of the eight soil types. Proteobacteria (29%), Acidobacteriota (22%), Actinobacteriota (15%), Bacteroidota (10%) and Verrucomicrobiota (7%) were the most abundant phyla across all soil samples. The

soil type explained the largest part of the variation in bacterial community composition (PERMANOVA R^2= 0.54, P < 0.001, Figure 3.3B), while both the incubation temperature (R^2 = 0.04, P < 0.001) and the sampling moment (R^2= 0.01, P < 0.001) had a significant effect on the bacterial community composition, as well as the interactions between all variables (Table 3.3). Despite significant differences in the amount of soil respiration across the temperature gradient at the C15 sampling moment, the soil bacterial community composition of all samples was not significantly related with the respiration amount at C15 (PERMANOVA; R^2= 0.004, P= 0.131). At C15, 6 out of 8 soil types showed a significant change in soil bacterial community composition with incubation temperature in a soil-specific PERMANOVA (R^2> 0.3, P<0.05, Figure 3.3a). The Shannon index of the soil bacterial communities did not correlate significantly with incubation temperature (mixed effects model, P= 0.76).

Lack of common temperature responsive species

While there were significant changes along the incubation gradient in overall bacterial community composition, no clear taxonomic patterns in abundance were shown across the soils at either phylum nor family level. Differential abundance analysis on ASV count data identified 240 ASVs at T100 and 111 AVSs at C15 that changed significantly in abundance along the incubation temperature gradient in at least one of the soil types (Figure 3.4). 19 of these ASVs showed significant differential abundance across the temperature gradient for both timepoints in at least one soil type. Only 7 of the ASVs (belonging to the orders Micrococcales, Acidobacteriales, Xanthomonadales, and Sphingobacteriales) responded to temperature in two or more soil types at C15 (Table 3.4).

T**able 3.4** Temperature responsive bacterial ASVs

ASV	Order	Family	Genus	Species	Observed in soils (n=)	Soils diff. abundant (n=)	Soils
1	Micrococcales	Microbacteriaceae	Unknown	Unknown	6	3	AB,TM,SA
2	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)	Unknown	Unknown	2	2	TH,TM
3	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	Unknown	3	2	TH,TM
4	Sphingobacteriales	env.OPS_17	env.OPS_17	uncultured_bacterium	5	2	FN,SA
5	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)	Occallatibacter	uncultured_Acidobacterium	2	2	TH,TM
6	Xanthomonadales	Rhodanobacteraceae	Rhodanobacter	uncultured_prokaryote	3	2	TH,TM
7	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	Unknown	3	2	TH,TM
8	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)	Occallatibacter	uncultured_Acidobacterium	2	2	TH,TM
9	Micrococcales	Micrococcaceae	Unknown	Unknown	3	2	FN,GN

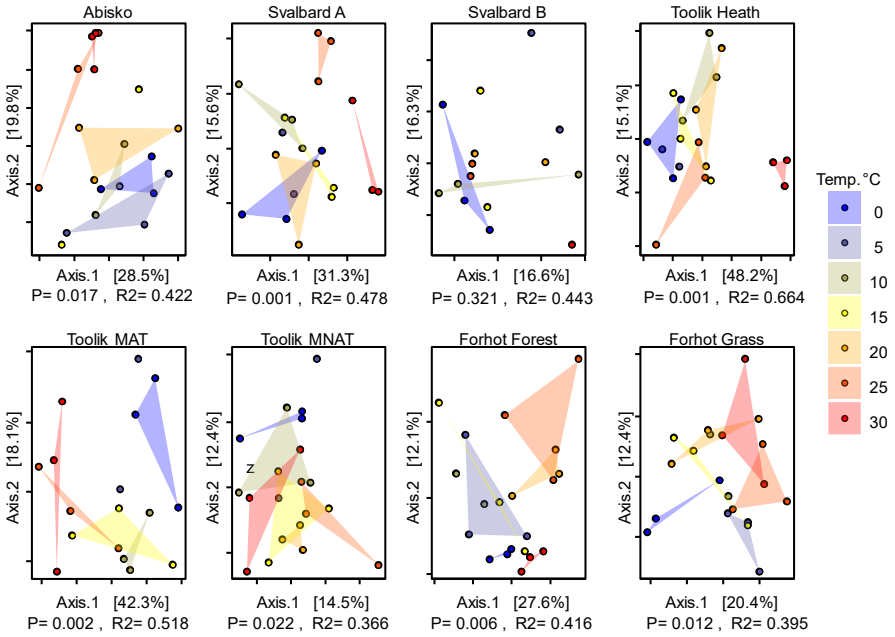


Figure 3.3a Bacterial community dynamics along the incubation temperature gradient. Principal coordinate analysis using weighted Unifrac distances computed from soil bacterial community profiles generated from 16S amplicon sequencing. Samples taken at C15 for each individual soil type are shown. Blue to red points and convex hulls indicate incubation temperature (0-30°C, n = 1 – 3 for each site x temperature combination). **b)** Principal coordinate analysis using weighted Unifrac distances computed from 16S amplicon profiles for all soil, incubation temperature and time points. The majority of variance is related to the soil type (indicated by the hull color).

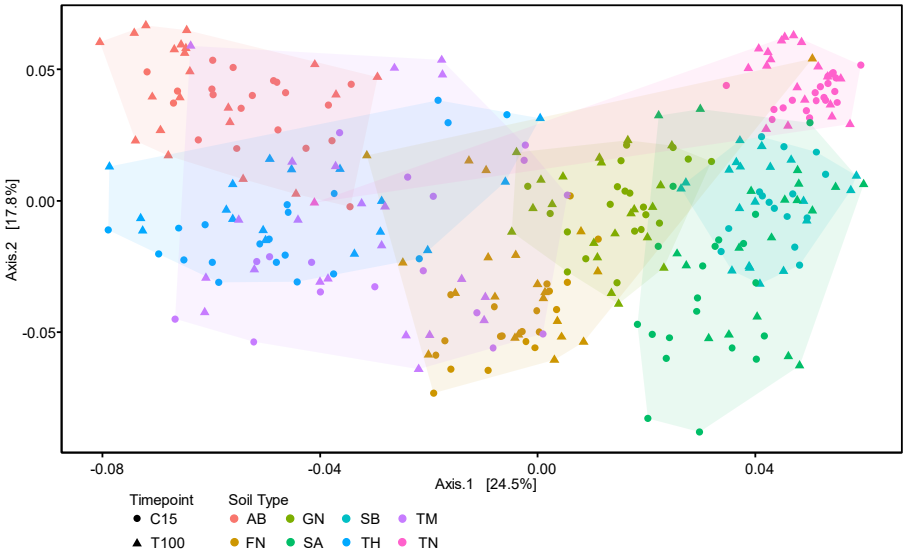


Figure 3.3b Bacterial community dynamics along the incubation temperature gradient. Principal coordinate analysis using weighted Unifrac distances computed from 16S amplicon profiles for all soil, incubation temperature and time points. The majority of variance is related to the soil type (indicated by the hull color).

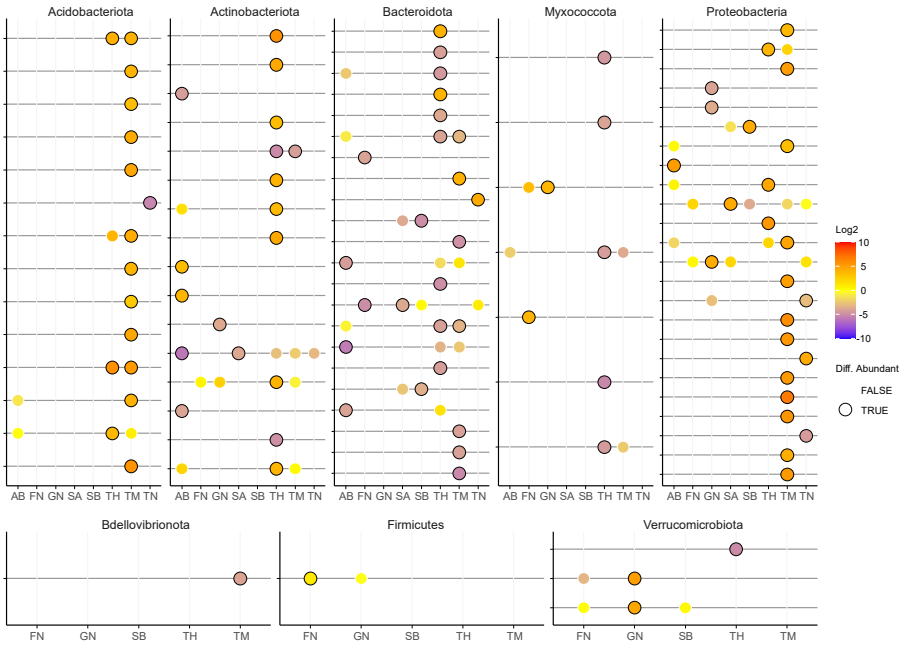


Figure 3.4 Differential abundance analysis of bacterial ASVs, shown in log2 fold difference per °C incubation temperature (color of points). Results are shown for C15 samples. Only ASVs significantly responding in at least one soil type are shown. For each ASV x soil combination: points with dark borders indicate significance (ANCOM-BC P < 0.05, Bonferroni FDR correction), points without borders are present but not significant, absence of points indicates ASV was not detected.

Discussion

In this study we examined the effect of incubation temperature on the temperature-growth relationships and community composition of Arctic soil bacterial communities. We provide evidence for temperature adaptation of the soil bacterial communities on the scale of weeks to months, as the community-aggregated optimal temperature for growth (T_{opt}) shifted in response to increasing incubation temperature at both sampling moments. Notably, there was no corresponding change in T_{min} for the same samples. These changes were presumably caused by a turnover in the community composition towards increased abundance of warm-adapted species since the soil bacterial community composition changed along the temperature gradient at both sampling moments for most soil types.

Temperature growth relationships show community adaptation to warmer temperatures

The observation that temperature-growth relationships changed all along the incubation temperature gradient contradicts part of our first hypothesis that such a shift would only occur at temperatures above the initial T_{opt}. It has been previously proposed that bacterial species die due to heat stress at temperatures above the T_{opt} of the soil bacterial community (Bárcenas-Moreno et al., 2009), which has been associated with an increase in maximum growth rates and a decline in species richness in short term incubation studies (Donhauser et al., 2020). In this present study four of the eight soil types (FN, SA, SB and TN) were exposed to temperatures more than 5 °C above their corresponding initial T_{opt} and did not show a corresponding abrupt change in either T_{opt} or T_{min}. The observed stability of T_{min} contradicts the findings of the majority of earlier incubation studies, which raises the question whether our result is attributable to technical and/or experimental design issues**.** An additional simulation power analysis showed that our study design was adequate for detecting the expected changes in T_{min} (Supplementary Methods). Moreover, the lack of an observation of increased maximum growth rates or decreasing species richness of the bacterial communities suggests that the “heat-induced death” mechanism was unlikely to be causing the observed patterns.

The non-effect of incubation temperature on T_{min} meant that, in our experiment, bacterial soil communities effectively broadened their temperature range of growth, through increases in T_{opt} and T_{max}. While previous work has proposed that

the interval between T_{max} and T_{min} is stable (Bååth, 2018; Li & Dickie, 1987), variation of temperature ranges of growth have been observed in response to soil temperature fluctuations in at least some cases (van Gestel et al., 2013). We observed a broader range between T_{min} and T_{max} with increasing incubation temperatures. Due to the difficulties of reliably measuring T_{opt} and T_{max} (Rinnan et al., 2009), not all previous studies have reported the full set of growth parameters, which complicates comparison (Rinnan et al., 2009; Birgander et al., 2013). However, it has been shown that alpine soil bacterial communities varying in T_{opt} 27.3 – 30.3 °C also increased in T_{opt} without changing T_{min} when incubated at 25 °C, while at 35 °C both T_{min} and T_{opt} changed (Donhauser et al., 2020). Recent work shows that Antarctic bacterial communities varied little in their range from T_{min} to T_{opt} between fluctuating thermal regimes in soil and stable maritime thermal regimes (van Gestel et al., 2020). More research is needed to develop a framework for understanding how the temperature range of growth is affected by environmental temperatures. In turn, such a framework could be important for understanding the mechanisms that lead to temperature adaptation of soil bacterial communities under different warming scenarios.

We hypothesized that soils from colder environments would respond more strongly to the incubation temperature gradient as these soils were exposed to temperatures further above the *in situ* thermal regime. However, despite the T_{opt} of *in situ* communities ranging from 22.5 to 31.5 °C across the 8 soils, there was a common slope of the response of the same parameter to the incubation temperature. In other words, the magnitude of the response of T_{opt} of Arctic soil bacterial communities to the incubation temperature was not related to the *in situ* soil temperature regime, and therefore resulted in T_{opt} varying between soils at the same incubation temperature. On the one hand this implies that all soils contain bacteria with a sufficient range of temperature traits to allow comparable responses to warming (C. Wang et al., 2021). On the other hand, it is possible that the shifts in T_{opt} of the soil communities require time scales greater than the length of this study, and that given sufficient time to equilibrate, all soils would eventually attain a temperature-specific T_{opt} (Nottingham et al., 2021). Therefore, more work on field warming experiments could help to define the roles of long-term dynamics and legacy effects on the adaptability of soil bacterial communities to soil warming.

The lack of threshold dynamics in the composition of the soil bacterial communities across the temperature gradient (Figure 3.3) indicates that the shifts in temperature-growth relationships were caused by a gradual turnover in the bacterial community. Such a pattern could emerge from species sorting due to environmental filtering (Leibold et al., 2004). Importantly, we observed that these compositional changes occurred in C15 samples, when differences in substrate availability between temperature treatments were minimized, indicating that direct temperature effects were at least partially responsible. While it is possible that temperature-growth relationships can change without compositional shifts, e.g. through genotypic (Chase et al., 2021) or phenotypic adaptation, these mechanisms have been considered less plausible in incubation experiments because of the insufficient duration for evolutionary processes, and because of the minor changes in temperature response typically associated with phenotypic changes (Bárcenas-Moreno et al., 2009). Our findings provide further evidence that community turnover is causing the shifts in temperature growth relationships, as has been previously been found in both incubation and field studies (Donhauser et al., 2020; Weedon et al., 2022).

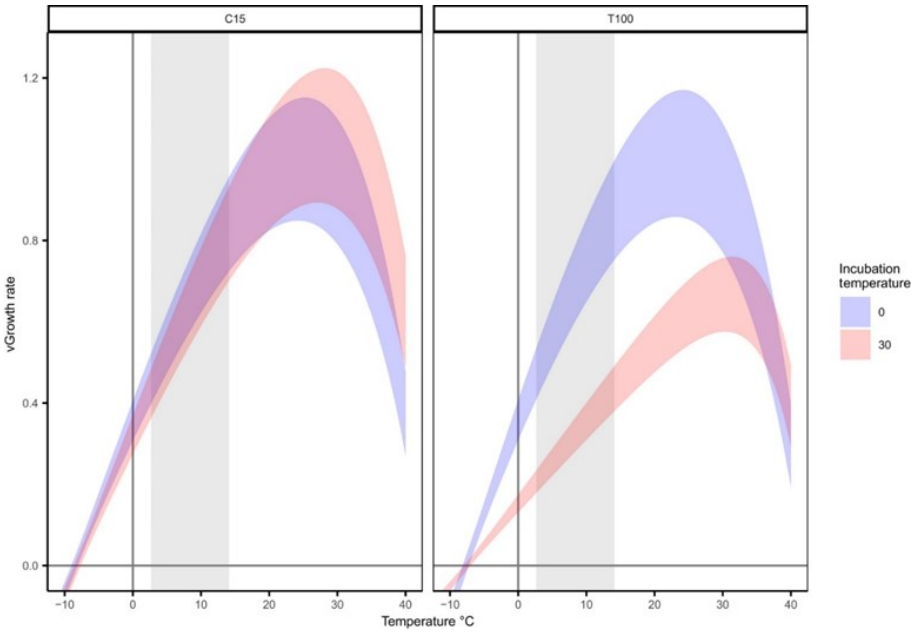


Figure 3.5. Estimated effect of soil incubation temperature on temperature-growth relationships of bacterial communities. Curves represent predicted responses of short-term growth rates to temperature between -10 and 40 °C. Separate curves are calculated for each sampling moment (left and right panels) and for soils incubated at 0 and 30°C (blue and red curves) using the parameter estimates for the Abisko soils. Bands show 90% confidence intervals estimated by bootstrapping fixed effects of incubation temperature and sampling moment from the mixed effect models for each of the 4 combinations of growth parameters (n=1000). Grey areas show the range of average daily summer soil temperatures over the 8 sites used in the present study.

Our comparison of C15 and T100 samples provides insight into drivers of the shifts in the temperature-growth relationships of soil bacterial communities under future warming. As for community composition, the changes in temperature-growth relationships appear to be at least partly driven by direct temperature effects, since incubation temperature effects on temperature-growth relationships were significant at C15. Nevertheless, the effect of incubation temperature on temperature-growth relationships was larger at T100 than C15, which refutes our second hypothesis. The sampling moment after 100 days was

substantially longer than previous studies, in which incubation periods of between 72 hours and 1-2 months are typical (Ranneklev and Bååth, 2001; Birgander et al., 2013; Donhauser et al., 2020). Such a longer incubation presumably allows for a greater number of generations within the bacterial community and consequently a greater degree of community turnover (compared to C15 samples) in the warmer treatments. On the other hand, limiting substrate availability could possibly drive the competitive benefits of bacterial species performing well at higher temperatures under resource limitations. In turn, this could lead to a larger effect of incubation temperature on the aggregate temperate growth relationships of soil bacterial communities. Overall, our results suggest that substrate limitation and/ or a larger number of generations are most likely responsible for the more pronounced temperature effects in the warmer treatments after 100 days. We are unable to test which of these mechanisms predominates, however the temperature-related decrease of maximum growth rates in T100 samples suggest that substrate limitation is playing a role (Figure 3.5).

The longer incubation period and separation of direct and indirect temperature effects in this study also allow us to make connections between observations from incubation and field studies**.** Long term warming studies under field conditions (>4 years) have shown that temperature-growth relationships can change without exceeding the T_{opt} of the initial soil microbial community (Nottingham et al., 2021; Weedon et al., 2022). Our study shows that this effect is also reproducible under incubation conditions and that this could be partially driven by substrate limitation and exposure time under warmed conditions. Indeed, the temperature adaptation of soil bacterial communities to temperature below T_{opt} can be a long-term process, for example, taking as long as 11 years after transplanting soil to a cooler climate (Nottingham et al., 2021). This indicates that, depending on the magnitude of the temperature change and new climate, temperature adaptation induced by soil warming might take more generations than previously assumed. Altogether, this shows that bacterial communities can adapt to relatively modest increases in temperature after extensive exposure. However, previous field studies with warming by open top chambers showed no significant change in temperature under moderate warming of 1-2 °C, which could be due to measurement error larger than the expected effect size (Rinnan et al., 2009, 2011). This study shows that more research is needed to evaluate the drivers of temperature adaptation by soil bacterial communities and whether temperature adaptation will occur under moderate soil warming in field conditions.

Compositional changes in response to warming – are there bioindicators?

The simultaneous shifts in temperature growth relationships and community composition under warmed conditions in this study support our third hypothesis (Donhauser et al., 2020; Weedon et al., 2022). Additionally, 4 of 8 soils showed strong similarity in the response to temperature in terms of community dynamics (Supplementary Methods). Since the incubation temperature could be an environmental filter for community assembly (Leibold et al., 2004), these shifts could indicate that the community turnover led to a dominance of warm-adapted species. It is likely that temperature responsive taxa can only be identified on a species or ASV level, as traits related to the thermal performance of microbial species are hypothesized to be phylogenetically conserved at a relatively shallow level (Martiny et al., 2015). In accordance with this, we found no universal temperature response when community data was analyzed according to bacterial phyla. We therefore used differential abundance analysis to identify which species were responsive to the incubation temperature at limited substrate effects (C15). We detected 111 ASVs at C15 as differentially abundant across the temperature gradient in at least one soil type, of which 69 ASVs were also differentially abundant at T100. Isolation of these temperature responsive ASVs will be needed to verify whether the ASVs are characterized by higher optimal growth temperatures than other community members. Previously it has been proposed that temperature responsive species in the bacterial community could be used as indicators for the estimation of community-wide temperature-growth relationships (Hicks et al., 2021). However, in this study only few ASVs were differentially abundant in more than one soil type. At C15, 7 ASVs responded in two or more soil types (Table 3.4). The soil specificity of temperature-responsive species suggests a limited utility of such 'bio-indicators' across soils. Indeed, in a biogeographic study only 15 OTUs (97%) were ubiquitously present across the 43 Arctic sampling sites (Malard et al., 2019). This high biogeographic heterogeneity combined with the aforementioned limited taxonomic signal of temperature responsive species implies that bio-indicator species might not be suited for all soils. Therefore, the determination of temperature-growth relationships of soil bacterial communities is likely to be more efficient through direct measurements such as the leucine assays employed in our study (Hicks et al., 2021).

Implications and conclusions

Our findings imply that temperature-growth relationships of Arctic soil bacterial communities will change under warmed conditions. However, we show that the

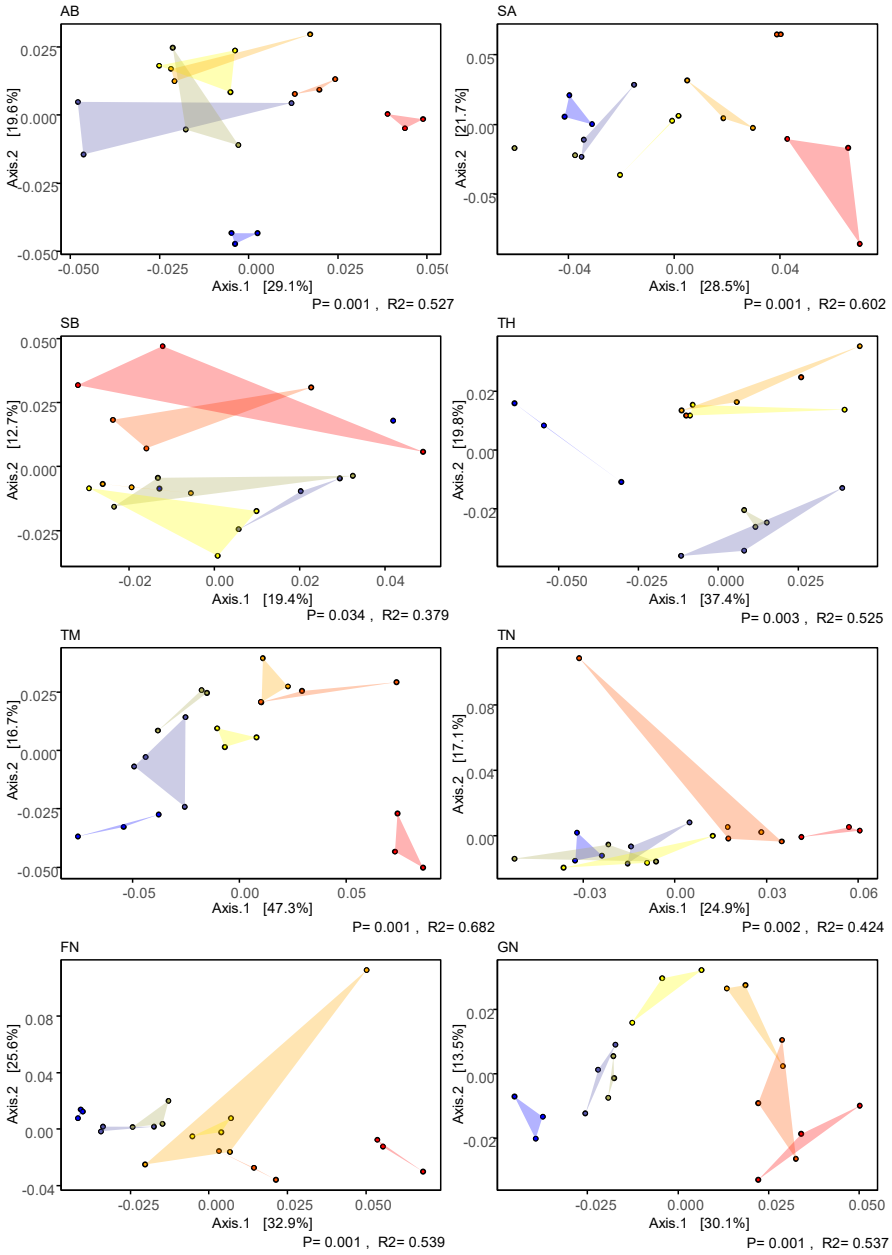
extent to which this occurs under field conditions will be determined by the degree of temperature adaptation, and the range of temperatures that soils are subject to under warming, as well as the dynamics of substrate availability. For example, based on our data, at C15 there are minimal differences in the predicted growth rates of the cold and warm exposed bacterial communities between 0-20 °C (Figure 3.5), which represents a scenario where substrate supply is comparable to the pre-warming state. However, if adaptation in the field is closer to the scenario related to T100 measurements, because of longer exposure and potential substrate limitation, this could induce substantial change in the growth rates at temperatures relevant to near-term warming in arctic soils. These results indicate that separation of these drivers will be important for understanding the potential for changes in growth rates of arctic soil bacterial communities under future climate conditions.

As follow-up to this study, will be important to assess whether other soil decomposer communities respond to soil warming as well. Fungal communities respond to temperature in similar way to soil bacterial communities (Pietikäinen et al., 2005; Bárcenas-Moreno et al., 2009; Birgander et al., 2018; Nottingham et al., 2019a), but so far no studies have shown shifts in the temperature-growth relationships of soil fungal communities (Birgander et al., 2018). It will also be important to assess the influence of shifts in microbial temperature-growth relationships on a broader range of soil biogeochemical processes, such as soil respiration and nutrient cycling. So far, studies have shown that implementation of microbial processes, such as altering the temperature sensitivity of the enzymatic parameters (Wieder et al., 2013) and/or the carbon use efficiency of soil bacterial communities (Allison et al., 2010; Wieder et al., 2013; García-Palacios et al., 2021), substantially influences model projections of SOC stock under warmed conditions. However, shifts in temperature-growth relationships have been shown to have only limited effects on soil respiration in a temperate forest soil, as the increased growth reduced under warmed conditions due to possible reduction in substrate availability (Rousk et al., 2012). Currently, less is known about the balance of warming and substrate-mediated feedbacks in Arctic organic matter-rich soils. Microbial growth rates in microbial-explicit SOC models are often defined by the uptake rate of substrates into the microbial biomass. Making this uptake rate temperature dependent, following the Ratkowsky equation for bacterial growth, would appropriately represent temperature-growth relationships in SOC models. These models could then be used to identify where

changing temperature-growth relationships will have large implications for SOC stocks on the global scale.

The temperature adaptation of Arctic soil bacterial communities, here shown under experimental warming, indicates that the microbial response to global warming could influence carbon cycling in Arctic terrestrial region. However, it remains a challenge to link the temperature adaptation to specific species that can be used as bioindicators for understanding and predicting the functional implications of temperature adaptation of soil communities. Furthermore, we propose incorporating shifts in temperature-growth relationships into earth-system models to assess the potential magnitudes of their impacts on soil carbon cycling.

Supplement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Axis.2 [19.6%]
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P= 0.001 , R2= 0.527
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P= 0.034 , R2= 0.379
TH
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TM
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P= 0.001 , R2= 0.682
TN
Axis.2 [17.1%]
Axis.1 [24.9%]
P= 0.002 , R2= 0.424
FN
Axis.2 [25.6%]
Axis.1 [32.9%]
P= 0.001 , R2= 0.539
GN
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Axis.1 [30.1%]
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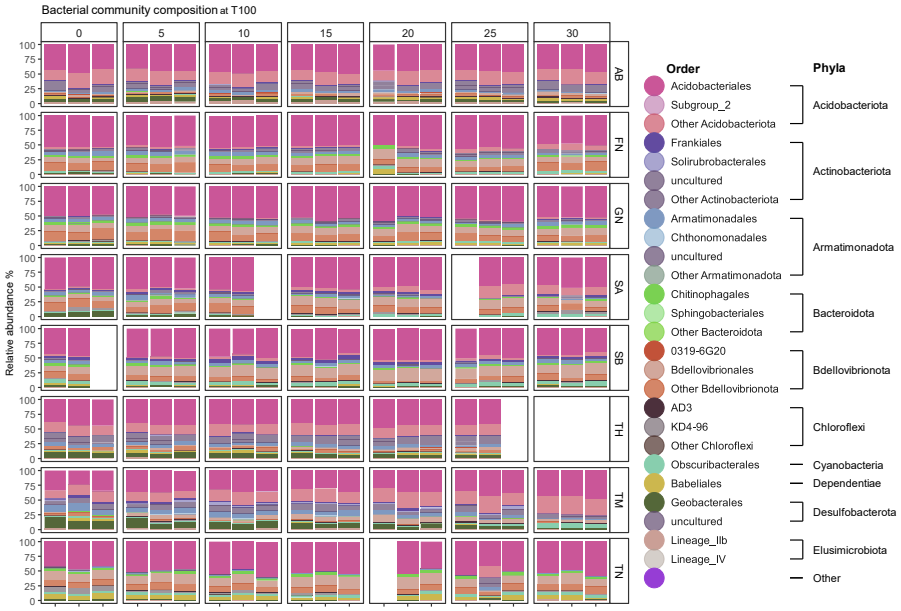
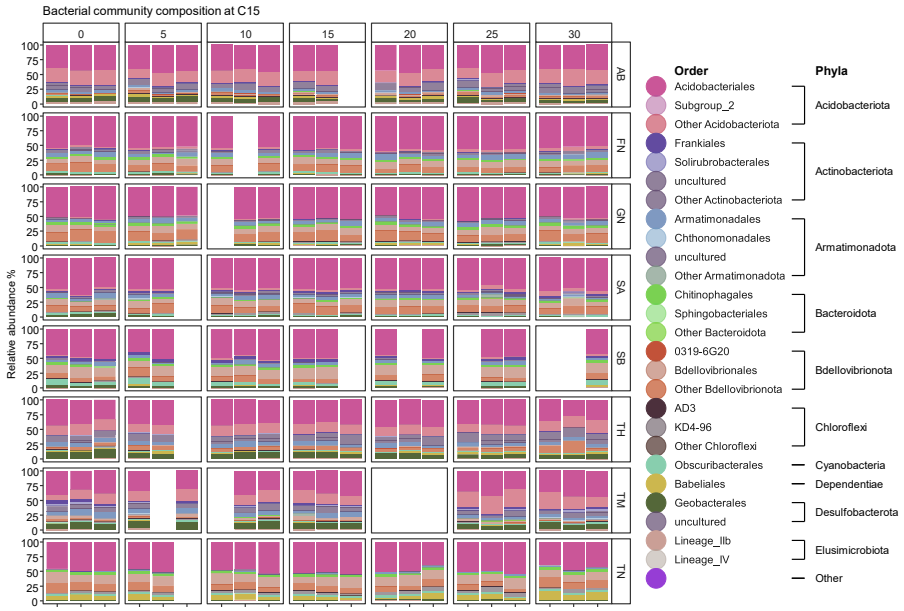
Supplementary Figure 3.1 Principal component analysis of the soil bacterial community composition at T100 for each individual soil type, blue to red hulls indicate the incubation temperature (0-30°C).



Supplementary Figure 3.2 Relative abundance of soil bacterial taxa on Order level for soil sampled at T100.



Supplementary Figure 3.3 Relative abundance of soil bacterial taxa on Order level for soil sampled at C15.

Supplementary Methods

Power analysis on Tmin measurements

We performed a simulation power analysis (Arnold et al., 2011) to evaluate the possibility that the observed null result was due to the relatively large sample variance of Tmin (mean CV = 14.9%) and modest amount of replication (n = 3 per soil and incubation temperature combination).Taking a Tmin increase of 0.8 °C per °C incubation temperature above the initial Topt reported by Birgander et al., (2018) as a starting point, we assumed an effect size of 4°C difference in Tmin between soils incubated > 5°C above and below the initial Topt. Simulations with alpha set at 0.05 showed that 80% power would already be expected above for a difference of Tmin of 1.5°C between samples incubated above or below (Figure SM3.1).

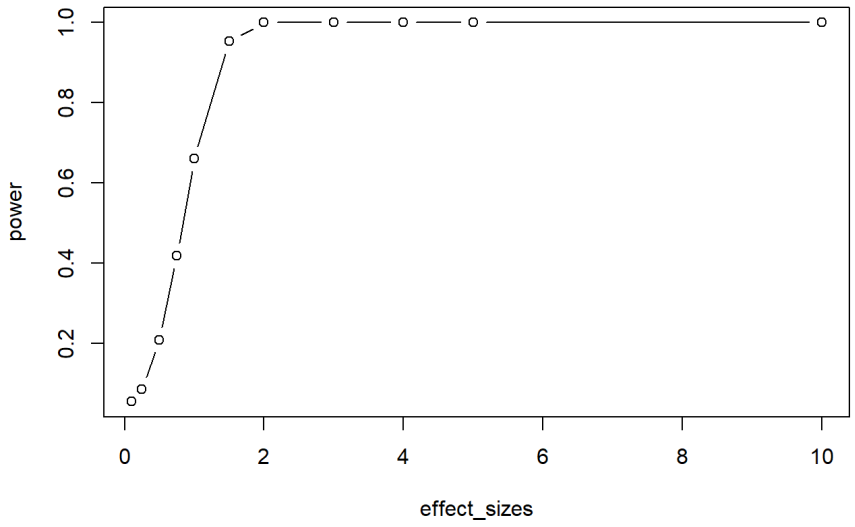


Figure SM3.1. Estimated power for effect size from 0 to 5 for change in Tmin above and below the initial Topt of incubated soils.

Comparison of community responses between soil types

The differential abundance analysis presented in the main text compares the response of bacterial communities to incubation temperature in terms of shared responsive species. At a more general level, it is possible to test the similarity or

divergence of community composition response to temperature. We do this by performing Mantel tests between the distance matrices of all 8 soil types to determine the correlation between community response to temperature between the soils. We converted the resulting correlation R^2 values to distances, $\sqrt{(2\ (1-R^2))}$ and performed hierarchical cluster analysis on the distance matrix with 'complete linkage' method. From this we conclude that at least four of the sites showed highly similar community-level responses to incubation temperature (Table SM3.1; $P<0.05$, $R2 > 0.6$). The remaining four were either not affected by incubation temperature or showed correlation in community response.

Supplementary Table SM2.1. Mantel test results for comparing the dissimilarity matrices of each soil type combination, values indicated R_2 and bracketed values are P-values.

	AB	**TH**	**TN**	**FN**	**GN**	**SB**	**TM**	**SA**
AB								
TH	**0.60 (0.01)**							
TN	0.28 (0.21)	0.00 (0.34)						
FN	0.10 (0.28)	-0.2 (0.87)	0.31 (0.05)					
GN	0.00 (0.52)	0.32 (0.18)	-0.2 (0.82)	-0.0 (0.63)				
SB	0.46 (0.08)	**0.78 (0.01)**	0.18 (0.28)	-0.1 (0.75)	0.18 (0.27)			
TM	**0.70 (0.00)**	**0.74 (0.01)**	0.13 (0.31)	0.04 (0.38)	0.31 (0.14)	0.56 (0.05)		
SA	**0.73 (0.01)**	**0.79 (0.02)**	-0.1 (0.59)	-0.1 (0.79)	0.42 (0.04)	0.61 (0.07)	**0.7 (>0.01)**	

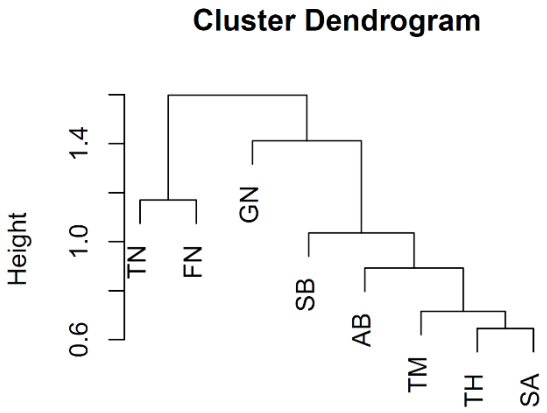


Figure SM3.2. Dendrogram on the similarity of community responses of soil bacterial communities to temperature gradient at C15 sampling moment. The length of tree represents the relative dissimilarity between soil types.in community responses to temperature.

Chapter 4

Limited utility of bacterial indicators for soil warming: a case study in an Icelandic grassland

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Abstract

Understanding when and where climate warming affects biological and geochemical processes in soil ecosystems is crucial for accurate predictions of the carbon feedback to climate change. Quantification of the abundance for specific bacterial taxa (indicator species) has been suggested to be informative for assessing whether soil ecosystems are responsive to soil warming. Here, we investigate whether indicator species can be identified over multiple years along a warming gradient in an Iceland grassland, for which a consistent compositional change in the bacterial community has been previously shown at 10°C of warming. To do so, we tested whether there are bacterial taxa consistently differentially abundant between ambient and 10°C warming. We found only a small group of taxa that were significantly responsive to 10°C warming across multiple years. These potential bacterial indicator species will, however, have limited use for detection of impacts by soil warming on soil ecosystems, as these taxa were not differentially abundant at other warming levels within our sampling site nor in previous warming studies. Bacterial taxa for which the thermal niche is known or that had been previously identified as warming responsive also showed no clear response to soil warming at the Icelandic grassland site. We therefore conclude that there is only a limited use of abundance data of bacterial taxa as indicators for soil warming.

Introduction

Global climate change is expected to have a large impact on the microbial communities of most ecosystems through alterations in the abiotic and biotic conditions that determine their composition and dynamics (Cavicchioli et al., 2019). The response of microbial communities to these perturbations has the potential to amplify the impact of climate change, due to the important role of microbes in biogeochemical cycles (Falkowski et al., 2008). For example, increased microbial decomposition of soil organic matter in terrestrial ecosystems under warmed scenarios has a large potential for contributing to increasing greenhouse gas emissions. Implementing the response of microbial communities to climate-driven environmental change into earth-systems models (ESMs) is therefore important for understanding the potential for such feedback dynamics.

Increasing temperatures can induce many changes to the properties of, and processes in, soil ecosystems. Soil respiration (Carey et al., 2016), microbial growth and biomass (Pold et al., 2017; Marañón-Jiménez et al., 2018; Purcell et al., 2021), soil aggregates (Poeplau et al., 2020) and soil carbon stocks (Melillo et al., 2017) have all been shown to be warming-sensitive. The occurrence of ecosystem changes caused by soil warming is often dependent on the magnitude and duration of warming and can be ecosystem-specific (Carey et al., 2016; Melillo et al., 2017; Romero-Olivares et al., 2017; Walker et al., 2020). Due to this complexity, predictions of soil process responses to warming are usually not possible from temperature data alone. It would therefore be useful to develop a comprehensive tool that indicates whether soil warming has induced one or more responses in the soil ecosystem that are relevant for biogeochemical processes. Due to the importance of bacterial communities in soil processes, these responses might be best illuminated by the dynamics of the composition of the soil bacterial community (Hermans et al., 2017).

It is currently debated whether information about the microbial community composition (i.e. the relative abundance of different community members) can improve predictions of microbially-driven soil processes (Graham et al., 2014; Fierer et al., 2021). It is not always straightforward to relate changes in the composition of soil bacterial communities directly to environmental changes, due to the many possible influences of biotic and abiotic drivers (Zhou and Ning, 2017; Delgado-Baquerizo et al., 2018b). This is exemplified by the fact that it is still unclear whether microbial community composition changes in response to soil

warming. Some studies show clear responses (DeAngelis et al., 2015; Radujkovic et al., 2017), while others do not detect significant changes (Schindlbacher et al., 2011; Weedon et al., 2017). Instead of focusing on the composition of the whole bacterial community, more information about the relevant responses of the soil system might be gathered from monitoring specific bacteria that are directly responsive to soil warming. An important first step is therefore to identify which bacterial taxa respond consistently to soil warming, and to subsequently investigate the specific conditions and underlying mechanisms associated with these changes. These warming-responsive species could then be used for the detection and monitoring of biologically-relevant changes in soils caused by rising temperatures.

The abundance of specific taxa that are sensitive to environmental change can act as a proxy for the state of ecosystems and ecological communities and has a long history in the broader fields of plant and animal ecology (Carignan and Villard, 2002; De Cáceres and Legendre, 2009). Such a "canary in the coal mine"-approach is commonly used for the detection of chemical pollution (Evers et al., 1998), assessment of biodiversity (Bal et al., 2018), ecosystem quality (Carignan and Villard, 2002; Yezerinac and Moola, 2006), and indication of climate change (Krajick, 2004). In a similar fashion, the abundance of appropriately selected bacterial taxa could thus potentially be used as an indicator of changing conditions and therefore changing functions in the soil ecosystem. For example, abundance data of specific taxa have been used for the assessment of soil pH (Willms et al., 2021).

Identification of bacterial indicator species can be technically and statistically challenging. Bacterial communities show a high species richness, especially in soil ecosystems where DNA-based methods typically find >1000 distinct taxa in a single sample (Thompson et al., 2017; Delgado-Baquerizo et al., 2018a). One way to select for indicator species is to identify the environmental preferences or niche of bacterial taxa. The niche space of species is commonly used for identifying indicators (Dufrêne and Legendre, 1997). The thermal niche of bacterial taxa based on culture or *in situ* growth-rate data has been reported in multiple studies and databases (Madin et al., 2020; Sato et al., 2020; C. Wang et al., 2021), suggesting that it might be possible to identify potential indicator species *a priori* based on the niche space of the soil bacteria. Alternatively, the change in

abundance of soil bacterial taxa in response to soil warming under field or lab incubation conditions (Oliverio et al., 2017) could be used.

Due to the commonly-used methods for characterization of bacterial community composition, typical data are inherently compositional (Gloor et al., 2017). This means that abundances of individual taxa are always measured as a proportion of the total community. Consequently, an increase in the relative abundance of one species will decrease the observed relative abundance of other species, even if the actual absolute abundance of these other species is constant. This makes correlating the abundance of specific bacterial taxa to associated environmental or functional variables statistically complicated (Lin and Peddada, 2020b). In recent years, numerous methods have been developed to identify which bacterial taxa are differentially abundant across bacterial communities (Kaul et al., 2017; Lin and Peddada, 2020a; Nearing et al., 2022). These methods can be used to, for example, identify species that increase or decrease in relative abundance in response to an experimental treatment or environmental gradient – while appropriately accounting for the compositional nature of the underlying abundance data, and controlling for the risk of false discovery that is inherent in any analysis with a large number of potential comparisons (Benjamini and Hochberg, 1995)

While several studies have identified sets of soil bacterial taxa that are responsive to warming (DeAngelis et al., 2015; Oliverio et al., 2017; Che et al., 2019; Weedon et al., 2022), these taxa should be validated across natural spatial and temporal variation for use as indicator species. Due to the seasonal and inter-annual variation in microbial community composition (De Gruyter et al., 2019; Carini et al., 2020), candidate indicator taxa should ideally be robust to potential temporal influences and therefore respond consistently across multiple time points. Similarly, to be useful for generalization, indicator taxa should show a similar response to soil warming across different sites, and ideally in ways that are independent of soil and vegetation characteristics. Although some research supports the ideas of a set of bacterial taxa that consistently respond to warming under a range of conditions (Oliverio et al., 2017), very few bacterial taxa are commonly shared amongst multiple soils (Delgado-Baquerizo et al., 2018a) and the taxa responsive to warming are sometimes unique between different soil types (Chapter 3). To identify useful indicator species, it is important to systematically test whether indicator species respond consistently to soil warming. Comparison of temperature-responding taxa detected at multiple sampling

moments at a single site would be useful for establishing the temporal consistency of indicator taxa. Additionally, comparison of datasets from multiple studies of soil warming effects, conducted at multiple sites, will help to identify ubiquitous bacterial taxa that are consistently responsive to warming. This set of comparisons would provide a first step to classification of indicator species in soil bacterial communities under warmed conditions. Once identified, isolation and further study of indicator bacterial taxa could illuminate why these taxa are responding to soil warming and subsequently how the abundance of individual taxa can be further developed as assessment tools for soil ecosystems.

This Study

We exploited a multi-year dataset on the bacterial community composition of soils subject to a range of warming levels in an Icelandic grassland (Sigurdsson et al., 2016) to detect bacterial taxa that are consistently responsive to soil warming across multiple years. Multiple studies at this site have indicated that soil warming induces responses for a range of ecosystem processes (Walker et al., 2020) and have consistently sampled soils at ambient and at 10°C warmed conditions. Soil bacterial communities show a divergent community composition and significant increase in a subset of rare taxa above 6-8°C (Radujkovic et al., 2017; Weedon et al., 2022). While 10°C of warming exceeds the currently projections for climate change (Collins et al., 2013), we assume that the previously documented responses at this temperature provide an appropriate test case for exploring the use of bacterial indicator species. In this study, we test 1) which bacterial taxa are consistently responsive to 10°C of *in situ* warming across 4 sampling moments, ranging from 4 – 8 years after the warming gradient was established (Figure 4.1A). To further validate the utility of these taxa as indicators of soil warming, we then assess 2) whether these bacterial taxa respond along the entire warming gradient for the years where data for more temperature elevation levels are available. We also test 3) the potential use of the indicator species for soil warming by comparing the response of the warming-responsive bacterial taxa identified at our study site to response of warming indicator species from previous studies, and 4) evaluate whether the thermal niche of corresponding bacterial taxa identified in previous studies correlates to their warming response.

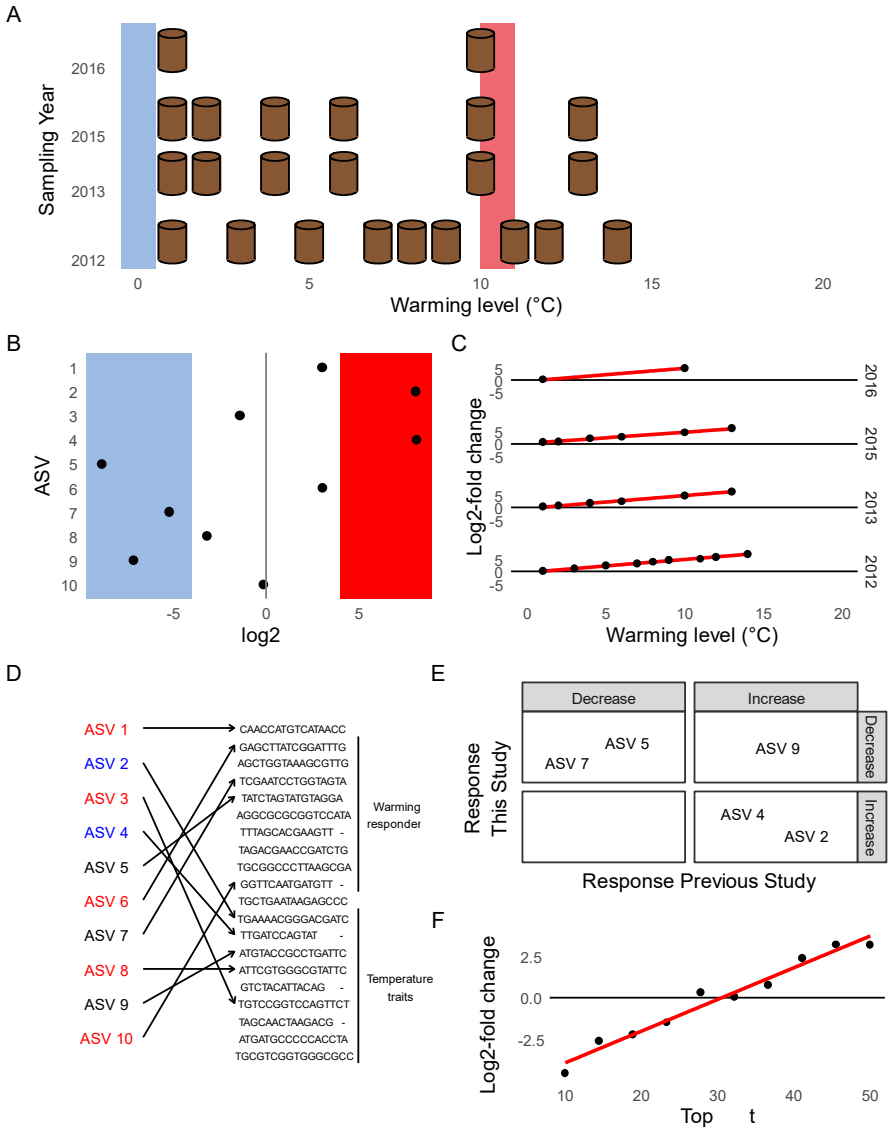


Figure 4.1 Overview of identification and validation of bacterial indicator species in this study. a) We sampled 0 and 10 °C warming levels (blue and red shades) at the FORHOT site from 2012-2016 and b) identified the ASVs that significantly decrease (blue) or increased (red) in abundance under warmed conditions. c) We validated the response over the other temperature steps of all years. d) We matched all ASVs to databases with bacterial taxa that are warming

responsive or have a known optimal growth temperate based on 99% sequence similarity. e) Validation of the robustness was done by comparing the response of ASVs in this study to other studies across studies, and by f) comparison of the known optimal growth temperature to log2 foldchange between ambient and +10°C warming.

Methods

Study site and sampling

We used samples collected at the FORHOT research site located in southwest Iceland. This site includes a small area of spruce forest (*Picea sitchensis*) surrounded by *Agrostis capillaris* grassland, both of which have been naturally warmed since an earthquake that occurred in 2008 (Sigurdsson et al., 2016). Geothermal warming from the bedrock has induced a stable natural warming gradient, heating soils to a range of temperature elevations from approximately 0 to +40 °C above ambient conditions (MAT 6.3°C ±0.3; Sigurdsson et al., 2016). In the autumn of 2012 plots were established at 6 warming levels (0,1,3,5,10,20 °C) along 5 transects. Previous sampling campaigns have assessed the response of bacterial community composition to warming along this gradient (Radujkovic et al., 2018; Weedon et al., 2022). Here, we sequenced DNA extracted from grassland soils obtained during sampling campaigns in 2012 (Weedon et al., 2022), 2013 (Radujkovic et al., 2017), 2015 (Radujkovic et al., 2017) and 2016 (Söllinger et al., 2022; Table 4.1). For samples from 2012-2015, DNA had previously been extracted using the MoBio Power soil kit following manufacturer's protocol and sequenced using the V3 region of the 16S rRNA gene (Radujkovic et al., 2017; Weedon et al., 2022). We extracted DNA for the 2016 samples using the same protocol. We sequenced the V4 region of the 16S rRNA gene for all datasets to directly compare the bacterial community composition across the entire dataset in one single sequencing run (Ge et al., 2014).

Table 4.1 Overview of sampling campaigns and samples

Study	Soil depth	Month	Year	Transect	Warming levels (^{0}C) above ambient
Weedon et al., (2022)	5-10 cm	May	2012	Initial transect	0, 2.0, 3.1, 5.3, 7.3, 10.1, 13.9, 20.2, 38.2

Radujkovic et al., (2018)	5-10 cm	May	2013	FORHOT	0, 0.5, 2.1, 3.9, 10.5, 17.3
Radujkovic et al., (2018)	5-10 cm	August	2015	FORHOT	0, 0.5, 2.1, 3.9, 10.5, 17.3
Söllinger et al., (2022)	0-10 cm	July	2016	FORHOT	0, 10.5

16S rRNA gene sequencing and bioinformatics

For the characterization of the bacterial community composition, the V4 region of the 16S rRNA gene was amplified by PCR using the 515 forward primer (5′-GTG YCA GCM GCC GCG GTA A-3′) and 806 reverse primer (5′-GGA CTA CNV GGG TWT CTA AT-3′; Caporaso et al., 2011). In the PCR there was an initial denaturation step of 1 min at 98°, followed by 24 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. The final PCR product was 50x diluted in sigma-sterilized water. In a second PCR of 8 cycles, the diluted amplicons were indexed with unique barcode primers using the same the PCR program. Ampure XP beads (Beckman Coulter) were used for purification of the indexed amplicons. Paired-end sequencing of the amplicons was done using a single MiSeq Illumina Sequencing runs V3-600 cycle chemistry. Raw sequences will be deposited on NCBI's Sequence Read Archive. In QIIME2 (Bokulich et al., 2018), demultiplexed sequences were truncated at 280 basepairs and dereplicated with DADA2 ("consensus" mode; max expected error =2 ; Callahan et al., 2016). The resulting amplicon sequence variants (ASVs) were aligned using MAFFT (Katoh and Standley, 2013). A phylogenetic tree was constructed with Fasttree (Price et al., 2009). Taxonomic classification of the ASVs was based on the SILVAv138 database (Yilmaz et al., 2014), using QIIME 2's scikit-learn naïve Bayes machine-learning classifier (Bokulich et al., 2018). ASVs matching mitochondrial and chloroplast sequences were discarded. We retrieved 903,919 sequences in total, 15 samples were excluded from analysis due to low sequence depth (<1000), after which the median sequencing depth was 12875.

Effects of temporal variation and +10°C warming on bacterial community composition

The R-packages '*phyloseq*' and '*vegan'* were used for statistical analysis. Across our dataset, soils were consistently sampled at ambient and +10°C conditions. Therefore, we performed multivariate analysis on the bacterial community composition to assess the effects of treatment and temporal variation at these

temperature levels. For multivariate comparisons of community composition between samples, we calculated the weighted Unifrac distances of the soil bacterial communities (Lozupone and Knight, 2005). In order to assess whether the beta-diversity was increased by the warming treatment, we calculated the beta-dispersion between the bacterial communities of ambient and at 10°C warmed conditions using betadisper function in R-package 'vegan' (Oksanen et al., 2007). We tested for differences in beta-dispersion between two ambient and +10° conditions using a permutational test. We performed permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) on the weighted Unifrac distances of the soil bacterial communities to test for the effects of sampling year and warming.

Indicator analysis

We determined which specific bacterial taxa were responsive to soil warming at the FORHOT grassland site by using differential abundance analysis on the ASV abundance data from the ambient soil and +10°C warmed soils in 2012, 2013, 2015 and 2016. We used ANCOMBC (Lin and Peddada, 2020a) on the bacterial community data using the warming level (0 or +10°C) as dependent variable and year of sampling as group variable of bias correction to determine the warming responsive bacteria. The Benjamini-Hochberg correction was used for multi-comparison P-value correction (Benjamini and Hochberg, 1995). To test the consistency of the response of the warming responsive taxa identified from the ambient vs 10 °C across a broader range of warming levels, we performed ANCOMBC on each annual dataset separately using each available warming level as the dependent variable (Table 4.1). For each ASV responsive to 10°C, we performed a linear regression per year with response variable of log2-fold change and the warming level as dependent variable. We considered an ASV to be a consistent responder when there was a significant log2 change in 3 out of the 4 years examined.

Matching published indicator taxa and temperature traits to taxa responsive to 10°C at FORHOT site

To establish the generality of the warming-responsive taxa identified from our site when compared to other environments, we conducted an analysis for cross-checking warming-responsive taxa from this study to the taxa that were responsive to previously identified as warming-responsive taxa or that have been associated with a particular thermal niche in published databases and studies

(Madin et al., 2020; Sato et al., 2020; C. Wang et al., 2021). First, we built a database of 16S rRNA gene sequences from taxa that have been previously described as either responsive to warming (Oliverio et al., 2017; Isobe et al., 2020; Nottingham et al., 2022; Chapter 3) or have previously described temperature traits such as the optimal growth temperature or temperature response (Madin et al., 2020; Sato et al., 2020; C. Wang et al., 2021). We retrieved the sequences of the indicator taxa from the publication in case published. When there were no sequences available, we requested the sequences or database identifiers from the corresponding author. We used Silva V128 database to retrieve the sequences of bacterial taxa when only the identifiers of this database were shared. To facilitate comparison with our dataset all 16S rRNA sequences were truncated *in silico* to the V4 region covered by the primer set we used for our sequencing, using 'cutadapt' in QIIME2. Sequences in our dataset and the reference dataset were matched by alignment of the sequences using 'usearch_local' in usearch10 with 99% sequence similarity (Edgar, 2010).

For the studies that identified bacterial taxa that are differentially abundant under warmed soil conditions we compared the direction of log2-fold change in abundance for each ASV in our dataset to the predicted direction of change in abundance from previous literature. We calculated the percentage of matching response by warming responsive taxa as between our study and previous study as a measure of predictive power. We tested for differences in mean change in abundance (log2-fold) between the groups of ASVs matched to increasing or decreasing bacterial taxa using two-sided student's t-test.

We also tested whether known temperature-traits, in this case the optimal growth temperature, of soil bacterial taxa were correlated with the response to 10 °C of soil warming. We hypothesized that bacteria that grow best at high temperatures would increase in abundance. To test this hypothesis, we performed a linear regression with the predicted optimal growth temperature as predictor variable and log2-fold change in abundance of the 99% similar ASVs as response variable, determined over multi-year comparison of soil bacterial communities at 0 and +10°C warming. We additionally tested for differences in mean log2 fold change between groups of ASVs that matched with the cold, moderate and warm responders from (C. Wang et al., 2021) using one-way ANOVA.

Results

Overall, the most abundant phyla in samples from ambient and +10°C across all were Proteobacteria (27.4%), Actinobacteria (18.1%), Acidobacteria (18%), Verrucomicrobia (7.6%), Chloroflexi (7%), Planctomycetes (6.6%) and Bacteroidetes (4.1%) (Figure 4.2). There was a significant difference between the bacterial community composition of the soils at ambient and +10°C warming conditions when all years were analyzed together (Figure 4.3a; Table 4.2, PERMANOVA R^2 = 0.12, P < 0.01). The beta dispersion between soil bacterial communities, described by the average distance of individual samples to their corresponding group centroid, increased from 0.10 to 0.13 between ambient and 10°C warmed soils (Figure 4.3b, Permutation test for homogeneity of multivariate dispersions; P < 0.05).

Table 4.2 Results of PERMANOVA on the 16S bacterial community composition between 0 and 10°C warming

	Df	SumOfSqs	R2	F	Pr(> F)
Temperature	1	0.053	0.125	3.573	0.002
Year	3	0.061	0.143	1.368	0.084
Residual	21	0.313	0.732		
Total	25	0.428	1		

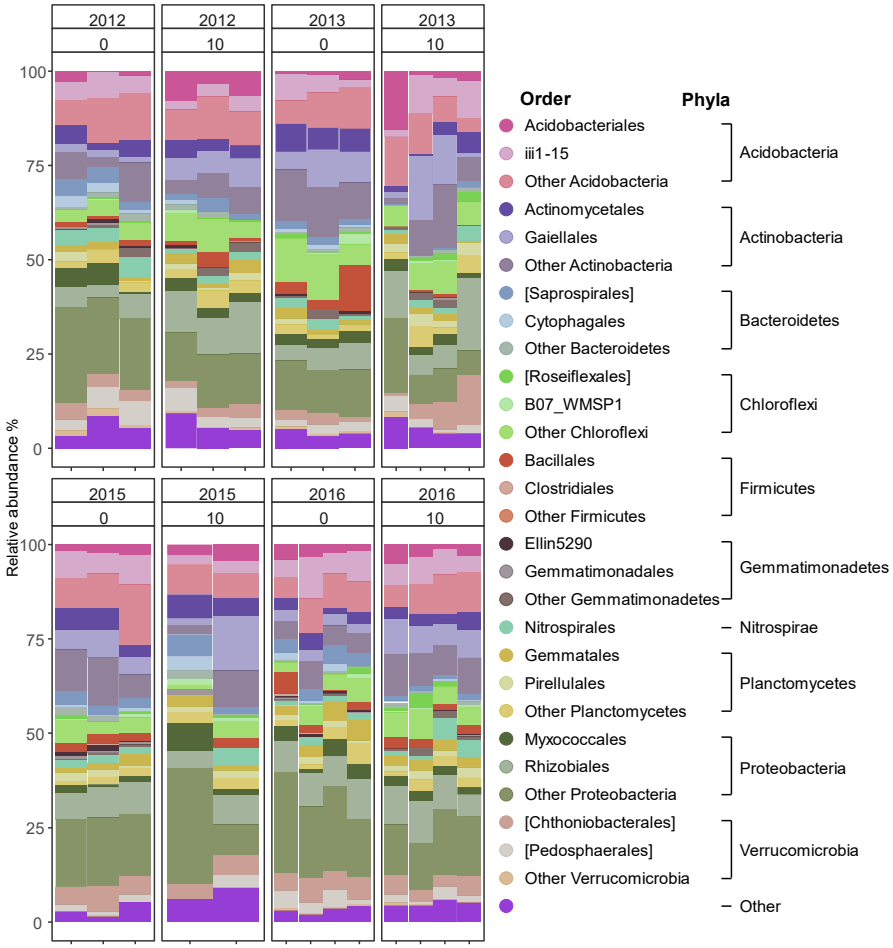


Figure 4.2 Relative abundance of the 10 most abundant Phyla for the soil bacterial communities at 0 and 10°C warming. Each panel shows a different sampling year and temperature level combination.

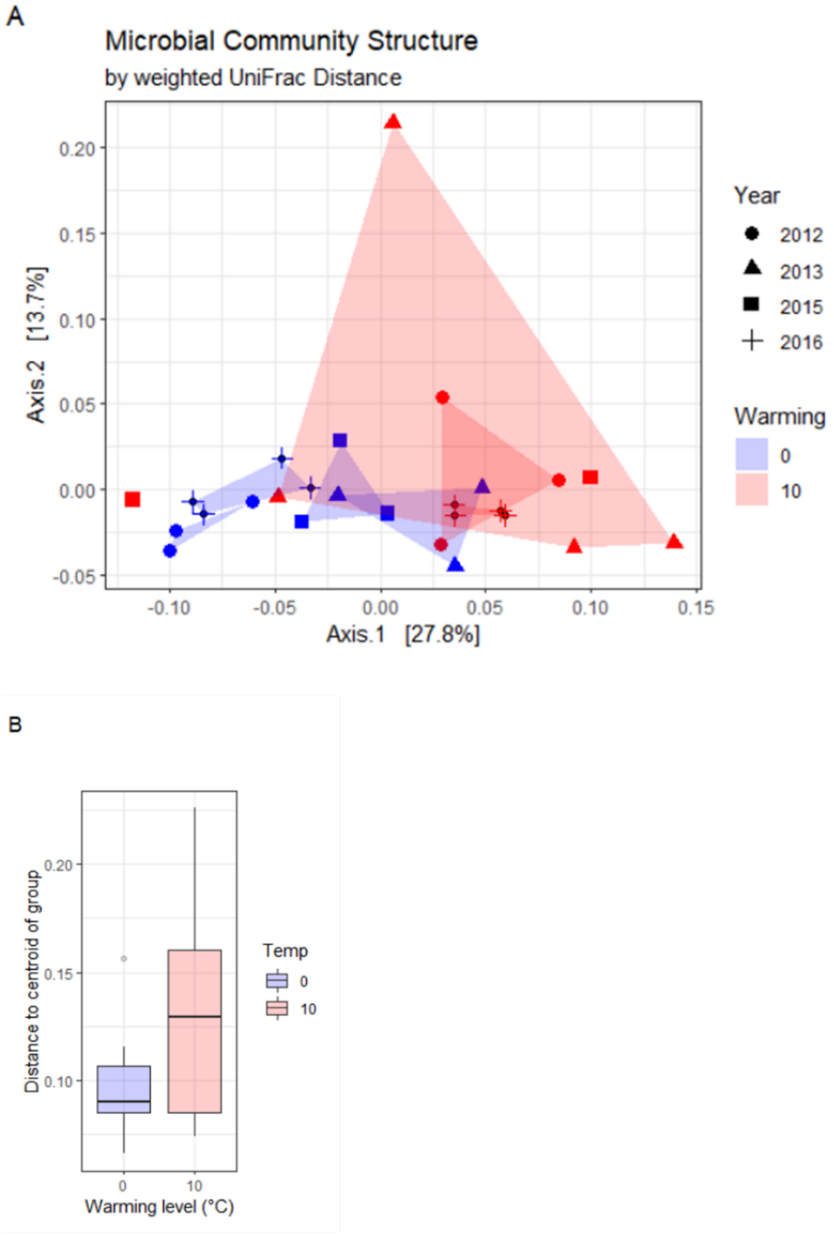


Figure 4.3 A) PCoA on the weighted UniFrac distances from the soil bacterial communities at ambient +6°C warming from 2012 to 2016. B) Beta-dispersion of the warming levels across al years.

Temperature responsive bacterial taxa across all years

Analyzing all years simultaneously, we identified 13 taxa that were differentially abundant between ambient and +10°C warming conditions (ANCOMBC, $P < 0.05$ after FDR correction). These taxa included ASVs assigned to the phyla of Actinobacteria, Chloroflexi, Nitrospirae, Planctomycetes, Proteobacteria, and Verrucomicrobia (Figure 4.4). Of these 13 ASVs, 10 were increasing and 3 decreasing. All of these taxa were present in either the ambient or +10°C warmed soils and absent in the other condition (Figure 4.5). The relative abundance, when present, for these responsive ASVs ranged from 0.079 to 0.44 %, with a median of 0.236%, compared to a mean relative abundance over all taxa of 0.023%.

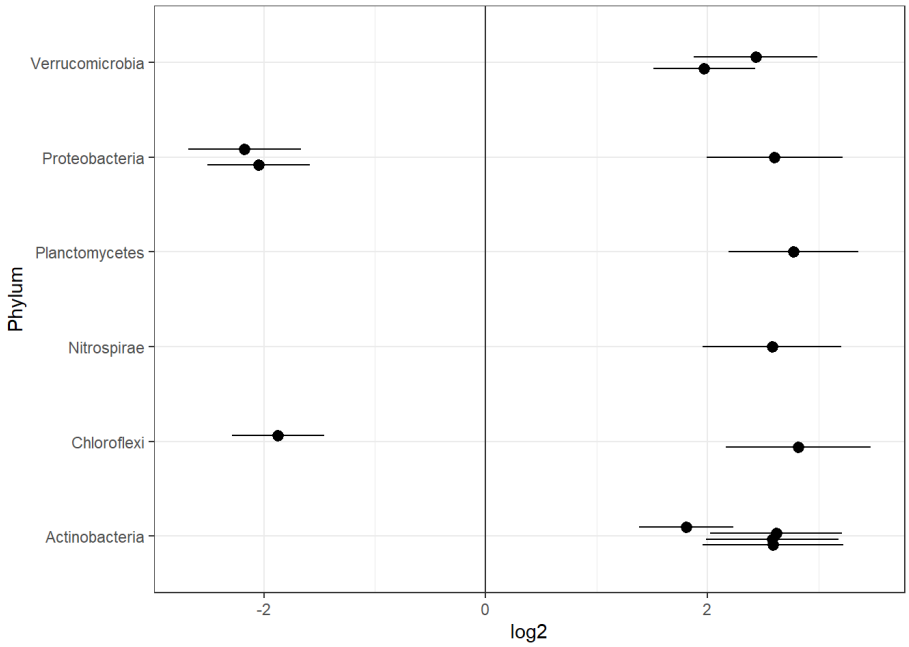


Figure 4.4 Log2 fold changes in abundance of the bacterial ASVs from ambient to +10 °C calculated across all years. Note that in all cases ASVs were absent at either ambient or 10°C warmed conditions (Figure 4.5), so fold-changes were not directly observed but rather derived as maximum likelihood estimates accounting for the total abundance in the dataset.

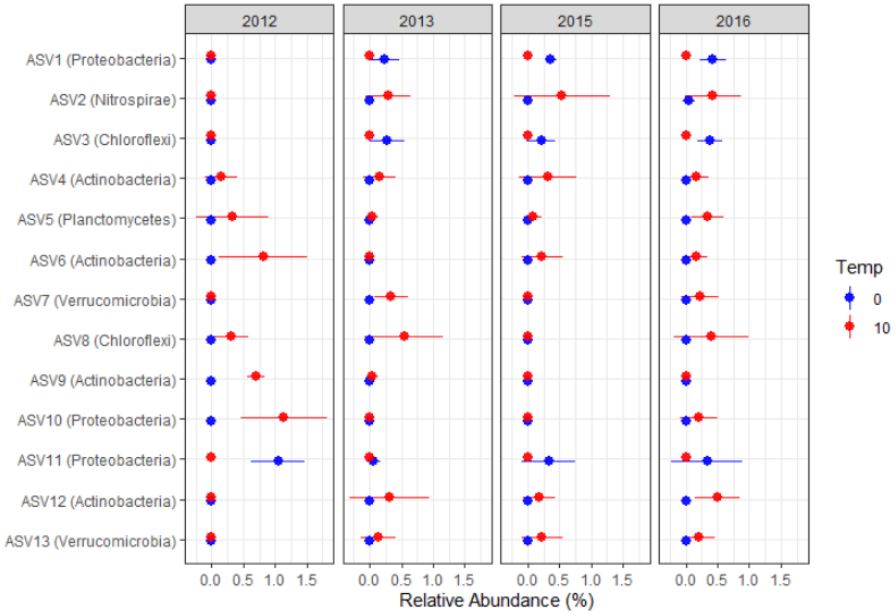


Figure 4.5 The change in relative abundance of the temperaure repsonsive bacterial ASVs at each year for ambient and 10°C warmed conditions at the Icelandic grassland.

Temperature responsive bacterial taxa across the entire warming gradient

In the years for which additional warming levels were studied, the 13 differentially abundant taxa identified in the ambient vs +10 analysis showed inconsistent responses when analyzed over more warming levels (Supplementary Figure 4.1). Only four of these taxa were consistent responders over time and the entire gradient: ASV1, ASV2, ASV3 and ASV11 (Supplementary Figure 4.1). Three of these ASVs decreased with soil warming, with ASV2 being the only ASV that increased in abundance with warming.

Validation of temperature responsive bacterial taxa

When comparing the community data from FORHOT to previously published studies, 471 ASVs showed >99% similarity with sequences of previously identified temperature-responding bacterial taxa (Supplementary Table 4.1). One of the ASVs that matched to previous responders showed significant changes in our dataset (Figure 4.6b) and increased in a similar way as previously reported (Figure 4.6b). However, this taxa were not part of the four taxa responding consistently

across the entire warming gradient. Previous identification as positive or negative responders to soil warming showed low predictability of the response at the FORHOT warming gradient, as 51% of the ASVs showed an opposite response compared to that in previous studies (Figure 4.6a). For the ASVs matched with sequences from Oliverio et al., (2017), both ASVs similar to reported positive and negative responders showed a mean increase in response to 10°C warming (Figure 4.6a), while the ASVs similar to the reported positive responders showed a larger increase than ASVs similar to reported negative responders, on average log2-fold change of 1.8 and 0.23 respectively (Student's t-test; $p<0.001$). For the ASVs matched to other reference databases with warming responsive taxa, we did not detect significant correspondence in patterns of response to warming (Figure 4.7; Student's t-test $P>0.05$ and test statistics).

ASVs matched to the trait-database

761 ASVs from the FORHOT dataset matched with 99% similarity to the sequences of the three trait-databases. For these ASVs there was no significant correlation between putative optimal growth temperature according to Madin et al., (2020) and Sato et al., (2020) and the change in abundance between ambient and 10°C warming based on observations at our study site (Figure 4.7; Linear regression models; $P>0.05$). There were significant differences in the log2-fold changes between the ASVs matching to cold, moderate and warm responders in the database of (Wang et al., 2021, ANOVA; $P< 0.05$). On average, ASVs matched to cold responders showed no response (mean log2 of 0.11 ± 0.21 s.e.), while moderate and warm responders tended to increase in abundance, log2 mean of 1.22 ± 0.28 and 0.88 ± 0.27 s.e. respectively (Figure 4.7C).

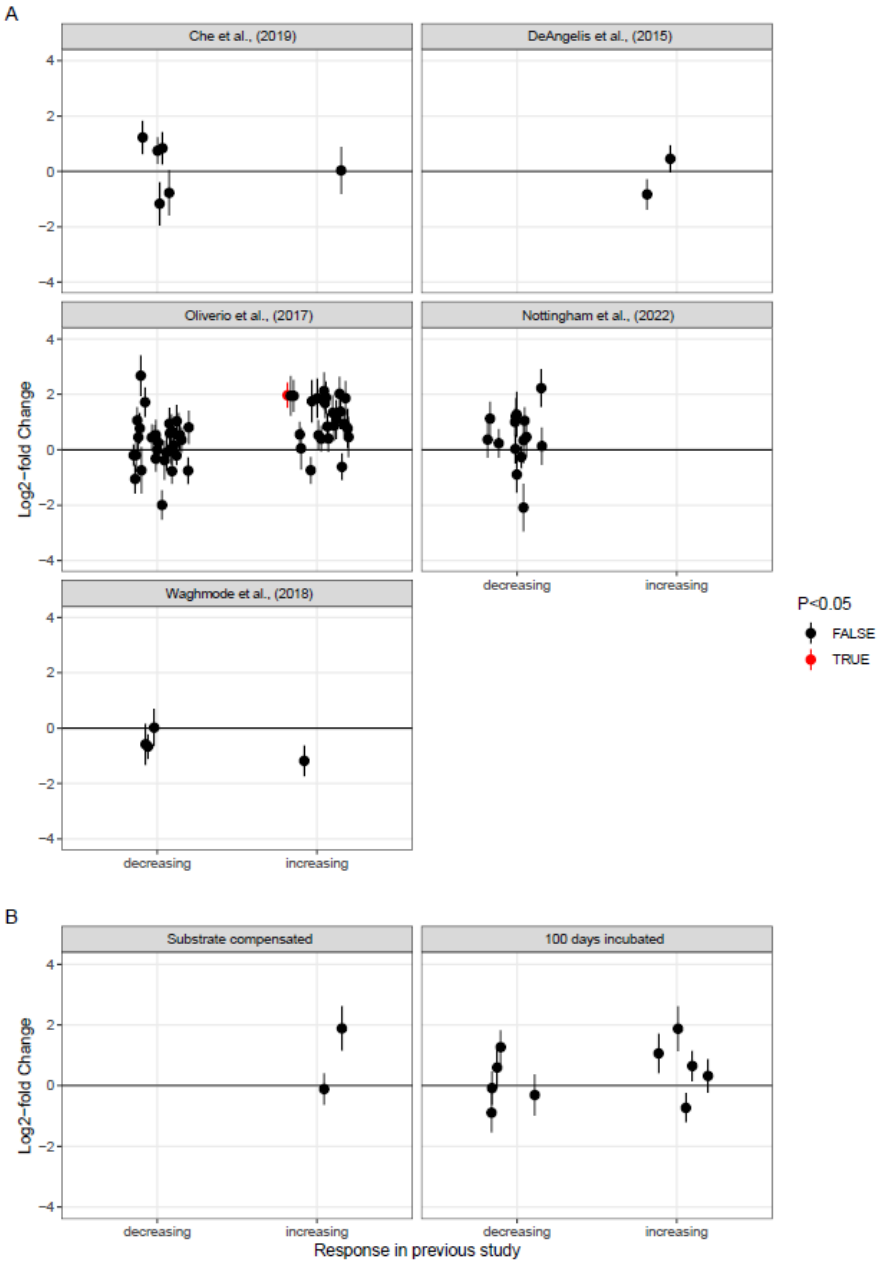


Figure 4.6 A) Change in abundance of ASVs that showed 99% similarity to bacterial sequences responsive to soil warming identified in previous studies, which are indicated by the panel. X axis

values indicate the direction of response to soil warming in previous study. **B**) Changes in abundance of ASV previously identified in Chapter 3 as responsive to warming at 100 days or at equal amount of respiration to compensate for difference in labile substrate.

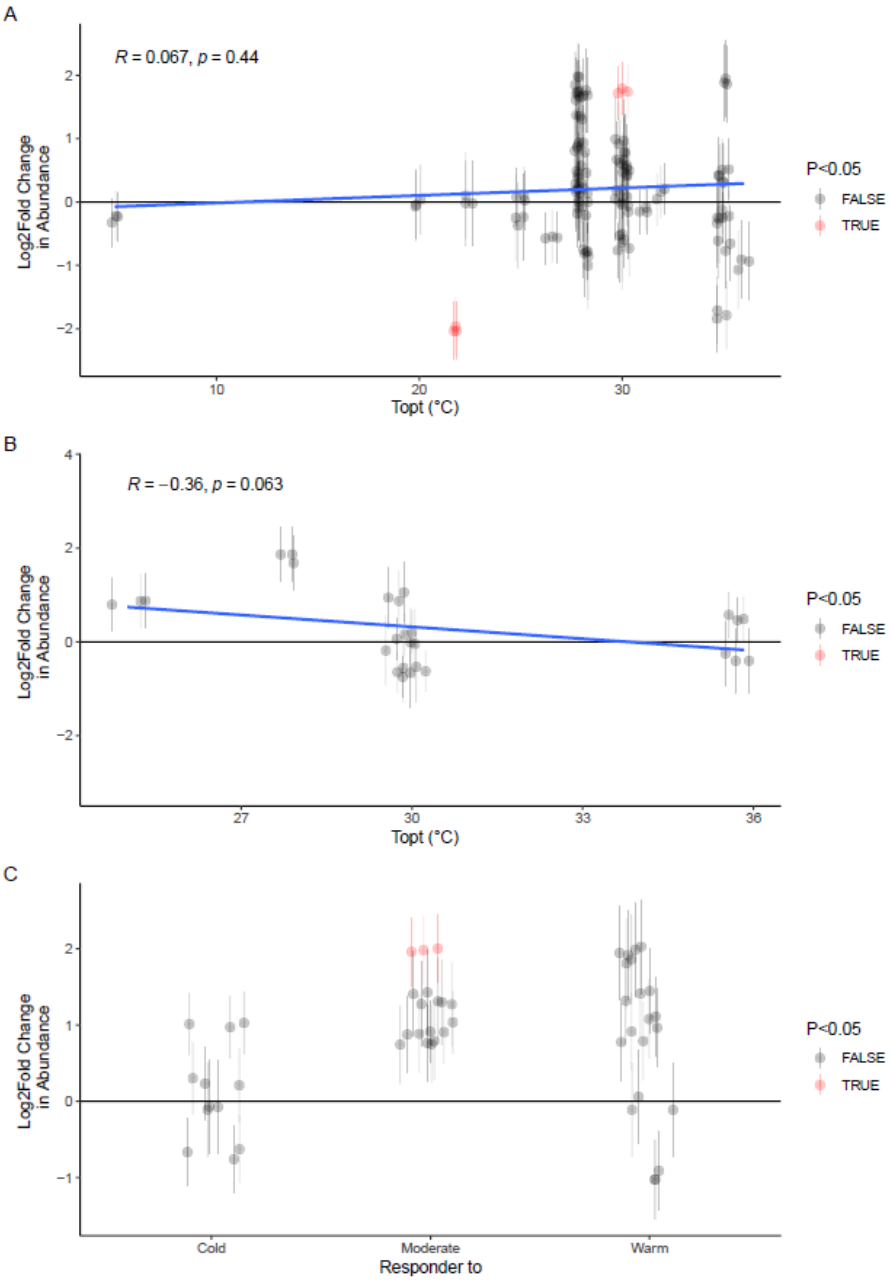


Figure 4.7 Change in ASV abundance between ambient and +10°C warming across all years over the temperature trait matching to the ASV by 99% sequence similarity in **A**) TEMPURA database (Sato et al.,

2020), **B**) Madin et al., 2020 and **C**) Wang et al., 2021. Fitted lines represent linear regression.

Discussion

In this study we explored the potential of bacteria taxa as indicator species for soil warming at an Icelandic grassland. We identified 13 bacterial taxa that were responsive to 10° C of warming in Icelandic grasslands across multiple years. This is a lower number of differentially abundant taxa than that have been previously identified at the same site from samples taken in 2012, where 303 OTUs were differentially abundant when measured over a broader range of warming (0 to +40 °C; Weedon et al., 2022). This discrepancy is not due to different analytical techniques (V4 region and ASVs instead of V3 and 97% OTUs), since a re-analysis of the 2012 samples along the whole gradient revealed 319 differentially abundant ASVs (data not shown). A more likely explanation would be a lack of consistent warming-responsive taxa over multiple years. Indeed, PERMANOVA indicated that both temperature and the sampling year explained similar magnitudes of variation between soil bacterial communities sampled at 0 and 10 °C warming from 2012-2016, which is commonly observed (Contosta et al., 2015). This relatively large inter-annual variation combined with decreased similarity in community composition under warmed conditions (Figure 4.3b; Guo et al., 2022), indicate that soil bacterial communities under warmed conditions share relatively low numbers of ubiquitous bacterial taxa responding to warming. This emphasizes that robust indicator species for soil warming might not be identified from a single sampling campaign.

Given that our indicator species were defined relative to a single temperature step, it is important to know whether the differentially abundant species at 10 °C of warming respond in a similar way at a broader range of temperature levels (Figure 4.1). As only 4 of the 13 ASVs showed a consistent response to the entire warming gradient, more complex dynamics in community assembly, such as indirect warming effects or species interactions, might interfere with the use of bacterial soil taxa responsive to a single warming level. Three of the four taxa that showed a consistent response to warming decreased from a mean abundance between 0.15 – 0.4 % to near or below the detection limit. This suggests potentially inhibited growth, or mortality due to high temperatures. Moreover, none of the four taxa matched to the bacterial sequences identified as temperature responsive in

previous studies. Overall, this suggest that there might be a limited generality of the warming responsive bacterial taxa identified in this study.

Previously identified temperature responders

In line with the lack of generality of the four taxa from the grassland site, there were almost no ASVs that matched with multiple reference databases. This indicate that warming responsive taxa might be unique to a particular ecosystem. We also show that almost all taxa that were differentially abundant in previous studies and did match to ASVs in our dataset, showed no significant response to 10°C of warming in the Icelandic grasslands. Since multiple comparison correction reduces the significance of differential abundance analysis, we compared the direction of warming response between the ASVs from this study and previous studies without regard to *P*-values. The previous response of ASVs was not informative for the direction of response to warming in the Icelandic grasslands. For example, ASVs that matched the bacterial taxa decreasing in abundance under warmed conditions in a tropical forest (Nottingham et al., 2022) mostly showed a positive response to warming in the soils of Iceland grasslands. This shows that the response in abundance of the 16S rRNA gene of bacterial taxa in a certain ecosystem gives little predictive power of response to warming for taxa corresponding to the same 16S rRNA gene in another ecosystem and highlights the importance of cross-checking indicator species between datasets.

The dataset of bacterial responders we gathered from previous studies included experimentally warmed sites in a temperate forest (DeAngelis et al., 2015), an alpine meadow (Che et al., 2019), a tropical forest (Nottingham et al., 2022) and soils collected along a 26°C gradient in mean annual temperature across North America (Oliverio et al., 2017). There are some indications that bacterial taxa have a preferred temperature range (Ratkowsky et al., 1983; C. Wang et al., 2021). If so, the local climate will filter for bacterial taxa with a certain temperature preference and thus warming will likely change the temperature-trait best suited to the environment depending on the local climate. Therefore, the bacterial taxa responsive to the climate at a given site might be different between sampling studies. Interestingly, Oliverio et al., (2017) were able to identify bacterial taxa that consistently changed in abundance for a large set of soils incubated at 12 and 28 °C and showed a correlation with mean annual temperature across soils collected along a large spatial gradient. Bacterial taxa in our dataset that matched to these responders showed a positive mean change in abundance to warming at our

sampling, regardless whether they matched to taxa that increased or decreased with warming for Oliverio et al., (2017; Figure 4.6a). This shows that, despite rigorous filtering for robust indicators, these previously identified indicators could not reliably predict responses to *in situ* soil warming. This might be explained by differences between lab incubation and soil warming in the field. As the authors note, increments in incubation temperature will also effectively change the availability of labile substrates due to differences in metabolic rates (Oliverio et al., 2017). Consequently, the response of individual taxa in incubation studies might be driven by factors other than temperature itself. While soil warming in the field might also change the availability of labile carbon (Kirschbaum, 2004; Marañón-Jiménez et al., 2018), it is unlikely that changes in the quantity and quality of labile substrate during warming experiments is similar in lab incubation and field warming (Feng et al., 2017). Moreover, ASVs responsive to warming in an incubation experiment with soils from the ambient Icelandic grassland site showed no apparent link between ASVs responding to soil warming in the field (Figure 4.6b), even when differences in labile carbon availability were compensated for by sampling strategy (Chapter 3). Altogether, the previous response of bacterial taxa to either *in situ* soil warming or warming in incubation experiment showed no clear link with the response to warming by bacterial taxa at the FORHOT site. We consequently find no support for ubiquitous bacterial taxa that respond to warming across sites (Weedon et al., 2022), which is exemplified by the lack of matching responders across different sites (Supplementary Figure 2).

Warming-responsive taxa might differ between climates

We evaluated whether information about the potential thermal niche of soil bacterial taxa would be predictive of their response to soil warming. We found no correlation between the response to 10 °C warming of the ASVs and their predicted optimal growth temperature (Figure 4.7; Madin et al., 2020; Sato et al., 2020). In these databases the optimal growth temperatures of bacterial taxa were collected from isolates under lab conditions, which may differ from the *in situ* soil environment This could lead to a mismatch between the measured thermal niche of a bacteria taxa in the lab and the realized thermal niche under *in situ* soil conditions (Deines et al., 2020; Yates et al., 2022). Interestingly, there was a significant difference between the average responses of the ASVs that matched to different groups of temperature responders as defined in the Wang et al., (2021) dataset. The large average increase in our dataset of taxa identified by Wang et al.,

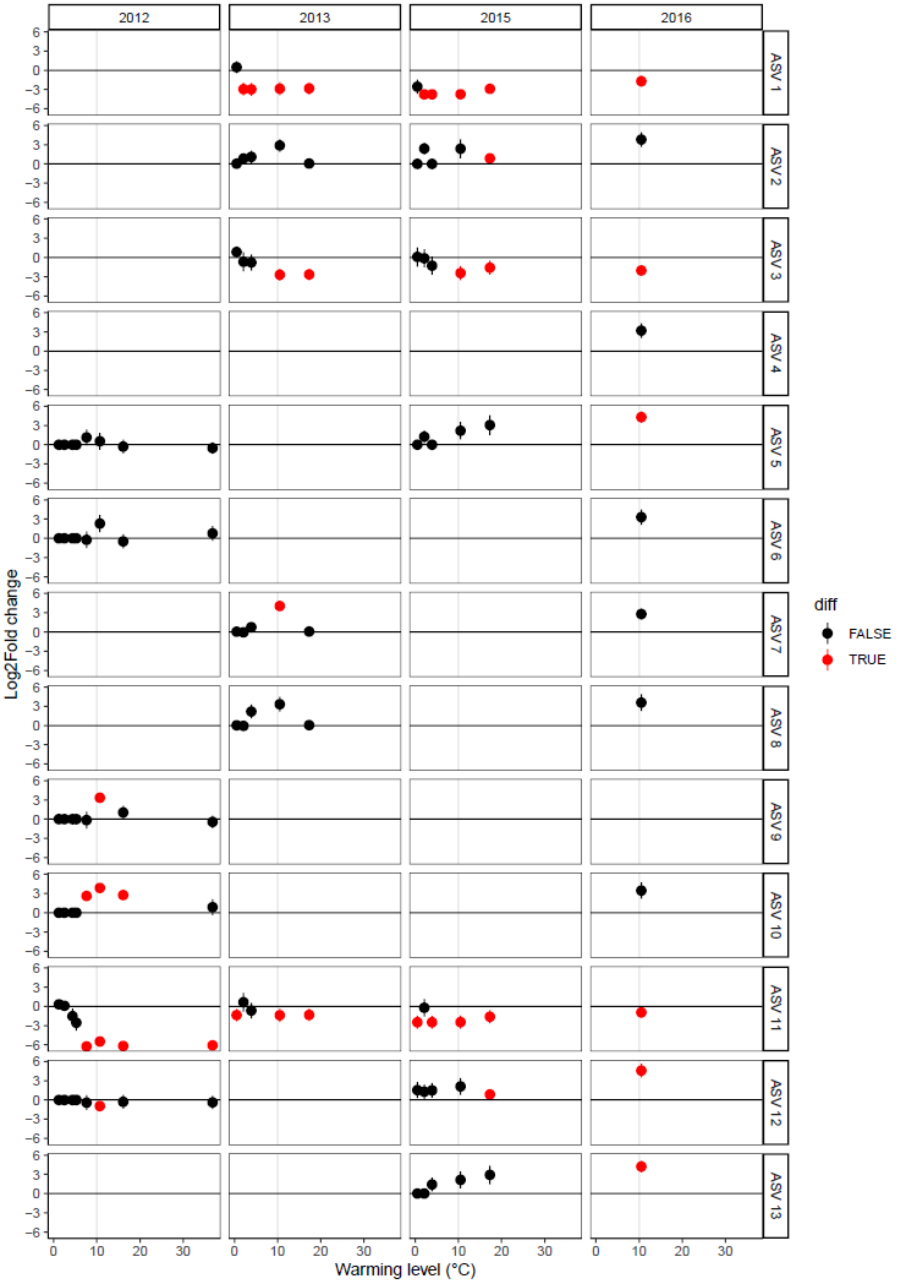
(2021) as 'moderate' and 'warm' responders supports the idea that bacterial taxa adapted to higher temperatures increase in abundance under warmed soil conditions. In contrast to the other trait databases, the trait data from (C. Wang et al., 2021) was obtained from ^{18}O-labeled H_2O quantitative stable isotope probing (qSIP) of microbial DNA to estimate the taxon-specific growth for natural soil bacterial communities incubated at various temperatures ranging from 5-35°C. This method circumvents biases introduced by isolation of soil bacterial into culture and likely estimates a more realistic range of growth rates for all soil bacteria present in the soil. To date, only one study has used ^{18}O-labeled H_2O qSIP to estimate the taxon-specific temperature sensitivity of soil bacterial communities. Therefore, we are unable to conclude whether this method provide better predictions for the *in situ* niche space of soil bacterial taxa, but this current observation might indicate that measures of the *in situ* thermal niche of soil bacteria might be most informative for their abundance response to soil warming.

Overall, our study shows that previous records of warming responses and temperature-traits for bacterial taxa give limited predictive power for the response of these taxa along an Icelandic warming gradient. The lack of a clear link between temperature traits/responses and the V4 region of 16S rRNA gene might be due to micro diversity among closely related bacterial taxa (Larkin and Martiny, 2017). Recent studies show that diversity at sub-species level of leaf litter bacteria encompass multiple local climate adaptations (Chase et al., 2018) and that these traits rapidly evolve under changing conditions (Chase et al., 2021; Scales et al., 2022). If such micro diversity would be apparent for a wide range of bacterial taxa, it could be that a diversity of thermal niches is associated with the same taxonomic classification. In turn, this would mean there would be no clear link between a thermal niche and a 16S rRNA sequence. This would limit the use of molecular methods to detect indicator species for soil warming. The lack of robust warming-responsive bacterial taxa showed limited support for bacterial indicator species to be used for soil warming.

Supplement

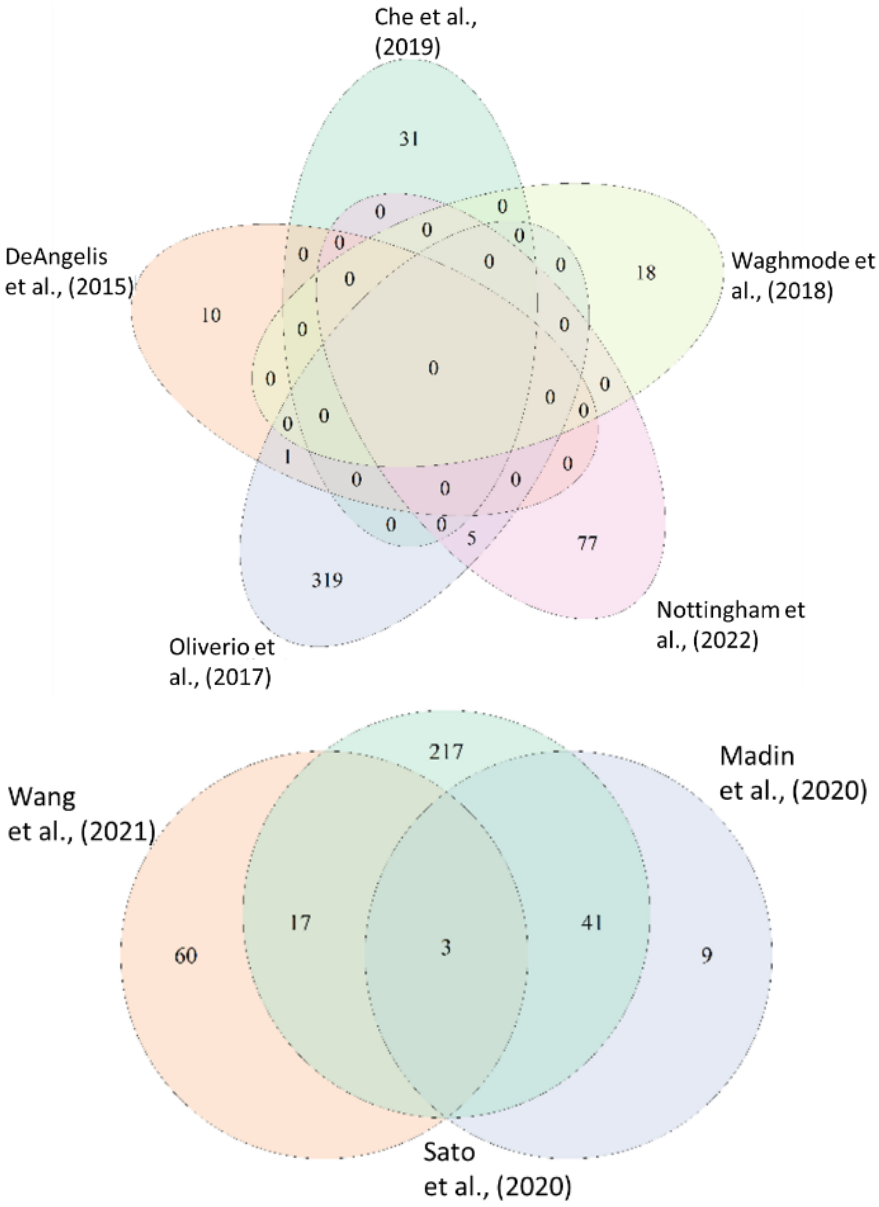
Supplementary Table 4.1 Overview of the number of previously identified bacterial taxa that matched with ASVS in our dataset across multiple filtering steps. *Sequences* indicate the initial number of sequences of the reference database, *matched* indicated the number of sequences from reference database to our dataset. *Matched ASVs* indicate the number of ASVs in our dataset that matched to the database sequences. *ASVs used in ANCOM BC* indicate the number of ASVs that were matched were abundant enough for differential abundance analysis.

Database	Sequences	Matched	Matched ASVs	ASVs used in ANCOM BC
Che et al., (2019)	20	7	31	6
DeAngelis et al., (2015)	10	3	11	2
Madin et al., (2020)	4663	36	53	71
Oliverio et al., (2017)	189	88	325	58
Nottingham et al., (2022)	130	29	82	15
Sato et al., (2020)	8644	122	278	46
Waghmode et al., (2018)	58	5	18	4
Wang et al., (2021)	1118	55	80	11



Supplementary Figure 4.1 The differential abundance in response to warming across the entire warming gradient for 13 identified ASVS

across multiple years. Colors indicate significant (red) or insignificant (black) change in abundance.



Supplementary Figure 4.2 a) Number of ASVs that matched to warming-responsive bacterial taxa from reference database by 99%

sequence similarity and **b)** databases with temperature-traits. Overlap in the Venn-diagram indicates that the same ASV from our dataset matched to multiple reference databases.

Chapter 5

A novel approach to modeling community-wide temperature responses of soil bacteria using thermal niche theory

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Abstract

Temperature drives the presence and abundance of different thermal niches in soil bacterial communities. Here, we explore whether implementing current knowledge about thermal niches for soil bacteria in a simplified trait-based model leads to realistic predictions of temperature-growth curves of soil bacterial communities based solely on soil climatic data. We found that our model could make predictions of temperature-growth relationships for soil bacterial communities from cold environments within the same magnitude of estimation error as previous statistical models. However, in temperate and warmer soils our model systematically overestimated the performance of thermophilic bacteria, thus leading to an overestimation of community-level temperature optima for growth. The model predictions fitted previously published temperature-growth relationships of soil bacterial best when there was a positive relationship between the maximum growth rate and optimal growth temperature (T_{opt}) of the modelled bacterial species (RMSE < 0.03). Despite some shortcomings we argue that our approach can substantially improve models for temperature adaptation of soil bacterial communities by using the distribution of temperature-traits. For example, our model provides an alternative explanation for the high T_{opt} that is commonly observed for soil bacterial communities, due to the breadth and maximum growth rate dependence of thermal niches. We address current knowledge gaps that could further improve these types of models. This in turn could help to tackle uncertainty in the feedback of soil carbon stocks to climate change.

Introduction

Bacterial species differ in their thermal niche, which can be described by temperature-related traits such as the temperature range or optimal temperature at which they can grow. Bacterial communities are comprised of bacterial species with different temperature traits (Yung et al., 2015; Wang et al., 2021; Smith et al., 2022). This distribution of temperature-traits within soil bacterial communities differs across climatic regions. Bacterial communities from cold ecosystems show a higher abundance of cold-responsive species than warm ecosystems (Wang et al., 2021). This indicates that soil temperatures will filter the bacterial community for bacterial taxa based on their temperature-traits and their performance under the corresponding soil climate (Bárcenas-Moreno et al., 2009). Accordingly, the temperature response of soil bacterial communities differs between climatic regions in terms of both growth and respiration (Bååth, 2018; Dacal et al., 2019; Wang et al., 2021). Therefore, the relationship between temperature and growth of bacterial communities might be predicted based on climate records, which is useful for predicting the response of soil bacterial communities and the biogeochemical cycles they mediate to future climate change (García-Palacios et al., 2021).

One line of evidence for the climatic influence on the temperature trait distribution of soil bacterial communities comes from studies showing that the relationship between temperature and the growth of soil bacterial communities correlates strongly with the their corresponding local climate (Bååth, 2018). This temperature adaptation of soil bacterial communities has also been observed to change after alterations of the temperature regime e.g. following experimental temperature manipulations, or transplant experiments (Bárcenas-Moreno et al., 2009; Rinnan et al., 2011; Rousk et al., 2012; Birgander et al., 2018; Nottingham et al., 2021). These changes often co-occur with a change in the relative abundance of different taxa within the bacterial community (Donhauser et al., 2020; Nottingham et al., 2022; Chapter 3; Weedon et al., 2022). This implies that altering soil climates will alter the temperature adaptation of soil bacterial community due to changes in the relative abundances of soil bacterial taxa that differ in their temperature-traits.

Besides the empirical link between climate and the temperature adaptation of soil bacterial communities, the mechanisms that drive the distribution of temperature traits in soil bacterial communities are so far unknown. Understanding these underlying mechanisms would help to explain patterns in climate-temperature

adaptation relationships and help to support reliable projections of changes under novel climatic conditions. One common observation is that the aggregate optimal growth temperature (T_{opt}) of soil bacterial communities reaches far above the *in situ* maximum soil temperature (Donhauser et al., 2020; van Gestel et al., 2020). For example, soil temperatures in subarctic soils typically do not reach above 12 °C at 5 cm depth, while soil bacterial communities sampled from the same depth show growth optima > 25 °C (Chapter 3). Maximum soil temperatures correlate strongly with temperature adaptation of soil bacterial communities (Chapter 2), which could indicate that soil bacteria are selected for the survival of periodic heatwaves. However, it has been observed that soil bacterial communities exhibit a broad distribution of thermal traits, including both species that grow best at low or at high temperatures (Wang et al., 2021; Smith et al., 2022). Therefore, it is unlikely that the maximum observed soil temperature solely explains the distribution of temperature-traits in soil bacterial communities. Consequently, other mechanisms, such as seasonality of the soil climate or difference in maximum growth rate between thermal niches, are likely to contribute to selection of bacterial thermal niches by soil climates. To model the temperature adaption of soil bacterial communities it is therefore necessary to include all possible mechanisms that influence their temperature trait distributions.

One possible explanation for the mismatch between community T_{opt} and *in situ* soil climate is the fluctuating nature of temperature. Soil temperature varies across short (diurnal) and long (annual) time-intervals, in contrast to other environmental factors that might drive community assembly such as pH, or organic carbon content. It could therefore be that temperature fluctuations promote the coexistence of bacterial species differing in temperature-traits. For example, in the ocean warm and cold adapted planktonic bacteria co-exist through fluctuating abundance between seasons (Yung et al., 2015). Recently, it has been shown that soil bacterial communities also shift in community composition amongst seasons (De Gruyter et al., 2019; Carini et al., 2020; Poppeliers et al., 2022). If fluctuating temperatures in soil also allow for the presence of multiple thermal niches in soil bacterial communities, this mechanism can influence the composition of the bacterial community and thereby the distribution of temperature traits in soil bacterial communities.

A second potential mechanism driving the mismatch in T_{opt} and climate might be the positive correlation between the maximum growth rates of organisms and

their optimal growth temperature (Figure 5.1b-d; Corkrey et al., 2016; Dell et al., 2013; Gillooly et al., 2001; Smith et al., 2019). This correlation is often referred to as the 'hot is better'-hypothesis and is likely imposed by thermodynamic constraints to growth (Angilletta et al., 2010). While the coefficient for this universal temperature dependence (UTD) of maximum growth rate varies between studies (Eppley, 1972; Gillooly et al., 2001; Kremer et al., 2017; Smith et al., 2019), there is a clear evidence for the hypothesis that species with higher optimal growth temperatures exhibit higher maximum growth rates. Overall, this indicates that warm-adapted species exceed the growth rate of cold adapted species at temperature below the optimum of both, which influences the relative performance of thermal niches within a bacterial community.

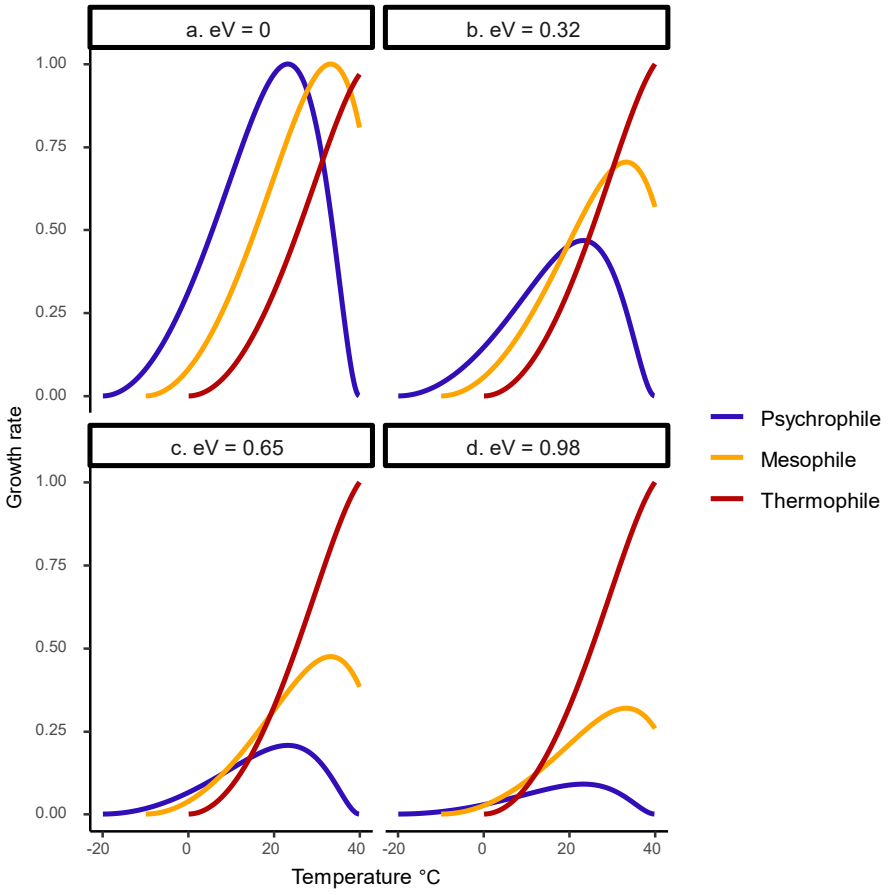


Figure 5.1 Temperature growth ranges from three temperature traits in the described models, here depicted under various eV values.

For synthetic bacterial communities it has recently been shown that the abundance of bacteria varying in thermal niches is influenced by environmental temperatures (Garcia et al., 2022). These results indicate that climatic variables might influence the distribution of temperature-traits and could thereby affect the temperature adaptation of bacterial communities. By simulating the growth over a typical soil temperature record for hypothetical species spanning a large range of thermal niches – from psychrophile to thermophile – it might be possible to predict the resulting temperature-trait distribution based on the relative performance of each species. The predicted temperature-trait distribution can then be used as a proxy for the overall temperature response of soil bacterial communities. Such predictions would also allow the exploration of several hypothesized mechanisms - such as variable thermal regimes or UTD of maximum growth rate - that might explain the temperature adaptation of soil bacterial communities. If indeed, such a model would provide accurate predictions of the temperature adaptation of soil bacterial communities, it could also provide predictions under various climate scenarios, including e.g., extreme weather events or disproportional warming in winters. In turn, this theoretical framework would provide testable hypotheses for the predicted traits of the community and its members under a given soil climate, and therefore partially overcome experimental limitations due to e.g. hampered cultivation of species within a soil bacterial community (Steen et al., 2019).

In this study, we explored whether current knowledge about the UTD of maximum growth rate, and the distribution of bacterial temperature-traits, can be combined with soil temperature records to predict the temperature adaptation, expressed as T_{opt}, of soil bacterial communities. To do this we modeled the growth of theoretical species varying in their thermal niches to evaluate the performance of each species as a function of temperature. Combining this performance data with the parameters of each species' thermal niche allowed us to compute the aggregated growth curve of soil bacterial community for a given temperature time series. We parameterized this model with previously published data on soil temperature records and corresponding growth curves of bacterial communities. Finally, we assessed the broader applicability of our approach by comparing model

predictions of temperature adaptation at local to global scales to observations from previous studies.

Methods

Modeling approach

In this study we constructed the temperature-trait distribution of soil bacterial communities by modelling the growth of species with different thermal niche as solely dependent on temperature over annual records. From this temperature trait distribution, we constructed the predicted community growth curve (Figure 5.2).

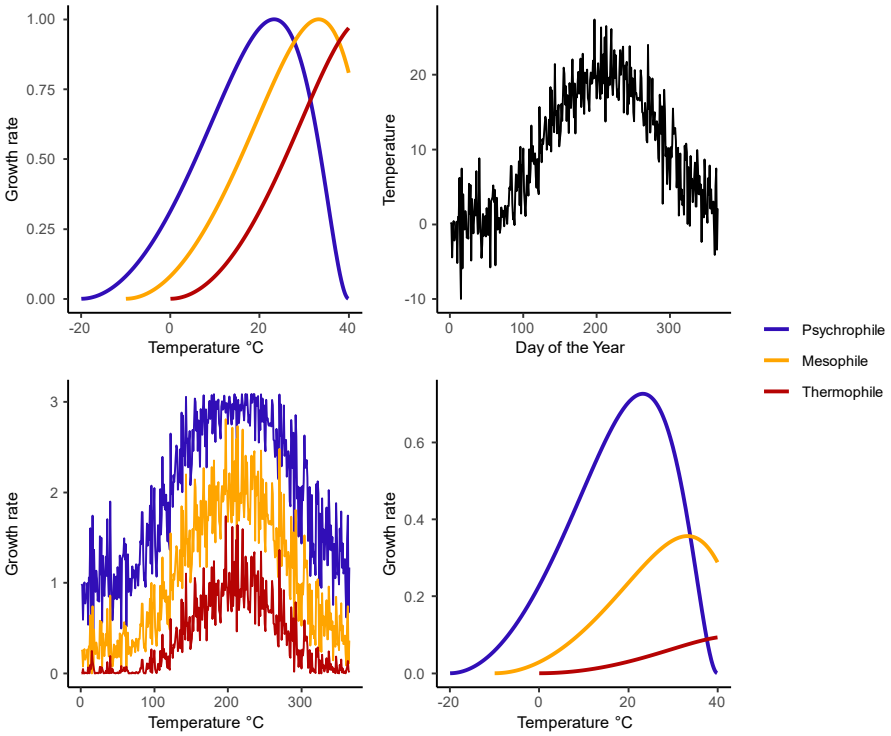


Figure 5.2 Projection of the temperature response for various thermal niches (A) over the annual soil temperature record (B) to predict their growth across the year (C) and predict their relative performance on a community level (D).

To do so, we first modelled the temperature-trait distribution by calculating annual growth of species with t_{opt} varying between -20 and 80 °C (Equation 5.1). To define the growth curve of each individual species' thermal niche (equation 5.4), we kept

the slope parameters constant and fixed t_{opt} at 70 % of the total thermal breadth (equation 5.2 & 5.3; Table 5.1).

For the i'th species in a total number of species set by the parameter *n,* the temperature traits were defined with equations 5.1 to 5.3. We divided the T_{opt} values evenly along the interval -20 to 80:

$$\mathrm{T}_{\mathrm{opt}_i} = -20 + \frac{100}{n}\, i \qquad (5.1)$$

in which t_{opt} is optimal growth temperature in ° C for species *i* (an integer valued index from 1 to n) and n is the total number of species in the model. The other thermal traits were given by:

$$\mathrm{t}_{min_i} = \mathrm{t}_{\mathrm{opt}_i} - 0.7\, t_{breadth} \qquad (5.2)$$

$$\mathrm{t}_{max_i} = \mathrm{t}_{\mathrm{opt}_i} + 0.3\, t_{breadth} \qquad (5.3)$$

in which t_{max} is maximal growth temperature. in which t_{min} and t_{max} are minimal and maximal temperature for growth and $t_{breadth}$ is a parameter defining the size of the temperature range over which a species can grow (t_{max} – t_{min}).

Table 5.1 Fixed parameters used in our model for equations 4 & 5

description	*parameter*	*values*	
slope parameter	**a**	0.05	
slope parameter	**b**	0.01	
optimal growth temperature	$\mathbf{T_{opt}}$	-20 - 80	°C
minimal growth temperature	$\mathbf{T_{min}}$	T_{opt} - 0.7 * $t_{breadth}$	°C
maximum growth temperature	$\mathbf{T_{max}}$	T_{opt} + 0.3* $t_{breadth}$	°C
base rate	**B0**	1	
Boltzmann constant	**k**	$8.617 * 10_{-5}$	eV K^{-1}

We calculated for each species the growth per day based on their corresponding temperature dependence of growth (see section 5.2.2). To calculate the distribution of the thermal niches, we calculated the fraction of annual growth for each individual species relative to the summed annual growth all species. To calculate the overall community temperature response, we multiplied the growth of each species by its corresponding fraction for temperatures ranging from -20

to 80 and then summed the total growth of the community at each temperature step (section 5.2.2). There were multiple tunable parameters, such as the dependence of maximum growth on t_{opt} (*eV*) and thermal breadth of the species ($t_{breadth}$), for which we ran a sensitivity analysis (section 5.2.3) and estimated the parameters that lead to best fits with existing datasets (section 5.2.4). Finally, we validated the model on the measured temperature adaptation of soil bacterial communities from previous studies not used in the parameterization and tested the accuracy of the model across a global scale (section 5.2.6).

Modelling the temperature dependence of bacterial growth

We used the Ratkowsky model to compute the microbial growth of bacterial species varying in temperature traits, as formulated in equation 5.4.

$$r_i(\mathrm{T}) = \sqrt{\mathrm{growth}_i(\mathrm{T})} = \mathrm{z}_i \mathrm{a}(\mathrm{T} - t_{min_i})(1 - e^{\mathrm{b}(\mathrm{T} - t_{max_i})}) \qquad (5.4)$$

in which r is the bacterial growth rate, T is the temperature in °C, t_{min} is the minimum theoretical growth temperature, t_{max} the maximum growth temperature, b and c are slope parameters; z is an additional parameter for the dependence of maximum growth rate on the optimal growth temperature for species *i*. For the slope parameters we set a at 0.05 and b at 0.1, where a is a dummy variable and b was estimated over previously published data (https://doi.org/10.6084/m9.figshare.19516777.v1). The dependence of maximum growth on optimum growth temperature was determined by z. We modeled the increase of the maximum growth rate with optimal growth temperature following the Arrhenius-Boltzmann equation (equation 5.5), such that z is equal to 1 for species with Topt of 10 °C.

$$\mathrm{z}_i = \mathrm{B0}\ \mathrm{e}^{-\mathrm{Ek}t_{opt_i}} \qquad (5.5)$$

where z is the growth rate at t_{opt}, E as the eV value, B0 is the base rate of 1, k is the Boltzmann constant (8.617 *10^{-5}eV K^{-1}) and t_{opt} the optimal growth temperature in Kelvin (see Table 5.1 for choices of constants and ranges for parameterization). B0 was dependent on the eV value and chosen to fit z = 1 for a T_{opt} value of 10°C.

Estimating the community response

We calculated the growth of each species over a temperature record following equation 5.4 and assessed the performance of the species by calculating the total growth m over the entire time interval d.

$$m_i = \sum_{j=1}^{d} r_i(t_j) \quad (5.6)$$

In which m is the total growth of a bacterial species summed for all daily soil temperatures t over the timepoints *j*, within the given time period d and the species-specific growth function is given by r (equation 5.4). We then calculated the participation factor of each temperature trait p by dividing the total annual growth of each species by total annual growth of the community.

$$p_i = \frac{m_i}{\sum_{i=1}^{n} m_i} \quad (5.7)$$

The temperature growth response curve for each species was multiplied by the participation factor at a given temperature from 0-40 °C, after which the participated growth curves were summed to obtain the community growth curve.

$$c(T) = \sum_{i=1}^{n} p_i \mathrm{r}_i(T) \quad (5.8)$$

Where c(T) is the community growth at temperature T.

Sensitivity analysis

We ran our model on the artificial temperature records for 1, 2, 3, 5, 9, 27, 100, 500 and 1000 number of species (*n*) to test whether the model behavior is stable in relation to species number. We ran the model at for values of 0, 0.32, 0.65, 0.98 for the dependence of maximal growth on optimal growth temperature and species' thermal breadth ($t_{breadth}$) for values between 10 and 60 °C with 10 °C interval (Table 5.2). We simulated an artificial daily temperature record for 1 year with a mean of 10 °C using equation 9.

$$\mathrm{T} = 10\sin(2\pi \mathrm{d}/365 - 90) + 10 + \mathrm{b} \quad (9)$$

where T is temperature in °C, d is day of the year, and b is added the standard deviation (s.d. = 3). We compared the total breadth, optimal temperature and width at 50% growth rate for the predicted community temperature response.

We then performed sensitivity analysis on the temperature scale of the input data, by calculating T_{opt} resulting from the artificial temperature record over *d*-values 1, 7, 28, 72, 156, and 365 days. We then determined the amplitude of the change in T_{opt} as a measure of sensitivity.

Parametrization of the coefficient for UTD of maximum growth rate and species thermal breadth

We performed a parameterization to determine which *eV*-value for the dependence of maximum growth rate on the optimum growth temperature and $t_{breadth}$-value lead to modeled growth curves that best agreed with previously measured community growth curves. We used a model with 100 species varying in T_{opt} between -20 and 80 °C (equally distributed) to predict the community response using the method described in section 5.2.2. There are only few studies that published datasets that include soil temperature records and bacterial community growth curves along large spatial gradient. In this study we used a previously published dataset on bacterial community-growth curves and temperature records from (sub-) arctic soils (Chapter 2) as a training dataset to parameterize our model. We ran the model for each soil temperature record of the training dataset, while varying values for dependence of maximum growth rate on the optimal growth temperature over *eV* between 0 and 1.5 (Gillooly et al., 2001), by steps of 0.01 and the thermal breadth of the species ($t_{breadth}$) between 10 and 60 by steps of 2 (Table 5.2). Next, we calculated the mean residual between the modeled and measured growth curves for each temperature record between 0 and 40 °C. For each soil in the training dataset, we selected best parameters based on the lowest mean residual. Final parameters of the model were chosen based on the mean parameters across the best parameters of each individual soil temperature record.

Validation of the final model

To test general use of our model across other biomes, we validated the model using the few other datasets that have published growth curves of soil bacterial communities and soil temperature records (Rinnan et al., 2009; Rousk et al., 2012; Birgander et al., 2013, 2018). For each dataset we extracted the daily soil temperature and community growth curves from the supplementary material of the paper or by extracting datapoints from the figures using Webplotdigitizer (Rohatgi, 2021). We then predicted the community growth curve using our model based on the soil temperature records. We calculated the estimation error of the predicted community growth curves compared to measured growth curves for each 0.1°C temperature step between 0-40°C as well as the estimation error between predicted and measured T_{opt}.

Since very few datasets are available that contain community growth curves of soil bacterial communities as well as soil temperature records, we evaluated whether our model is useful on a global scale using the LTER soil temperature dataset and compared the predictions of T_{opt} by our model to previously published estimates of climate-T_{opt} relationships (Rinnan et al., 2009; van Gestel et al., 2013). To estimate the variation a global scale, we used soil temperature records from 13 sites in the Long-Term Ecological Research network that reaches across North America, from desert shrublands to boreal forests (Supplementary Table 5.1; Anderson, 2022; Andrus, 2022; Chapin et al., 2022; Collins, 2021; Daly & McKee, 2022; Doran & Fountain, 2022; Groffman et al., 2022; Jesse, 2022; John J. et al., 2022; Ramirez, 2022; Robertson, 2020; Seeley, 2021) .

Results

Sensitivity analysis of the model

Sensitivity analyses were performed to assess the effect of varying the number of species (*n*) and thermal breadth ($t_{breadth}$) values. This showed that model predictions of the growth curve were unstable when only few numbers of species were used, $n < 9$ (Figure 5.3a). For example, at $t_{breadth}$ = 10°C for temperature-traits bi- or multimodal community growth curves were observed for less than 27 species (Supplementary Figure 5.2). Predictions for T_{opt} of the community were stable when 100 or more species were used in the model (Figure 5.3a; Table 5.2). The absolute thermal breadth of the community was dependent on $t_{breadth}$ and did not differ between specified *eV*-values (Figure 5.3c). In contrast, the width of the predicted community growth curves, here expressed as the range between the two temperature points where the growth is 50% of the maximum growth, increased with larger $t_{breadth}$ and *eV* values (Figure 5.3d).

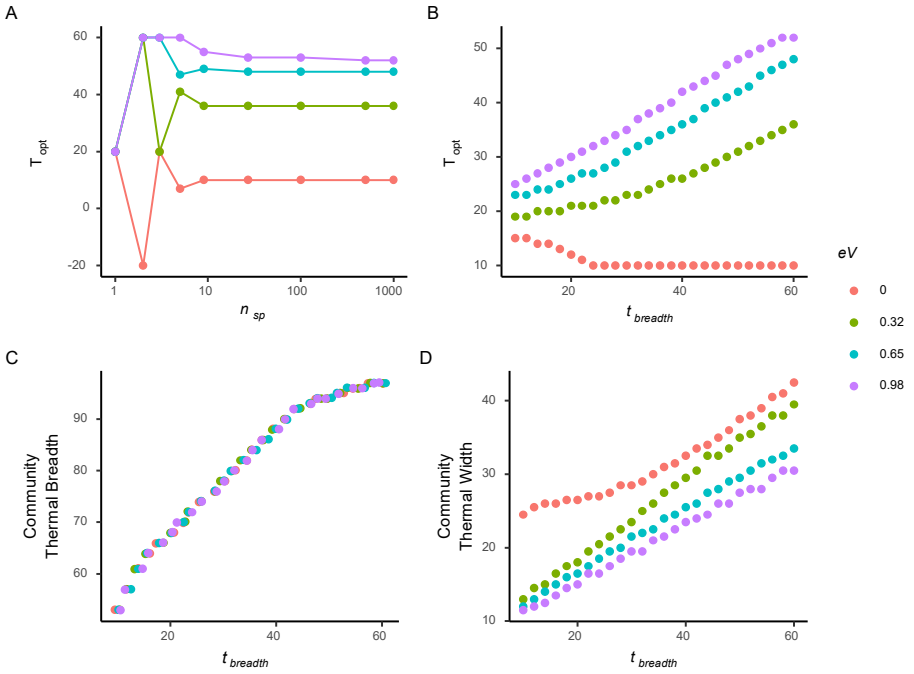


Figure 5.3 Sensitivity of the model output in terms of computed a) optimal growth temperature at species' thermal breadth of 60°C, and, b) optimal growth temperature, **c)** thermal breadth and **d)**

width of the predicted community growth curves over varying values for $t_{breadth}$ and for multiple *eV* values at n = 100.

Table 5.2 Respective range for the estimation of parameters during sensitivity analysis and parameterization over training set.

description	*parameter*	*values*	*step*	*final value*
number of species	***n***	1-1000		100
timeframe in days	***d***	1-365		365
thermal breadth of individual species	$\boldsymbol{t_{breadth}}$	10-60	2	46.7
dependence of maximum growth on T_{opt}	***eV***	0-1.5	0.01	0.69

The model predictions of T_{opt} were relatively sensitive to $t_{breadth}$ (Figure 5.3b). Only at *eV* of 0 the predicted T_{opt} was stable between $t_{breadth}$ values of 21 and 60 °C. In contrast, the predicted T_{opt} differed between *eV* 0.32 and 0.98, but this variation between *eV* values was relatively small and stable across a range of $t_{breadth}$ (at $t_{breadth}$ 60°C T_{opt} varied between 36 and 52°C). Since the resulting community growth curves and corresponding Topt-values were dependent on the *eV* and $t_{breadth,}$ we included $t_{breadth}$ and *eV* for parameterization of the model over the training dataset.

Over the simulated temperature data of equation 5.3, the model predicted a stable T_{opt} 35 °C with no amplitude for a timeframe of 365 days. For shorter time frames, the amplitude of T_{opt} predictions varied between 7 °C for 156 days and 15.5 °C for 1 day as timeframe (Figure 5.4), with T_{opt} varying between 11 and 40 °C for 1-day timeframes.

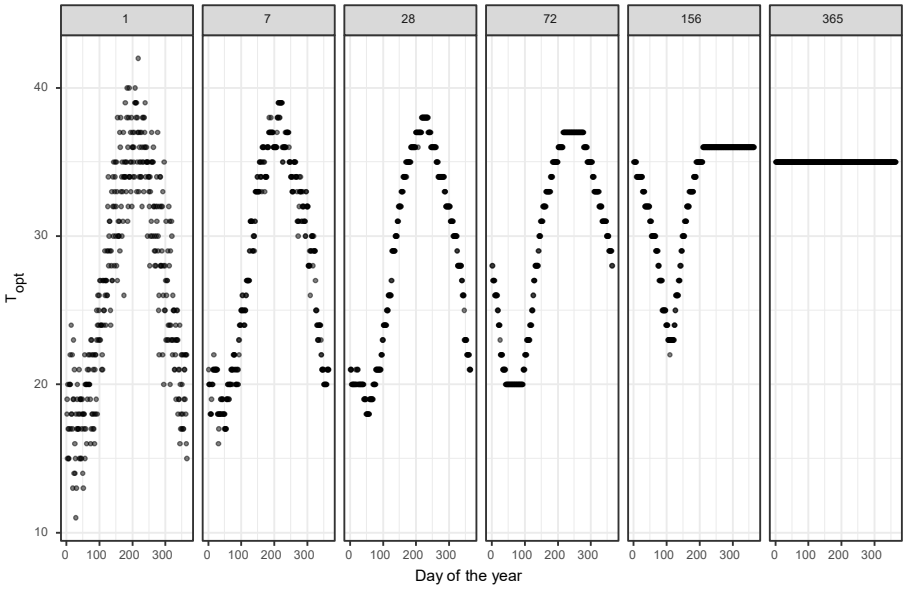


Figure 5.4 Sensitivity analysis for the number of days used from for the records on daily soil temperature to determine Topt value of the community growth curve. Individual points indicate the T_{opt} calculated for each day, amplitude in T_{opt} values shows the relative sensitivity to annual temperature fluctuations.

Parameterization of the final model

Given the sensitivity of the model to the *eV* and $t_{breadth}$ parameters, we estimated their values using data from 11 sites across the (sub) arctic. Parameters values for eV and $t_{breadth}$ that gave the lowest RMSE for each individual site varied between 0.54 and 0.79 for the *eV* value (mean = 0.69) of the dependence of maximum growth on optimal growth temperature (Figure 5.5a). For $t_{breadth}$ the optimal parameters varied between 40 and 54.4 between the sites (mean = 46.73; Figure 5.5a). The mean of the best fitted values of *eV* and $t_{breadth}$ were used in our final model. Across the training datasets of temperature curves the RMSE of the final model was 0.02 on average. The predicted T_{opt} from the final model was on average 0.38 °C higher than the T_{opt} of the measured community growth curves with a standard deviation of 2.17 (Figures 5.5b & 5.7).

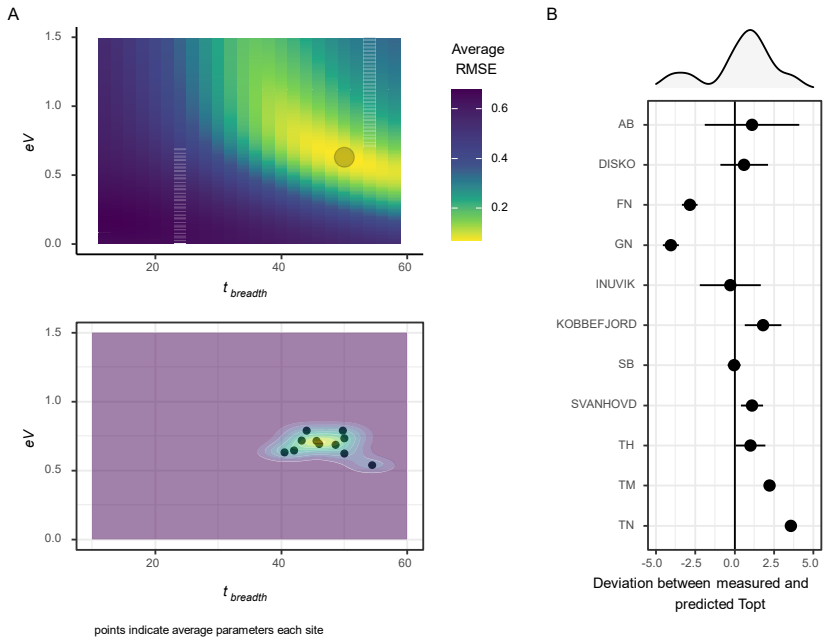


Figure 5.5 a) Upper panel shows the average RMSE across all community growth curves of the training dataset across set of *eV* and *t_breadth* values. Lower panel shows a 2 dimensional density plot of the best fitted parameters for each individual sampling site based on lowest RMSE. **b)** estimation error in the predicted Topt and the measured Topt for each sampling site (error bars indicate standard deviation amongst annual soil records used)

Validation of model

For the temperature records of the previously published community growth curves, the final model showed a mean prediction error of 3.34 (s.d. = 4.8) for T_{opt} and mean RMSE varying between 0.14 and 0.28 for the temperature-growth curves between different studies (Figure 5.6). Within the validation set, multiple studies included experimental warming sites for which the final model predicted no difference in Topt between control and 1 year of winter warming for Birgander et al., (2018), but an increase from 37 to 42 °C in T_{opt} was predicted under +5°C warmed conditions for Rousk et al., (2012) (Supplementary Figure 5.3).

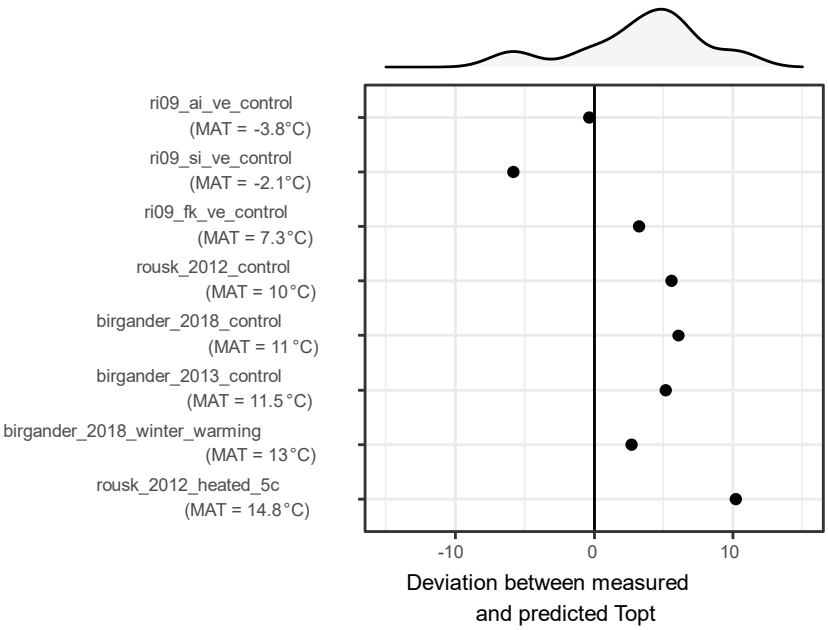


Figure 5.6 Deviation between the predicted and measured Topt for community growth curves of the bacterial communities extracted from literature, ranked at increasing Mean Annual Temperatures (MAT).

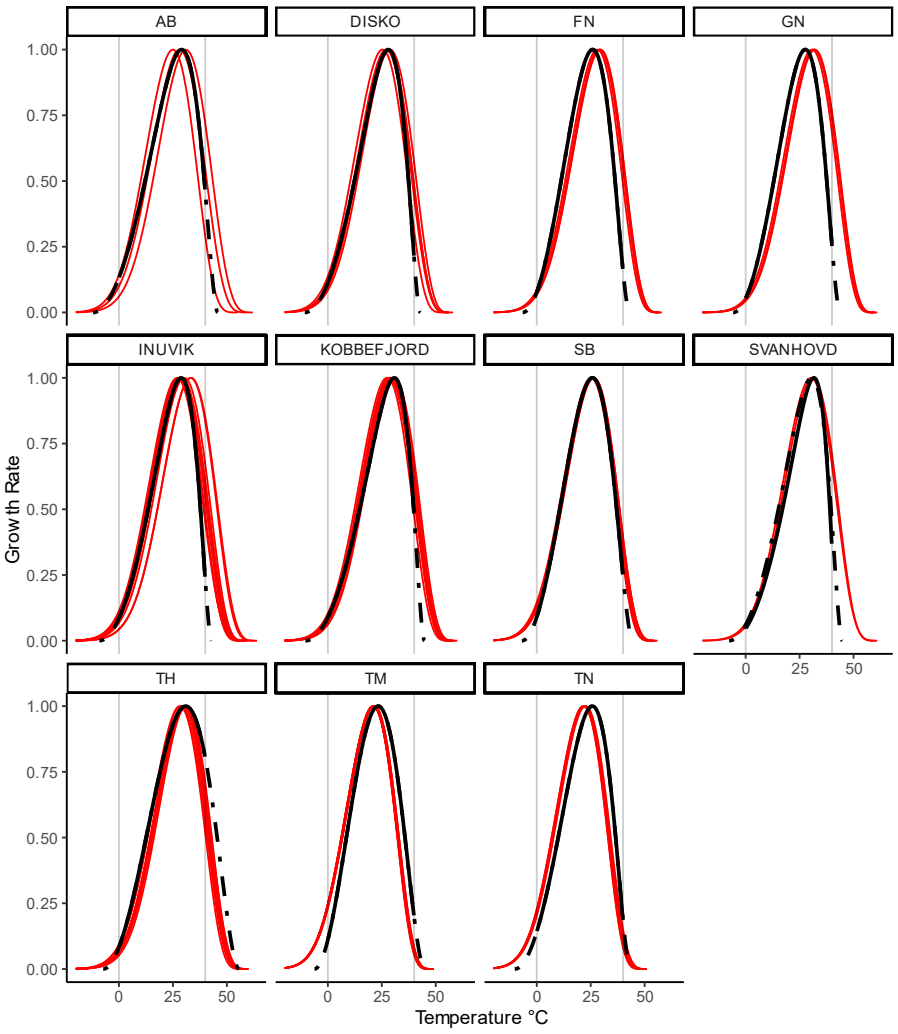
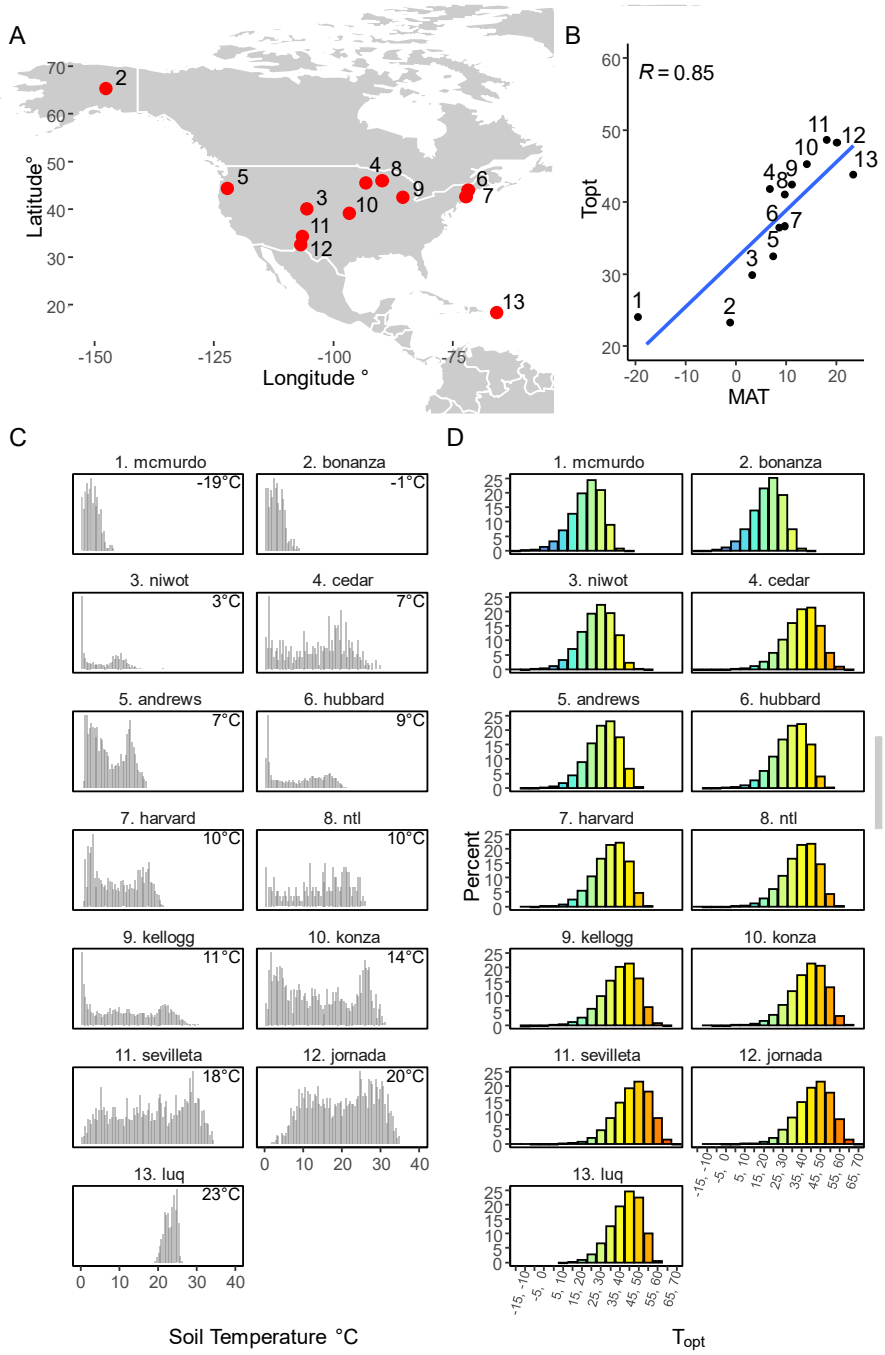


Figure 5.7 Measured and the predicted community growth curves for the soil bacterial communities of the training data set (Chapter 2; Table 2.1), respectively in black and red. Grey vertical lines and solid black lines indicate the range over which the community growth curves were measured.

Assessing model performance over large geographical scales

Across the temperature records of the LTER sampling sites we found the predicted T_{opt} to vary between 22 °C in the boreal forest at Bonanza Creek and 50 °C at desert

shrublands in Jornada. Overall, T_{opt} was predicted to increase 0.67 °C per °C in MAT (Figure 5.8b). The distribution of temperature traits followed a unimodal function typically ranging over 40 ° C of T_{opt} values with the group of species exhibiting a T_{opt} > 20°C above the mean annual temperature of the soil comprising the largest part of the community (Figure 8d). The predicted temperature-trait distribution differed between the sites. For example, at desert shrublands at Jornada site the model predicted species with T_{opt} 50 - 55 °C to be most abundant (21.1 %), while for Antarctic dry valleys of McMurdo species with T_{opt} 20 - 25 °C were most abundant (24.3 %) (Fig. 5.8c). Mean annual temperature was not predictive of the temperature-distribution of soil bacterial communities. For example, the predicted temperature-trait distribution for Harvard (MAT 9.8 °C) showed an abundance below 1% for species with T_{opt} > 55, where Kellogg (MAT 11.2 °C) showed abundance for these species present of 4.7 %.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2. bonanza
-1°C
3. niwot
3°C
4. cedar
7°C
5. andrews
7°C
6. hubbard
9°C
7. harvard
10°C
8. ntl
10°C
9. kellogg
11°C
10. konza
14°C
11. sevilleta
18°C
12. jornada
20°C
13. luq
23°C
Soil Temperature °C
D
Percent
Topt

Figure 5.8 Overview of sampling sites in North America (1. McMurdo is located in Antarctica and not displayed), **B)** Topt predicted by the final model over the mean annual soil temperature of all LTER sites. **C)** Distribution of soil temperature above 0° for LTER sites and mean annual soil temperature in top right corner. **D)** Predicted distribution of species abundance (%) varying in optimal growth temperature for LTER sites. Each bar represents the predicted relative abundance of taxa within 5 °C intervals of T_{opt}.

Discussion

In this study we presented a novel approach that shows how knowledge about potential thermal niches of soil bacteria can be used for making predictions about the distribution of temperature traits in soil bacterial communities. We developed a simplified trait-based model for estimating the temperature-growth relationships of soil bacterial communities based on soil temperature records using only a few assumptions about thermal niches within soil bacterial communities. The predictions of our model produced similar features compared to previously measured community temperature-growth responses. However, our model tended to overestimate the optimal growth temperature for warm environments. First, we will discuss the underlying assumptions of our model approach and estimated parameters of our model. Then, we will evaluate the accuracy and limitations of our current model. Last, we address knowledge gaps that could improve our model and contribute to improved understanding of the temperature adaptation of bacterial communities to climate change.

The coefficient (eV) for the universal temperature dependence of maximum growth rate

Our model included an explicit assumption about the dependence of species-specific maximum growth rates on the corresponding optimal growth temperature (equation 5.5). Parameterization of the model showed that no dependence ($eV = 0$) led to a poor fit of the predicted community growth curves to the training dataset. Instead, an eV value of 0.69 was estimated, which shows support for the 'Hot is Better'-hypothesis. This eV value was closer to the eV value proposed by the Metabolic Theory of Ecology (0.6-0.7; Gillooly et al., 2001; Savage et al., 2004) than to the values estimated from recent empirical data for mesophilic heterotrophic bacteria (eV 0.98; Smith et al., 2019). Approaches such as our model, which estimate the Topt-μ dependence using whole community data growing in *in situ* conditions may contribute to the explanation of this discrepancy between

studies. Specifically, since our model shows a lower *eV*-value for the Topt-μ dependence, it could be the case that the Topt-μ dependence of soil bacteria is lower than what is currently estimated using datasets from experiments with growth data primarily from bacterial isolates (Smith et al., 2019).

Predicted thermal breadth of temperature-traits

A second assumption of our model was that the community growth curve is an abundance-weighted sum of the growth curves of its individual members. Parameterization showed that the model was best fitted to the measured growth curves when species-level thermal breadth was 46 °C across the training set, which is within the range of thermal breadth measurements obtained from bacterial isolates (17-51°C; Ratkowsky et al., 1983). At the aggregated community level, the thermal breadths predicted by the model were wider (ranging from 68 to 80°C) than both the thermal breadth measured for bacterial communities of (sub-) arctic soils in the training dataset which showed a thermal breadth of 49 - 53°C, as well as those obtained in previous studies used for validation (Figure 5.6; Birgander et al., 2013, 2018; Rinnan et al., 2009; Rousk et al., 2012; van Gestel et al., 2013). This discrepancy could be attributed to the difference in determination of the thermal breadth between lab assays as compared to the approach used to determine this using our model. For measured community growth curves the Ratkowsky equation is fitted, after which the thermal breadth can be calculated from the range between extrapolated cardinal points T_{min} and T_{max}. In contrast, the community growth curve predicted in our model is not strictly a Ratkowsky relationship (equation 5.4), but rather a summation of the temperature growth curves for all species. This can lead to a broader breadth of the community growth curve in the model, especially when a wide range of thermal traits are present in the modeled community. This mismatch between model and experimental data can be partly resolved by extrapolating the cardinal points T_{min} and T_{max} for the predicted community growths over the range that growth curves are typically measured which narrows the predicted thermal breadth (Supplemental Figure 5.4). Since the RMSE of predicted community growth curves was calculated over the temperature range where temperature growth curves were measured (0 – 40 °C), the parameterization of the model was unaffected by the artificially large thermal breadth of communities predicted by our model.

The sensitivity analysis on the model highlighted that the performance of species with different temperature-traits was highly dependent on $t_{breadth}$. This can be

explained by the fact that the thermal breadth is directly related to temperature at which a certain thermal niche is most competitive relative to other thermal niches. For example, when the thermal breadth is narrow (e.g., < 30 °C), thermophilic species (T_{opt}>40°C) grow slower than psychrophilic species at low temperatures regardless of the T_{opt}-μ dependence (Supplementary Figure 5.1a). As thermal breadth increases, there is an increasing overlap in the temperature range in which cold and warmed adapted species can both grow. At eV-values >0 and a wide thermal breadth of e.g., 60°C, a species can competitively outperform other species at temperatures below its T_{opt}. Therefore, there is a mismatch between the temperature at which a species outcompetes other species and its own optimal growth temperature (Angilletta, 2009; Martin & Huey, 2008). In our model the $t_{breadth}$ and *eV* value induce a mismatch for all species of roughly 10°C between the temperature of their maximal relative fitness (i.e., growth higher than all other species) and their and optimal growth temperature (Supplementary Figure 5.1b). This means that for a soil temperature of 30°C the fastest growing bacteria have a T_{opt} of ~40°C. This emergent property of our model might explain the commonly observed phenomenon of an optimal growth temperature of soil bacterial communities far above their climate regime (van Gestel et al., 2020). Previously, it has been proposed that high T_{opt} of soil bacterial communities might be explained by a thermal-safety margin of individual species (Bárcenas-Moreno et al., 2009; Birgander et al., 2013). This means that species would die due to heat-related shock when they are exposed to temperatures above their optimal growth temperature (Deutsch et al., 2008). This emergent mismatch provides an alternative explanation for the high T_{opt} of soil bacterial communities other than the thermal safety margin of soil bacterial species.

To estimate the relative fitness of thermal niches it is relevant to know whether there are constraints posing limits to thermal breadth in bacterial species. In our model we assumed that thermal breadth was equal across all species, but this is not realistic (Ratkowsky et al., 1983). It has been hypothesized that a larger thermal breadth will reduce the maximum growth rate. This "jack of all trades is master of none" hypothesis has been refuted for most ectothermic macro-organisms (Huey and Hertz, 1984), but an evolutionary tradeoff between maximum growth rate and thermal breadth might exist for bacteria (Mongold et al., 1996; Bennett and Lenski, 2007). So far, such a tradeoff has not been observed for bacterial taxa isolated from soils (Smith et al., 2022). However, if general patterns between thermal

breadth and maximum growth rate exist, implementation of this tradeoff could improve the accuracy of the model.

Accuracy of the model

Our model was able to capture the shape of measured community growth curves when inspected visually for the training dataset (Figure 5.7). As an alternative to estimating temperature-growth relationships with this first-principle model, predictions about the T_{opt} of the soil bacterial communities across the Arctic can be made based on the statistical relationship between the T_{opt} of soil bacterial communities and maximum daily soil temperature (Chapter 2). When comparing the two approaches, the statistical model had a smaller estimation error than our model for predicting T_{opt}, with a standard deviation of residuals of 1.83 and 2.17 respectively. However, our model provides accurate estimates for growth over the entire temperature range, which is beneficial for modeling the growth of soil bacterial communities under varying conditions.

Model performance over large geographical scales

On the global scale, our model predicted an increase in T_{opt} of 0.7 °C per °C MAT, which is higher than previously suggested by Bååth (2018; 0.3 °C per °C MAT), but within the range of previous estimates (Chapter 2; Birgander et al., 2013; Rinnan et al., 2011). This might be explained by the relative performance of the model amongst climatic zones. For example, our model predicted T_{opt} of 24 °C for the McMurdo valley in Antarctica, which matches the with measured T_{opt} of 24 -28 °C for Antarctic soil bacterial communities (Rinnan et al., 2009). At the other extreme, the model predicted the highest T_{opt} for the Jornada LTER site in the Chihuahuan desert (54 °C). Previous work has shown a T_{opt} of 42.9 °C for soil bacterial communities at 0-5 cm depth from the Big Bend National park in the Chihuahuan desert, roughly 550 km away from Jornada (van Gestel et al., 2013). The large deviation between predicted and measured T_{opt} for warmer soils was also observed for the validation dataset, for which temperate soils showed a larger estimation error for T_{opt} and RMSE than the colder soils (Figure 5.6). As the model parameterization was performed exclusively on (sub-) arctic soil bacterial communities, there is a large risk that the model was overfitted to cold environments.

The reduced accuracy of our model for temperate and warm soils might be explained by the assumption that maximum growth rate increases monotonically

over the entire span of optimal growth temperatures used in the model. In the model T_{opt}-values of the species varied from -20 to 80 °C with increasing maximum growth rates along the entire range. However, for thermophilic bacteria (T_{opt} > 40 °C) there is evidence for equalization of fitness across these thermal niches, such that there is no increase in maximum growth rate with higher T_{opt}-values (Corkrey et al., 2016; Smith et al., 2019). If this is the case, our model overestimates the performance of thermophilic bacteria, which will lead to an overestimation of T_{opt} in the predicted community temperature response. Moreover, our parametrization of the model might be affected by the discrepancy. The model parameterization was performed on (sub-) arctic soils, where it is likely that thermophiles contribute only very little to the community growth curve (Figure 5.8). We therefore believe that the *eV*-value was realistic for soils in our training dataset. For improvement of the accuracy of the model it will be important to include data from warmer soils and constrain the model parameters based on empirically based values for eV and the thermal breadth for the entire range of thermal niches as well as impose an accurate inflection point for the relationship between T_{opt} and maximum growth rate.

More realistic modelling

It is important to make accurate predictions of the temperature adaptation of soil bacterial communities to correctly estimate the soil carbon feedback to future climate conditions (García-Palacios et al., 2021). While our model provides a novel method for the estimation of the temperature adaptation of soil bacterial communities, there are still many ways to improve the accuracy of its temporal and global predictions. For example, implementing *in situ* measurements of the variance in species' thermal breadth as well as the coefficient and infliction point of Topt-μ dependence will be especially valuable to verify the parameters of our model for realism. This could be measured by taxon-specific stable isotope probing of soil bacteria (Koch et al., 2018).

The sensitivity analysis on the time window used in the model highlighted the limited ability of our current model to capture realistic seasonal dynamics for the temperature-growth curves of soil bacterial communities compared to other studies (Kritzberg and Bååth, 2022). Implementing realistic turnover rates of the bacterial species could potentially give insight into the time needed for soil bacterial communities to adapt to new soil climates after changes in temperature regime, and could be calibrated with data from transplantation experiments e.g.

(Nottingham et al., 2021). Current estimates of turnover times for bacterial community biomass vary largely from 2 to 169 days (Caro et al., 2022). The large deviation in growth rates might be explained by other environmental factors such as soil moisture, plant-soil interactions, pH, substrate quantity and quality (Rousk and Bååth, 2011), which means that implementing growth rates into our model would like require more environmental data about the soils.

Our model currently ignores many other important factors that could influence the growth of individual bacterial species such as species-interactions, substrate availability, dynamic soil variables and carrying capacity. These factors might affect the temperature adaptation of soil bacterial communities in case they influence the growth of certain thermal niche. One way to implement such factors would be via the use of generalized Lotka-Volterra models (Wade et al., 2016; Zaccaria et al., 2017; Abreu et al., 2019; Lax et al., 2020). We propose to explore the potential of species-interactions and carrying capacity to stabilize the overall community temperature adaptation across the year via modeling approaches. In turn, predictions from more dynamic models could be validated by experiments (Wilpiszeski et al., 2019) using synthetic microbial communities under varying climatic conditions. Recent studies show that the isolation of bacteria differing in temperature-traits from soil is possible by incubation of soils at different temperatures (Smith et al., 2022). Synthetic composure of microbial communities that consist of bacterial taxa that vary in temperature-traits (Garcia et al., 2022) can further elucidate how temperature adaptation of bacterial communities is influenced by biotic and abiotic factors (Burman and Bengtsson-Palme, 2021).

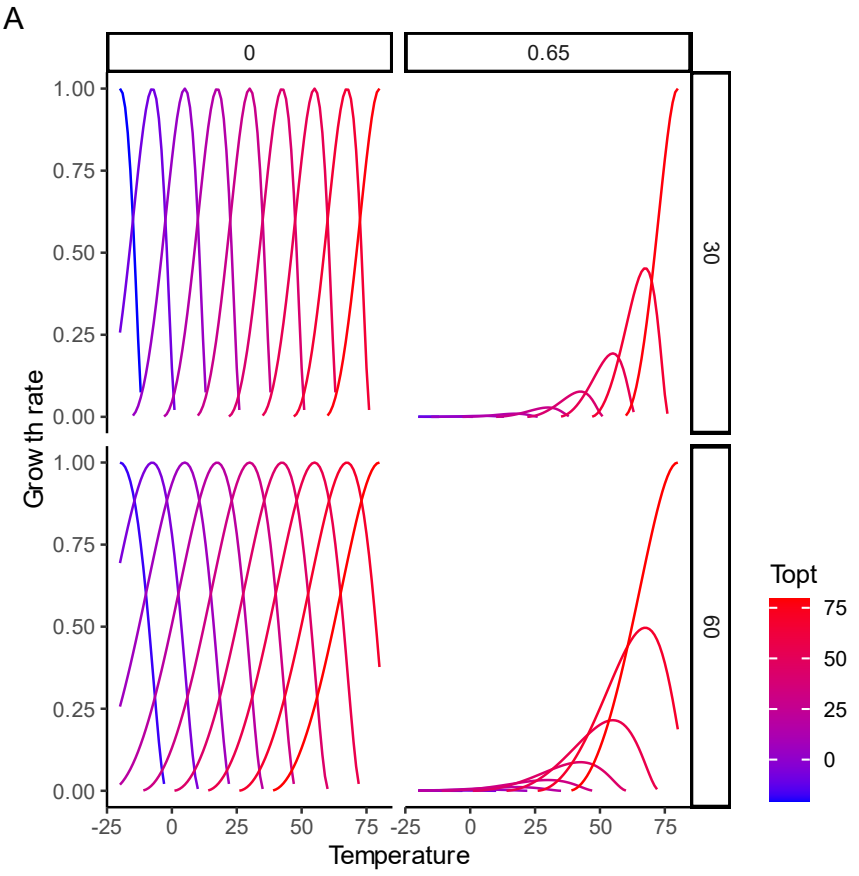
Conclusions

Our model provides a framework for the determination of the possible thermal niches that soil bacterial communities comprise and predict growth curves for soil bacterial communities based on times series of soil temperature. The parameterization of our model supported the 'Hot Is Better' hypothesis and thereby provides an alternative explanation for the high T_{opt} that is commonly observed for soil bacterial communities. While our model was unable to capture realistic seasonal fluctuations in the temperature growth curves of the soil bacterial communities, this could be achieved in future developments of the model by incorporating more realistic behavior by adding growth rates, competition and other environmental variables such as substrate availability and soil moisture.

Supplement

Supplementary table 5.1 Description of the sites used from the Long-Term Ecological Research Network. Nd = not described

	Short name	Full Name	Latitude, Longitude	Depth (cm)	Description
1	**mcmcurdo**	Lake Vanda Meteorological Station	-77.5232 161.6868	5	Antarctic dry valleys
2	**bonanza**	Bonanza Creek	65.19275 -147.499	30	Alpine tundra
3	**niwot**	Niwot Ridge LTER	40.035 -105.531	5	Subalpine forest
4	**cedar**	Cedar Creek	45.44138 -93.1629	Nd	Temperate grasslands
5	**andrews**	Andrews Forest	44.34393 -122.095	10	Douglas-fir-western hemlock forest
6	**hubbard**	Hubbard Brook	43.94618 -71.7148	5	Northern hardwood forest
7	**harvard**	Harvard Forest	42.53508 -72.1742	10	Temperate forest
8	**ntl**	Northern Temperature Lakes LTER	45.925 -89.734	5	Temperate forest
9	**kellogg**	Kellogg Biological Station	42.42027 -85.3669	5	Spruce forest
10	**konza**	Konza LTER	39.1083 -96.6118	25	Prairie
11	**sevilleta**	Sevilleta	34.20143 -106.415	8	Semi-arid grasslands
12	**jornada**	Jornada Basin LTER	32.53043 -106.804	5	Warm desert shrublands
13	**luq**	Luquillo LTER	18.3239 65.8183	Nd	Tropical rainforest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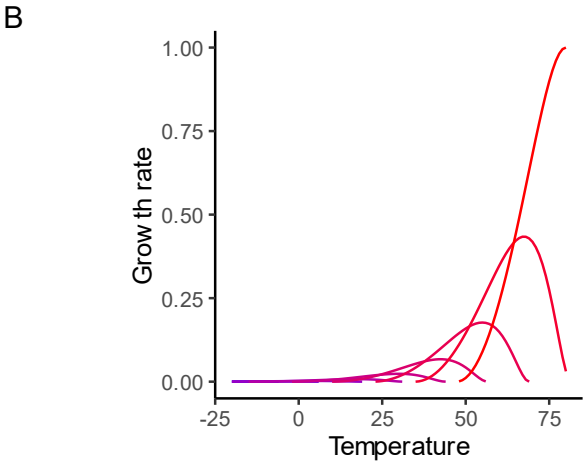
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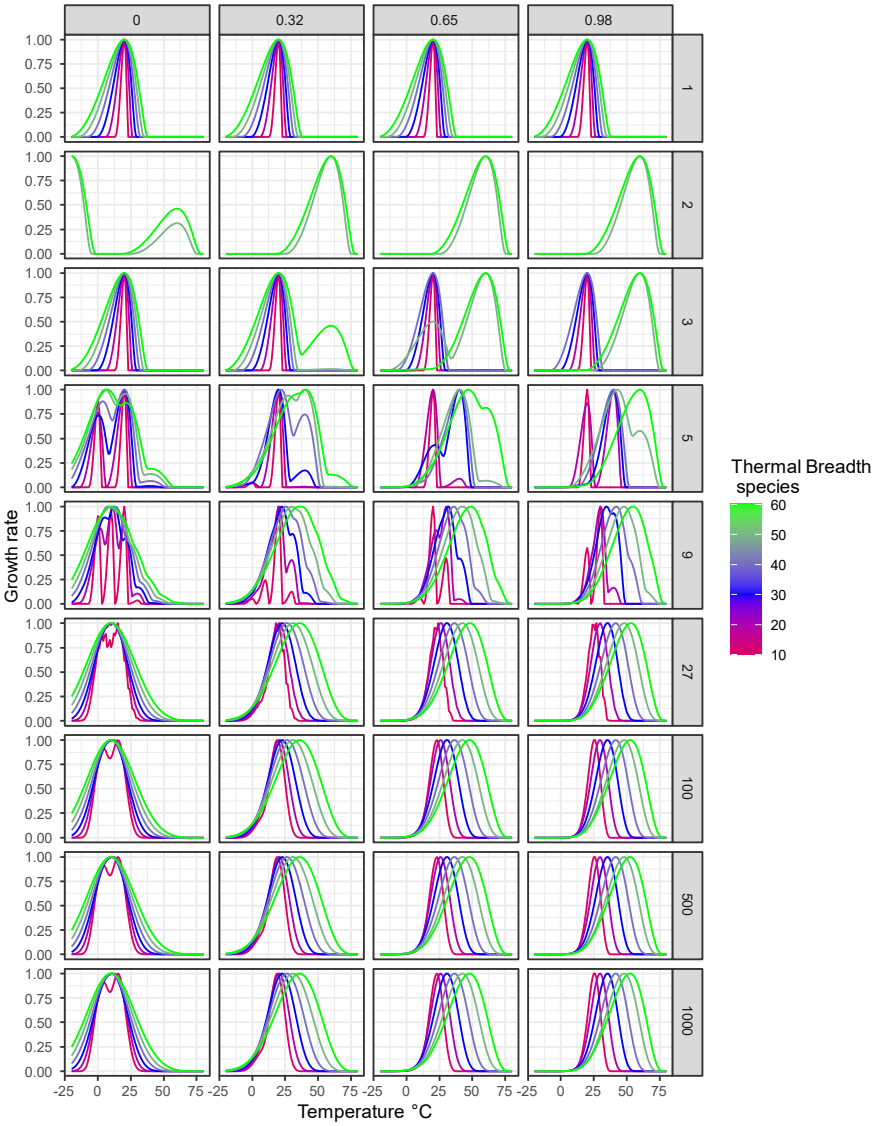
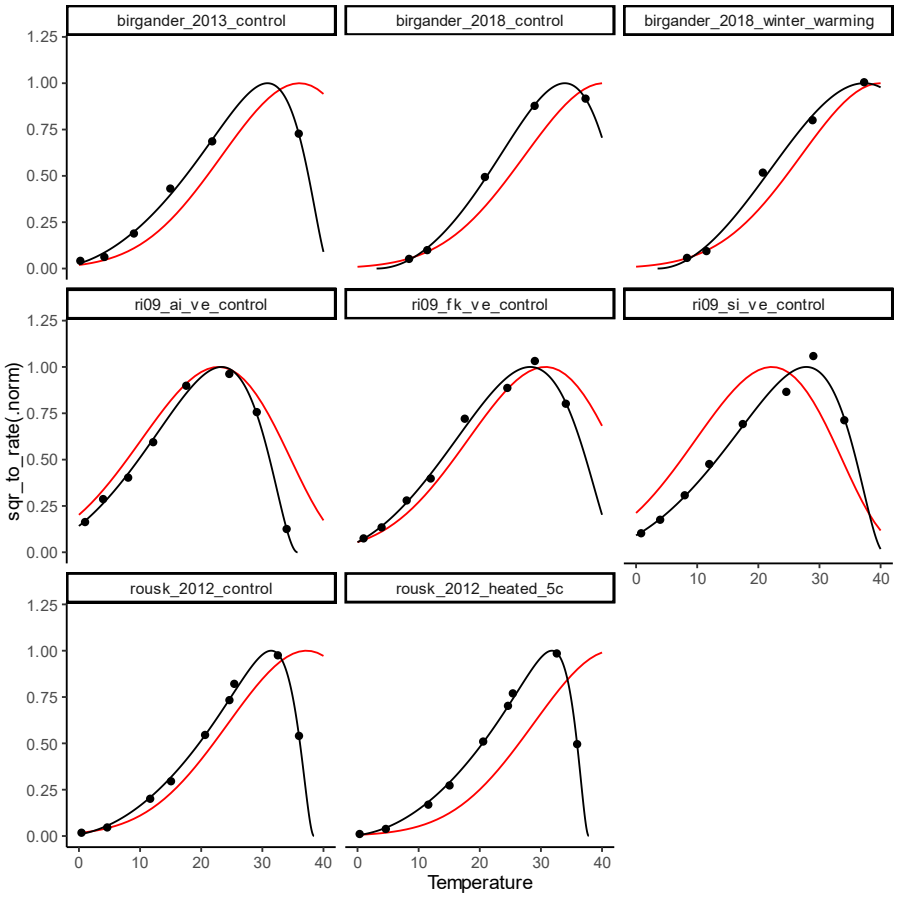
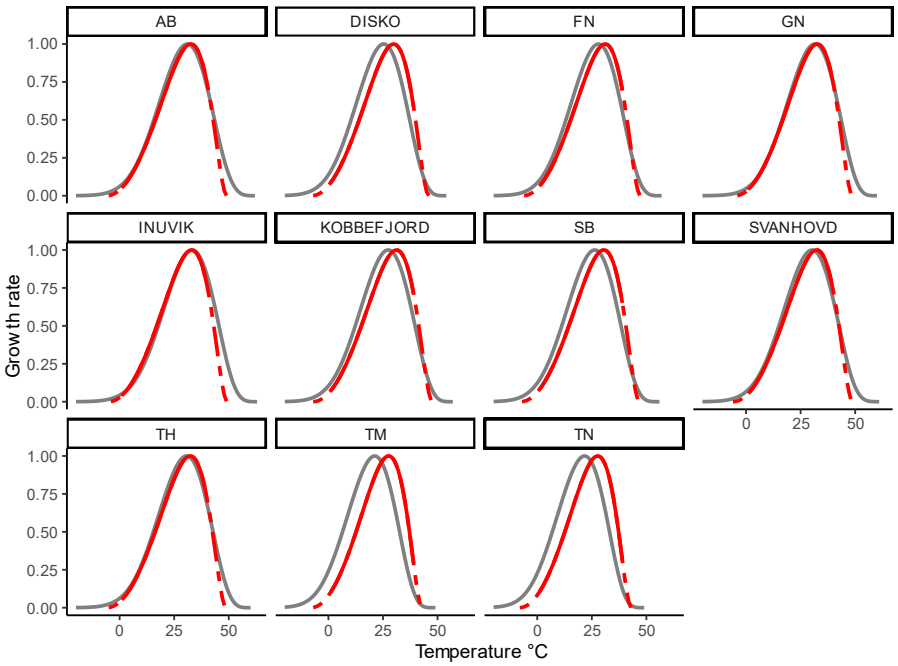
Supplementary Figure 5.1 The maximal fitness for each thermal niche arising from the interaction between thermal breadth and t_{opt}-μ dependence under multiple scenarios (a) and in our model (b).



Supplementary Figure 5.2 Community growth curves computed for the virtual temperature records of equation 3 and varying in model parameters for sensitivity analysis on n, tbreadth and eV (see sect 5.2.4).



Supplementary Figure 5.3 Measured and the predicted community growth curves for the bacterial communities extracted from literature, respectively in black and red. Black is a single Ratkowsky model fit to community-level Leucine growth assay data. Red = model predictions for overall community growth and are derived from weighted sums of species-specific Ratkowsky curves.



Supplementary Figure 5.4 Predicted growth curves (grey) for each site in the training dataset and the corresponding refitted Ratkowsky model for growth rates between 0 and 40 °C (red).

Chapter 6

General Discussion

Introduction

The aims of this dissertation were to: (1) evaluate the current temperature adaptation of soil bacterial communities in the Arctic region; and (2) determine the key drivers for accurate predictions of bacterial temperature adaptation under future climate change. While the high temperature sensitivity of the functional response of arctic soil bacteria is commonly recognized (Karhu et al., 2014), the temperature adaptation of bacterial communities themselves has so far not been evaluated across the entire Arctic region. Shifts in the temperature adaptation of arctic soil bacterial communities have the potential to alter decomposition rates of soil organic matter under future climate change. I therefore assessed the current temperature adaptation of soil bacterial communities in the Arctic (**Figure 2.2**) and identified maximum soil temperature as the main driver of their temperature adaptation (**Chapter 2**). I then showed that the temperature adaptation of arctic soil bacterial communities shifts in response to experimental warming treatments (**Chapter 3**). Lastly, I evaluated methods for the prediction of the temperature adaptation of soil bacterial communities under current and future climates (**Chapters 4 and 5**). Below I further discuss the main findings of this thesis and their potential implications.

Temperature adaptation of bacterial communities in arctic soils will alter under future climate change

Soil bacterial communities in the Arctic are hypothesized to be adapted to low temperatures (Bååth, 2018). This is exemplified by observations from the sub-Arctic which show that arctic soil bacterial communities are better adapted to low temperatures than bacterial communities from temperate or warmer regions (Rinnan et al., 2011; Cruz-Paredes et al., 2021; Weedon et al., 2022). In **Chapter 2**, I showed that the optimal growth temperature (T_{opt}) of soil bacterial communities varies greatly across the Arctic region, from to 23.4 to 34.1 °C, and was influenced by the local climate. The temperature adaptation of soil bacterial communities in the Arctic is similar to that of soil bacterial communities in the Antarctic and adapted to lower temperatures than communities from warmer regions (Rinnan et al., 2011; van Gestel et al., 2013), confirming the general character of the adaptation to colder soil climates. To predict whether the temperature adaptation of arctic soil bacterial communities will change under warmed conditions, I used a large-scale geographical gradient to assess what the most important driver is for temperature adaptation across the Arctic. Along the temperature gradient, the

optimal growth temperature of soil bacterial communities increased with higher *maximum* soil temperatures. This contrasts with previous observations, wherein the temperature adaptation of soil bacterial communities was correlated to *mean* annual soil temperature (Rinnan et al., 2009; Nottingham et al., 2019a). Our results thus support the emerging view (van Gestel et al., 2020) that the mean annual temperature is not the best predictor of the temperature adaptation of bacterial communities in cold environments. Due to the limited bacterial growth at temperatures below 0 °C, winter temperatures might have little effect on the temperature adaptation of bacterial communities (van Gestel et al., 2020). Therefore, temperatures above 0 °C are most relevant for the temperature adaptation of soil bacterial communities (Berglund and Rousk, 2018; van Gestel et al., 2020). **I conclude that increasing summer temperatures, especially high maxima caused by heat waves, will likely be the most important climatic factor determining temperature adaptation in a warmed Arctic.**

By experimentally increasing soil temperatures, researchers can validate whether shifts in the temperature adaptation of soil bacterial communities can potentially be induced by climate change. Previous incubation studies showed that soil bacterial communities only shift in their temperature adaptation when exposed to temperatures above their initial T_{opt} (Bárcenas-Moreno et al., 2009; Birgander et al., 2013; Donhauser et al., 2020). Therefore, I hypothesized that future warming would not be able to induce shifts in the temperature adaptation, as the optimal growth temperature for arctic soil communities typically reaches ~ 20 °C above the maximum soil temperature. In **Chapter 3**, I used an incubation experiment to test whether bacterial communities in arctic soils can shift their temperature adaptation when exposed to temperatures varying between 0 to 30 °C. T_{opt} of the soil bacterial communities for 8 (sub-) arctic soils increased along the incubation temperature gradient. The changes in T_{opt} occurred at incubation temperatures below the *in situ* T_{opt} of the soil bacterial communities. These results thus refute the hypothesis that shifts in the temperature adaptation of soil bacterial communities only occur above the initial T_{opt} of a bacterial community (Bárcenas-Moreno et al., 2009; Birgander et al., 2013). The discrepancy with earlier findings is likely explained by the longer incubation times used in our study. These results also allow us to reconcile the different observations made in soil incubation studies and field warming studies. Under field conditions, warming can change the temperature adaptation of soil bacterial communities without temperature reaching above the T_{opt} of a bacterial community (Nottingham et al., 2019a;

Weedon et al., 2022). Our results show that this phenomenon can be reproduced in lab incubation when soils are incubated for time periods longer than 3 months. **Consequently, I conclude that shifts in the temperature adaptation of soil bacterial communities in the Arctic can occur at each level of warming regardless of their T_{opt}. This implies that the temperature response of e.g., soil decomposition rates might change due to shifts in the temperature adaptation of soil bacterial communities.**

To evaluate whether shifts in the temperature adaptation of soil bacterial communities will impact soil carbon cycling in the Arctic, accurate predictions of the exact magnitude of the change in temperature adaptation are essential. Previous work has shown an increase in T_{opt} of 0.17 °C per °C increase in mean annual temperature for Antarctic soil bacterial communities (Rinnan et al., 2009). Similarly, the incubation experiment (Chapter 3) showed a change in T_{opt} that varied between 0.07 ° and 0.27 °C per °C of incubation temperature, depending on the sampling moment. I observed a larger change in T_{opt} for soils that were incubated for longer time periods. This could mean that shifts in temperature adaptation of the soil bacterial communities were not completed over the time period of the experiment. For example, shifts in the temperature adaptation of soil bacterial communities took 2-11 years to complete in experiments that transplanted soils along an altitudinal gradient (Nottingham et al., 2021). Therefore, the total change in temperature adaptation is likely best estimated from large scale natural temperature gradients, such as sampled across the Arctic in Chapter 2. **I therefore conclude that the T_{opt} of arctic soil bacterial communities is likely to respond more strongly to warming than previously thought, with an estimated increase of 0.63 °C per °C increase in maximum soil temperature (Chapter 2).**

The impact of shifts in the temperature adaptation of soil bacterial communities on soil carbon cycling in the Arctic is likely to be dependent on the warming level, current soil organic matter stocks and the composition of the microbial communities present in the soils. Previous work has indicated that the impacts of shifts in temperature adaptation of soil bacterial communities can be diminished by e.g. substrate depletion (Rousk et al., 2012), as this reduces growth and respiration of soil bacteria. Substrate depletion under warmed conditions has also been observed in some studies at a sub-arctic grassland (Marañón-Jiménez et al., 2018; Walker et al., 2018). However, at other arctic sites, increased respiration

under warmed conditions was sustained over a period of at least 8 years (Dorrepaal et al., 2009), indicating that substrate depletion will not necessarily occur all throughout the Arctic. Collectively, these studies show that substrate depletion could overrule the effects of a shift in temperature adaptation. Moreover, the contribution of the community's temperature adaptation to the warming response of soil decomposition rates will depend on the soil organic carbon stock and the availability of labile carbon. The incorporation of more biological realism in earth system models has shown great improvement of predictions on soil carbon cycling (Graham et al., 2014; Wieder et al., 2015). **I therefore urge researchers to model the possible impacts of shifting temperature adaptation of soil bacterial communities on global soil carbon cycling, which has so far not been done** (García-Palacios et al., 2021)**.** This could be achieved by updating current models for soil carbon cycling that incorporate microbial dynamics, for example in the model of Allison et al., (2010). Sensitivity analysis of implemented models could highlight where and when shifts in the temperature adaptation will influence the soil decomposition rates within the Arctic.

A new theoretical framework for temperature adaptation

The results from Chapter 3 contradict the current theoretical framework for temperature adaption of soil bacterial communities, which predicts that warming-induced shifts will only occur at temperatures above the community's T_{opt} (Chapter 1, section 1.3). Therefore, an updated theoretical framework is needed to understand and predict shifts in the temperature adaptation of soil bacterial communities, which incorporates observations made across both incubation and field warming studies. Since shifts in the temperature adaptation of soil bacterial communities are more responsive to warming than previously thought, I tested the potential mechanisms that control temperature adaptation. In accordance with recent studies (Donhauser et al., 2020; Nottingham et al., 2022; Weedon et al., 2022), I show in **Chapter 3** that shifts in the temperature adaptation co-occur with changes in the composition of bacterial communities. In the incubation experiment, soils were incubated in sterile closed containers which limited the introduction of external warm-adapted species from distant soil bacterial communities through dispersal. This supports the hypothesis that the temperature adaptation of soil bacterial communities is dependent on its community members and warming will select for species that perform well at these increased temperatures. In accordance with our hypothesis, recent studies

show that soil bacterial communities are comprised of a large variety of temperature traits among their community members (C. Wang et al., 2021; Smith et al., 2022). This variety of temperature traits can explain how the temperature adaptation of soil bacterial communities shifts when exposed to a changed soil climate (Nottingham et al., 2021, 2022) and can change seasonally (Kritzberg and Bååth, 2022). **From these observations, I propose that the temperature adaptation of soil bacterial communities is directly influenced by the temperature traits of their community members, which supports the previous theoretical framework (Bárcenas-Moreno et al., 2009). Consequently, shifts in the temperature adaptation of a soil bacterial community under altered soil climates are caused by community turnover, since the distribution of temperature traits within the community alters. In contrast to the previous hypotheses (Birgander et al., 2013), I propose in the new emergent theoretical framework that the large variety of thermal traits present within soil bacterial communities allows community turnover to occur for each level of warming, which results in shifts in the temperature adaptation of soil bacterial communities (Figure 6.1). Consequently, I propose that there is no community-specific threshold beyond which warming can induce shifts in the temperature adaptation of soil bacterial communities (as observed in Chapter 3).** The ability of researchers to detect changes in the temperature adaptation of soil bacterial communities under altered soil temperatures will depend on the exposure time, *in situ* climate and current temperature adaptation of soil bacterial communities.

As discussed in **Chapter 3**, phenomena other than community turnover could also contribute to the shifts in temperature adaptation of soil bacterial communities. For example, rapid evolution of bacterial species under changing climatic conditions can occur when they are exposed to changing climates (Chase et al., 2021). Moreover, phenotypic plasticity could also contribute to rapid adaptation to changing soil temperatures on the scale of individual species, such as the use of isomeric enzymes that vary in temperature optima (Pinney et al., 2021). However, other lines of evidence show that bacterial communities comprise a diversity of bacterial species that exhibit various temperature preferences (Smith et al., 2022) and that shifts in the temperature adaptation of soil bacterial communities can take up to a decade to complete (Nottingham et al., 2021). **Therefore, I conclude that environmental filtering of species is likely the key mechanism of the temperature adaptation of soil bacterial communities**

(Figure 6.1), since it has been observed for all studies that evaluated the effects on both bacterial community composition and temperature adaptation (Chapter 3; Bárcenas-Moreno et al., 2009; Donhauser et al., 2020; Nottingham et al., 2022; Weedon et al., 2022).

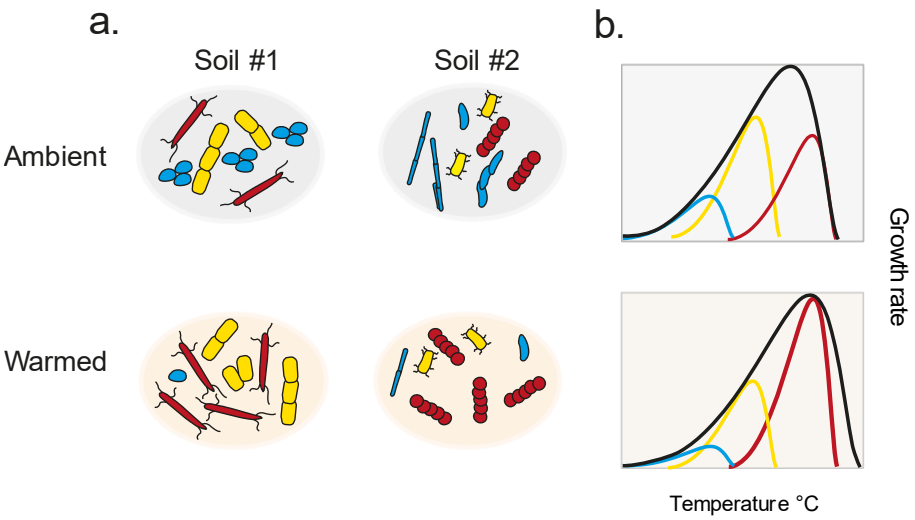


Figure 6.1 Conceptual diagram illustrating the change in community composition of two soil bacterial communities exposed to ambient and warmed conditions, where (a) warm-adapted soil bacteria are more abundant under warmed conditions and (b) by their individual temperature traits (colored lines) alter the temperature adaptation of the soil bacterial community (black line). Note that similar bacterial communities that differ in the present species can both similarly shift in temperature adaptation due to the wide variety of temperature traits among the community members.

Prediction of shifts in temperature adaptation

From Chapter 2 and 3, I concluded that temperature adaptation in the Arctic is likely to change under climate warming. Since increasing temperatures are likely to induce species sorting for bacterial taxa that perform well under these conditions, the temperature adaptation of a soil bacterial community might be predicted from theoretical considerations of which combinations of thermal traits would perform best under a given temperature regime. Conversely, the

presence/abundance of species with a particular temperature preference might be related to soil climate. In Chapter 4 and 5, I evaluated (1) whether there are bacterial species that could be used as indicators of increased soil temperatures and (2) if the current understanding of the underlying mechanisms for temperature adaptation, as well as theory about bacterial thermal niches could be used to predict the temperature adaptation of soil bacterial communities.

Due to the concurrent changes in bacterial community composition with shifts in temperature adaptation, the abundance of specific bacterial species might be used as indicators of soil warming or temperature adaptation. This has previously been done for soil bacteria responding to warming in temperate forests (Oliverio et al., 2017). Overall, the composition of bacterial communities changes in response to soil temperature (**Chapter 2, Chapter 3 and Chapter 4**), lending support to the idea that bacterial indicators for soil warming might indeed be identified for arctic soil bacterial communities. In **Chapter 4**, I sampled soils across an Icelandic grassland temperature gradient, where a shift in the bacterial community temperature adaptation had previously been shown (Weedon et al., 2022). I then identified potential bacterial indicators for soil warming. Across the multiple year dataset, there was only a small group of differentially abundant bacterial taxa that were consistent across warming levels and years. These bacterial taxa did not match with the 16S rRNA sequence of warming responsive bacterial taxa identified in previous studies. Furthermore, the differential abundance of identified bioindicators in the Icelandic grassland did not match with bacterial taxa that were responsive to the incubation temperature gradient in **Chapter 3**. Overall, I found a lack of generality and consistency in the response of warming responsive bacterial taxa. **I therefore conclude that the utility of the 16S rRNA gene sequences of bacterial taxa for the detection of soil warming and shifting temperature adaptation for soil bacterial communities is very limited. Instead, I conclude that the temperature adaptation of soil bacterial communities is therefore better predicted from the annual records of daily soil temperatures (Chapter 2 and 5).** Nonetheless, characterization of bacterial community composition can still be still useful to elucidate the mechanisms how bacterial communities respond to changing soil climates. With the information gathered from compositional changes as observed in Chapter 3, I propose that hypothesis-driven mechanistic frameworks (section 6.3) about how and when soil bacterial communities change could improve our predictions about the

temperature adaptation of soil bacterial communities (Prosser, 2020), as shown in **Chapter 5**.

Model-based approaches for predictions of temperature adaptation require explicit assumptions about the mechanisms that drive soil bacterial community temperature adaptation. By implementing experimental results on temperature adaptation and the current knowledge of thermal niche theory, I show in **Chapter 5** that predictions of temperature adaptation for soil bacterial communities can be made accurately, relative to statistical models, such as those used in Chapter 2. Our simple trait-based model provided both insights into the likely thermal niche traits of bacterial taxa and a possible alternative mechanism for the emergence of the high T_{opt} paradox. The model supports the 'Hot is better' hypothesis, implying that there is a fundamental correlation between maximum growth rate and T_{opt} of individual taxa. This correlation influences the temperature-growth relationships of soil bacterial communities, such that soil bacterial communities grow optimally at temperatures far above the temperatures of the corresponding soil climate, a phenomenon that is commonly observed (**Chapter 2**). **I encourage researchers to use trait-based approaches to predict the adaptation of bacterial communities to environmental conditions. The use of trait-based modeling showed an alternative explanation for observed phenomena and highlighted traits of high value for modeling, such as the thermal breadth** (Lajoie and Kembel, 2019)**.** The model provided testable predictions on the expected temperature trait distribution and temperature adaptation of soil bacterial communities based on a given soil climate, which can be verified by experimental work. This can, for example, be achieved by testing the T_{opt}-μ dependence on bacterial isolates from soils or *in situ* taxon-specific quantitative stable isotope probing. Additionally, our model indicates that thermal breadth might be an important but overlooked trait of bacterial species that greatly influences the trait distribution and community-aggregated temperature response curve of bacterial communities. Inaccuracy of our model might be explained by its relative simplicity, since it only incorporates soil temperature data. Further improvement could be made by the implementation of dynamics of bacterial growth-death processes, substrate supply, other environmental conditions (such as soil moisture, pH, etc.) and biotic interactions amongst bacterial taxa and/or with other organisms.

Conclusions and future perspectives

In this dissertation, I show that soil bacterial communities from the sub to High Arctic will change in their temperature adaptation under changing climate conditions. I did this with by the first-ever study to span a large-scale temperature gradient in this region, and by using a combination of measurements and experimental approaches that highlighted the mechanisms underlying these changes. The experimental work from this dissertation reconciles contrasting observations previously made in incubation studies and field warming studies and shows that the shift in temperature adaptation of soil bacterial communities under warmed conditions occurs regardless of the communities' T_{opt}. Furthermore, I propose that the lack of change in temperature adaptation observed in incubation studies is more likely due to short-term incubations rather than to the previous hypothesis that changes in temperature adaption will only occur above communities' T_{opt}. While I show that these changes are related to shifts in the community composition, there is limited utility of species abundance data for estimating the temperature adaptation of soil bacterial communities, as the warming-responsive bacterial taxa differed widely amongst the soils I studied. The key finding from the dissertation, that arctic soil bacterial communities will shift their temperature adaptation in response to warming, will need further validation by field warming experiments and will also require further studies on the contribution of these shifts to soil carbon decomposition processes under future climates.

An important outcome of this thesis is the potential for trait-based modeling for soil bacterial communities. However, newly gathered insights into the potential role of thermal niches and community assembly processes still need experimental validation in order to further develop theory and predictive power for the temperature adaptation of soil bacterial communities. A next step could be to use high-throughput cultivation at various temperatures to isolate soil bacteria that vary in temperature traits (Smith et al., 2022) in order to estimate the thermal breadth values for a large number of species. Moreover, by combining bacterial species with various temperature traits, artificial bacterial communities can be constructed that differ in temperature adaption in *in vivo* soils. These can then be used to test for differential effects on carbon cycling between the communities. Overall, these approaches would provide data and evidence that support the further development of trait-based modelling approaches for predictions of the temperature adaptation of soil bacterial communities. These trait-based models

could in turn inform earth-system models for representation of adaptation of soil bacterial communities to their local climate.

Summary

The microbial organisms that inhabit soil play an important role in the decomposition of plant litter and root exudates. Since soils form a large global stock of organic carbon, the microbial control over the formation, stabilization and mineralization of soil organic carbon has an important influence on global carbon cycle. For example, higher soil temperatures can increase the activity of soil microbial organisms, such that there is a potential feedback to climate change when increased microbial decomposition of soil organic stocks exceeds increased photosynthetic rates. Globally speaking, climate change is currently most rapidly warming the region where most soil organic carbon is located, namely the Arctic region. This large increase in air temperature can cause arctic ecosystems to turn in from carbon sinks to carbon sources due increased microbial decomposition of thawed soil organic matter.

The microbial organisms in arctic soils are typically well adapted to low temperatures and are highly responsive to temperature increases. So far, it unknown whether microbial communities will respond differently to temperature when they have been exposed to warming for an extended period of time. A change in temperature adaptation of microbial communities might affect how fast they will decompose soil organic matter in a warmed Arctic. Currently, the potential change in temperature adaptation of soil bacterial communities is under scientific debate, due to contrasting observations made in field and laboratory settings. However, it is essential to understand how and when soil microbial communities adapted to the temperatures they are exposed to, in order to predict how microbial communities respond the changing environmental conditions.

The overall aim of this thesis was to evaluate the current temperature adaption of soil bacterial communities in the Arctic and to investigate whether the temperature adaptation of arctic soil bacterial communities shifts when exposed to warmed conditions. Therefore, I used both a large natural temperature gradient and an incubation study with controlled temperatures to assess how bacterial soil communities change their temperature adaptation. In Chapter 2, the optimal growth temperature of bacterial communities increased when comparing samples from the colder sites to warmer sites. While soil bacterial communities in the Arctic experience very low temperatures during winter, I observed that tone of the most important factors for their temperature adaptation was the mean maximum soil temperature. I concluded from this study that increasing temperatures – especially summer temperatures – will likely alter the temperature adaptation of bacterial communities in arctic soils. To confirm this hypothesis, I conducted an incubation study with 8 soils collected from the (sub-) Arctic and exposed them to different temperatures, ranging

between 0 and 30°C. When the soils were incubated, the bacterial communities altered their temperature adaptation depending on the incubation temperature. For example, after 100 days the optimal growth temperature increased from 25 °C for soils incubated at 0°C, while soil incubated at 30°C showed an optimal growth temperature of the bacterial communities of 30°C. It is likely that soil bacterial communities will alter their temperature adaptation in response into to current warming conditions of the Arctic.

The change in temperature adaptation of soil bacterial communities under warmed conditions will likely influence soil carbon cycling in the Arctic under future climatic conditions. To improve predictions on the current and/or future temperature adaptation of soil bacterial communities, I furthermore focused on understanding the mechanisms that influence the temperature adaptation of soil bacterial communities and which methods we can use to predict the temperature adaptation accurately. In the third chapter, I found that the induced change in temperature adaptation of soil bacterial communities was accompanied by a change in the overall composition of the bacterial community. Therefore, I hypothesized that the individual response of bacterial species to soil warming might reflect the temperature adaptation. Thus, the abundance of particular bacterial species in a soil sample could be potentially used for estimating the temperature adaptation of soil bacterial communities. Throughout chapter 2 and 3, there were only a few limited number of bacterial species that I observed in multiple soil types. In chapter 4, I further evaluated the use of bacterial species abundance as indicator of bacterial communities responding to soil warming. Along a natural warming gradient in the south west of Iceland, I show that, while there are changes in the composition of bacterial communities of grassland soil in response to soil warming, there are only very few bacterial species that respond to the warming through multiple and multiple levels of warming. From this study I concluded that there is indeed limited use of bacterial abundance data in predicting the temperature adaptation and response to warming for soil bacterial communities. Overall, I did not observe bacterial species that are both 1. common amongst many soil types and 2. respond consistently to soil warming.

Throughout this thesis, multiple hypotheses about the how and when soil bacterial communities will alter their temperature adaptation in response to their climate are discussed, refuted and synthesized. In chapter 5, I used a trait-based model approach to test whether the current theoretical framework around thermal traits of soil bacterial species can explain the relationship between temperature adaptation of soil bacterial communities and the temperatures that they are exposed to. The model made relatively accurate

predictions about the temperature adaptation of soil bacterial communities. Furthermore, I discuss which traits related to the thermal niche of a bacterial species are relevant for improved modelling. Finally, I present a synthesis of the findings from this thesis in Chapter 6. Bacterial communities will likely alter their temperature adaptation in response to long term exposure to warming. It will be important to further assess the influence of these changes on the functioning on soil bacterial communities in the Arctic. From the experimental work in this thesis, the view emerges that changes in the temperature adaptation of soil bacterial communities are likely due to the changes in the abundance of bacterial species, most likely related to their ideal temperature range. Accurate predictions for the current and future temperature adaptation of soil bacterial communities will require more in-depth knowledge of the traits that bacteria possess related to temperature.

Samenvatting

Micro-organismen in de aardbodem spelen een belangrijke rol in de afbraak van plantenmateriaal, zoals bladeren en wortelexudaten. De aardbodem is een belangrijk en dynamisch reservoir van organisch koolstof bevattend materiaal en micro-organismen hebben een grote invloed op de vorming, stabilisatie en afbraak hiervan. Zo kunnen micro-organismen bijvoorbeeld bij een hoge temperatuur het organische bodemmateriaal sneller afbreken. Wanneer de afbraak van organisch materiaal sneller verloopt dan dat er nieuw bodemmateriaal bijkomt, kan dit proces klimaatverandering versnellen door de CO2 die daarbij vrijkomt. Van al het organisch bodemmateriaal wereldwijd, bevindt ongeveer de helft zich in het Arctisch gebied, waar ook de grootste opwarming plaatsvindt. Door de snelle opwarming kunnen de ecosystemen in het Arctisch gebied snel veranderen van reservoir voor opname van CO2 naar een belangrijke bron van uitstoot van CO2.

De micro-organismen in arctische aardbodems zijn over het algemeen goed aangepast aan de lage temperaturen van het bodemklimaat, en zijn erg gevoelig voor hogere temperaturen. Tot nu toe is het nog onbekend of deze microbiële gemeenschappen anders reageren na langdurige blootstelling aan een opgewarmd bodemklimaat. Een verandering in de temperatuuradaptatie van microbiële gemeenschappen zou de snelheid van de afbraakprocessen kunnen beïnvloeden in warmer Arctisch gebied. Er is op dit moment nog geen duidelijke consensus of bacteriële gemeenschappen hun temperatuuradaptatie veranderen, omdat er tegenstrijdige waarnemingen zijn gedaan tijdens experimenten in het veld en in gecontroleerde incubatie-experimenten in het laboratorium. Om de respons van bacteriële gemeenschappen in arctische aardbodems nauwkeurig te kunnen voorspellen, is het belangrijk om te begrijpen hoe en wanneer bacteriële bodemgemeenschappen zich aanpassen aan temperatuurverandering.

Het doel van de dissertatie was om de huidige temperatuuradaptatie van bacteriële gemeenschappen te bepalen in arctische aardbodems en om te onderzoeken of deze temperatuuradaptatie verandert wanneer het Arctisch gebied opwarmt. Ik heb daarvoor gebruik gemaakt van een studie over een grootschalige temperatuurgradiënt en een incubatie-experiment waarbij ik de temperaturen kon bepalen. In Hoofdstuk 2 laat ik zien hoe de optimale groeitemperatuur van bacteriële bodemgemeenschappen toeneemt in de warmere gebieden van het Arctisch gebied. De belangrijkste factor daarbij is de maximale temperatuur van het bodemklimaat. Ik concludeer in Hoofdstuk 2 dat

het erg waarschijnlijk is dat bacteriële bodemgemeenschappen veranderen in hun temperatuuradaptatie naarmate de bodem warmer wordt. In Hoofdstuk 3 test ik dit in een incubatie-experiment door acht bodemtypes op te warmen tussen 0 en 30°C. Tijdens het incubatie-experiment veranderde de optimale groeitemperatuur van de bacteriële bodemgemeenschappen aan naarmate de temperatuur waaraan zij werden blootgesteld. Zo groeiden de bacteriën die werden blootgesteld aan 0°C het beste bij 25°C, terwijl bacteriën die blootgesteld werden aan 30°C het beste groeiden bij 30°C. Deze aanpassing aan het klimaat waaraan bacteriële gemeenschappen werden blootgesteld geeft duidelijk weer dat het erg waarschijnlijk is dat de temperatuuradaptatie van bacteriële gemeenschappen zal veranderen naarmate het Arctische gebied opwarmt.

De verandering in temperatuuradaptatie van bacteriële gemeenschappen in arctische aardbodems onder verwarmde condities heeft mogelijk invloed op de koolstofcyclus in het Arctisch gebied. Om de mate van verandering correct te kunnen voorspellen heb ik in Hoofdstuk 3 en 4 gefocust op mogelijke methoden om deze verandering te kunnen meten en voorspellen. In het derde hoofdstuk vond ik dat de verandering in temperatuuradaptatie gepaard gaat met een verandering in de samenstelling van de bacteriële gemeenschappen. Ik nam daarom de stelling aan dat de aanwezigheid en hoeveelheid van individuele bacteriële soorten misschien de temperatuuradaptatie van de gehele gemeenschappen zouden kunnen verklaren. Daardoor zou het mogelijk kunnen zijn om temperatuurgevoelige bacteriesoorten te gebruiken als indicator van opwarming en de huidige temperatuuradaptatie van bacteriële gemeenschappen. Er waren echter maar weinig bacteriële soorten die in meerdere grondsoorten voorkwamen (Hoofdstuk 2), en geen bacteriële soorten die in meerdere grondsoorten op een veranderend bodemklimaat reageerden (Hoofdstuk 3). Om het gebruik van bacteriële indicator te onderzoeken bestudeerde ik daarom de bacteriële gemeenschappen van graslandschap in IJsland met een natuurlijke temperatuurgradiënt. Ondanks de grote verschillen in samenstelling van de bacteriële gemeenschappen tussen de onverwarmde en verwarmde grond, vond ik slechts een klein aantal bacteriële soorten dat consistent reageerde op de temperatuurverschillen. Hierdoor concludeerde ik dat de kwantificering van mogelijke bacteriële indicators geen voorspellend vermogen heeft om de temperatuuradaptatie van de gemeenschap te kunnen bepalen.

In deze thesis worden meerdere hypothesen over hoe en wanneer bacteriële gemeenschappen veranderen in hun temperatuuradaptatie besproken, weerlegd en opgesteld. In Hoofstuk 5 testte ik het huidige theoretisch kader door met een model de correlatie tussen het bodemklimaat en de optimale groeitemperatuur van de gemeenschap te verklaren. In dit model beschrijf ik de verschillende eigenschappen die voor een bacterie een bepaalde temperatuurs-niche vormen, zoals de minimale, optimale en maximale temperatuur, en ook de maximum groeisnelheid van de bacterie. Ik laat zien dat op basis van de huidige hypothesen met het model een vrij nauwkeurige voorspelling van de optimale groeitemperatuur van de gemeenschap gemaakt kan worden.

Ten slotte presenteer ik in Hoofdstuk 6 een synthese van de bevindingen in dit proefschrift. Bacteriële gemeenschappen zullen waarschijnlijk hun temperatuur-adaptatie veranderen na langdurige blootstelling aan opwarming. Verder onderzoek naar de invloed van deze veranderingen op het functioneren van de bacteriële bodemgemeenschappen in het Arctisch gebied is nodig, met name in de context van de koolstofcyclus. Uit het experimentele werk in dit proefschrift komt naar voren dat veranderingen in de temperatuuradaptatie van bacteriële bodemgemeenschappen waarschijnlijk het gevolg zijn van veranderingen in de abundantie van bacteriesoorten, hoogstwaarschijnlijk gerelateerd aan hun temperatuureigenschappen. Nauwkeurige voorspellingen voor de huidige en toekomstige temperatuuradaptatie van bacteriële gemeenschappen zullen meer diepgaande kennis vereisen van de temperatuureigenschappen van de betreffende bacteriënsoorten.

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About the Author

Photo by Logan Sharma

Ruud Rijkers was born on 25th of August 1993 in Rijswijk, The Netherlands.

He started a Bachelor of Applied Sciences at Hogeschool Leiden in Biology and Medical Sciences in 2010, in which he focused on microbiology during a minor. As an intern at the laboratory of Prof. Mary Firestone at UC Berkeley he had his first exposure to microbial ecology. He continued his studies with at Radboud Nijmegen with consecutive premaster and master in Biology with a specialization track in microbiology. During his master's he researched methane cycling in chemostat cultures and permafrost settings during internships at Radboud University Nijmegen and Deutsche GeoForschungsZentrum GFZ Potsdam.

In 2017, Ruud started a PhD at the Vrije Universiteit Amsterdam in the Systems Ecology group of Prof. Rien Aerts, of which this PhD thesis is the final result. Under the supervision of Dr. James T. Weedon, he explored the temperature adaptation of soil bacterial communities for a NWO Polar grant #866.16.042. He has recently started as a postdoctoral researcher at Stockholm University. In the group of Dr. Birgit Wild, he will investigate the contribution of plant priming to the potential destabilization of soil organic carbon stocks in the Arctic.

List of Publications

Publications from this thesis

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Other publications

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