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Thermal adaptation of soil microbial growth traits in response to chronic warming

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ABSTRACT Adaptation of soil microbes due to warming from climate change has been observed, but it remains unknown what microbial growth traits are adaptive to warming. We studied bacterial isolates from the Harvard Forest Long-Term Ecological Research site, where field soils have been experimentally heated to 5°C above ambient temperature with unheated controls for 30 years. We hypothesized that Alphaproteobacteria from warmed plots have (i) less temperature-sensitive growth rates; (ii) higher optimum growth temperatures; and (iii) higher maximum growth temperatures compared to isolates from control plots. We made high-throughput measurements of bacterial growth in liquid cultures over time and across temperatures from 22°C to 37°C in 2–3°C increments. We estimated growth rates by fitting Gompertz models to the growth data. Temperature sensitivity of growth rate, optimum growth temperature, and maximum growth temperature were estimated by the Ratkowsky 1983 model and a modified Macromolecular Rate Theory (MMRT) model. To determine evidence of adaptation, we ran phylogenetic generalized least squares tests on isolates from warmed and control soils. Our results showed evidence of adaptation of higher optimum growth temperature of bacterial isolates from heated soils. However, we observed no evidence of adaptation of temperature sensitivity of growth and maximum growth temperature. Our project begins to capture the shape of the temperature response curves, but illustrates that the relationship between growth and temperature is complex and cannot be limited to a single point in the biokinetic range.

IMPORTANCE Soils are the largest terrestrial carbon sink and the foundation of our food, fiber, and fuel systems. Healthy soils are carbon sinks, storing more carbon than they release. This reduces the amount of carbon dioxide released into the atmosphere and buffers against climate change. Soil microbes drive biogeochemical cycling and contribute to soil health through organic matter breakdown, plant growth promotion, and nutrient distribution. In this study, we determined how soil microbial growth traits respond to long-term soil warming. We found that bacterial isolates from warmed plots showed evidence of adaptation of optimum growth temperature. This suggests that increased microbial biomass and growth in a warming world could result in greater carbon storage. As temperatures increase, greater microbial activity may help reduce the soil carbon feedback loop. Our results provide insight on how atmospheric carbon cycling and soil health may respond in a warming world.

KEYWORDS soil, soil warming, climate change, microbial evolution, microbial ecology

The Earth's climate is warming, and the cascading stressors from warming may have irreversible effects on microbes and the ecosystem functions that they drive. Between 2011 and 2020, Earth's land temperatures increased by 1.59°C, which is the largest rise in temperature in the last 2000 years (1). Temperature impacts the rates of biological processes (2) and can result in thermal adaptation or acclimation (3). The

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ability of microbes to adapt to environmental change may alter ecosystem function (4). Because soil microbes drive biogeochemical cycles and mediate atmospheric carbon fluxes (5, 6), we need to understand the effects of long-term warming on soil microbes. Healthy forest soils are characterized by high concentrations of organic matter and abundant and active microbial communities (7). The activity of these microbes contributes to new organic matter deposition and soil health (8). Soils serve as a large carbon sink, and healthy soils absorb more carbon than they release. This reduces the amount of carbon dioxide (CO_2) emitted to the atmosphere and buffers against climate change (9). Microbial adaptation in response to warming due to climate change could thus impact microbial traits associated with soil carbon cycling (10).

When microbes adapt, populations acquire new traits that fundamentally change how microbial systems respond to changes in the environment. Microbial adaptation can be defined as irreversible changes in microbial traits that increase fitness. Adaptation does not specify a mechanism and can be used to define changes in traits in response to environmental changes at the community level (4). Phylogenetic comparative methods (PCMs) can be used to test for evidence of adaptation of microbial traits. PCMs test for differences among species while accounting for phylogenetic relatedness (11–13). In this study, we used phylogenetic generalized least squares (PGLS), which transforms trait data based on phylogenetic distance among species then tests for differences in traits. Such trait transformation allows comparisons among species as if they were independent groups (11). While PCMs test for evidence of adaptation of microbial traits, they do not test for evolutionary adaptation associated with heritable mutations. Using PGLS, we determined whether long-term soil warming is associated with adaptation of microbial growth traits.

To study the impacts of long-term warming on soils, a 30-year field experiment is ongoing at the Harvard Forest Long-Term Ecological Research (LTER) site in Petersham, Massachusetts. Here, experimental soils are heated 5°C above ambient temperature throughout the year since 1991 to simulate the effects of climate change. Five degrees of warming was chosen as a worst-case scenario for the rise in soil temperatures by the year 2100 (1); control soils received no warming treatment. Increased rates of decomposition following 30 years of warming has led to 34% loss of soil organic matter and increased flux of CO_2 to the atmosphere in the heated versus control plots (14). An isolate screen and metagenomic analysis showed that the ability of soil microbes to degrade complex carbohydrates also increased in response to rising temperatures (6). This was preliminary evidence of adaptation to long-term warming and suggests the potential for adaptation of other microbial traits (6, 14). Given that microbial growth and activity contribute to soil health, we sought to characterize whether microbial growth traits show responses to long-term soil warming and whether they are adaptive.

We selected Alphaproteobacteria as the focus of our study because they tend to be dominant in soils, and because they showed increased absolute abundance in heated plots compared to control plots in a previous community-level experiment of soil microbes (15). We hypothesized that (i) growth of Alphaproteobacteria from warmed plots is less temperature-sensitive than that from control plots; (2) optimum growth temperature of Alphaproteobacteria from warmed plots is higher than that from control plots; and (iii) maximum growth temperature of Alphaproteobacteria from warmed plots is higher than that from control plots. Given that microbes in heated soils have been exposed to higher temperatures for 22–23 years at the time of isolation, we expect them to have adapted microbial growth traits that are advantageous in warmer temperatures (16). However, if warming does not result in adaptation of these microbial growth traits, this would suggest that changes in soil carbon dynamics may be a result of other factors such as nutrient availability, changes in microbial biomass and carbon use efficiency, or thermal acclimation (14).

To directly measure the adaptation of bacterial growth traits due to chronic warming, we measured growth over time and across temperatures for Alphaproteobacteria isolated from the warmed and control soil plots. We estimated the intrinsic growth rate

for each replicate isolate at each temperature (17). The Ratkowsky 1983 model (18) and a modified version of Macromolecular Rate Theory (MMRT) (19, 20) were fitted to data for growth rate over temperature for each isolate to estimate temperature sensitivity of growth, optimum growth temperature, and maximum growth temperature. We chose the Ratkowsky 1983 model because it is a widely accepted model for bacterial growth over temperature and the MMRT model because of its underlying thermodynamic theory and application in soil microbial communities. While we chose to fit both the Ratkowsky 1983 and MMRT models, the objective was to present both fits rather than select one. There lacks a standard approach to modeling such data. Furthermore, fitting both models and estimating several parameters allows for increased versatility in the data set which can be used for future work. Finally, we used phylogenetic comparative methods to test for adaptation of soil microbial growth traits (11–13).

MATERIALS AND METHODS

Isolate selection

All organisms were isolated from soils collected from the Harvard Forest long-term warming study, located at the Harvard Forest LTER site in Petersham, MA (21). The site is a mixed hardwood forest with paper and black birch (*Betula papyrifera* and lenta), red maple (*Acer rubrum*), black and red oak (*Quercus velutina* and *rubra*), and American beech (*Fagus grandifolia*) dominant tree species. Soils are coarse-loamy inceptisols. Eighteen 6×6 m^2 plots were randomly assigned one of three treatments: (i) plots with buried electrical cables, heating soils 5°C above ambient temperature throughout the year; (ii) disturbance control plots with the same as set up as the heated plots, but without electrical power; and (iii) undisturbed control plots. Soils are heated 5°C above ambient temperature by way of electrical cables buried 10 cm below the soil surface. This temperature was chosen as a worst-case scenario rise in soil temperatures by the year 2100 (1).

We selected 23 strains of Alphaproteobacteria (Table 1) from our lab culture collection originating from either the heated ($n = 8$) or control plots ($n = 15$). Bacteria were isolated from soils using several cultivation methods (Table S1) and cryopreserved at −80°C. Isolates were grown on 10% Tryptic Soy Agar until we could identify distinct colony morphology. We genotyped isolates by sequencing their full-length 16S ribosomal RNA. We extracted genomic DNA using CTAB-lysozyme extraction protocol (22). 16S rRNA was amplified on an Eppendorf AG 22331 Hamburg using the 27F and 1492R primers. We used a 25-µL final reaction volume with 0.125 µL Invitrogen Taq, 10 µL $MgCl_2$ 10× PCR buffer, 0.75 µL 50 mM $MgCl_2$, 1 µL of each primer, 2 µL of dNTP mix, and 1 µL of template for amplification reactions. We performed PCR amplifications using 35 cycles of 94°C (45 s), 50°C (30 s), and 72°C (120 s), followed by a final extension of 72°C (10 min). We used agarose gel electrophoresis to verify amplifications. DNA purification and Sanger sequencing were performed by Genewiz at Azenta Life Sciences (15).

Genome sequencing

To extract genomic DNA for genome sequencing, strains were grown in 10% Tryptic Soy Broth, and pellets were extracted using the DNeasy Blood and Tissue kit (Qiagen). We let cultures grow until the late exponential phase (OD_{600} nm 0.6–0.8). One day before extractions, we added 100 µL of 10% glycine to culture tubes for a final concentration of 1%; this helped to prevent cells from adhering to one another and forming clumps as cultures reached the late exponential phase. DNA was eluted in TE buffer, quantified by Qubit, and transferred to the freezer for long-term storage.

Genomes were sequenced by the United States Department of Energy's Joint Genome Institute (JGI), the University of Massachusetts Medical Center, or in-house using an Oxford Nanopore Technologies (ONT) MinION (Table S1). Illumina sequencing technology was performed at JGI and UMass Medical Center according to standard

TABLE 1 Taxonomic identification, soil warming treatment, and genome assembly accession numbers for Alphaproteobacteria isolates included in this study

Isolate	Taxonomic identification	Warming treatment	Genome assembly accession number
19YEA23	*Ochrobactrum* sp. 19YEA23	Warm	GCA_029909865.1
24YEA27	*Gemmobacter* sp. 24YEA27	Control	GCA_030052995.1
28DA2	*Rhizobium* sp. 28DA2	Warm	GCA_030550875.1
28YEA48	*Beijerinckia* sp. 28-YEA-48	Warm	GCA_900104955.1
AN5	*Rhizobium* sp. AN5	Control	GCA_900215255.1
AN63	*Rhizobium* sp. AN63	Warm	JAVFDK000000000
AN64	*Rhizobium* sp. AN64	Control	JARWMJ000000000
AN67	*Rhizobium* sp. AN67	Control	GCA_029909905.1
AN68	*Rhizobium* sp. AN68	Control	JARWMI000000000
AN69	*Rhizobium* sp. AN69	Control	JARWMG000000000
AN6A	*Rhizobium* sp. AN6A	Warm	GCA_900215635.1
AN70	*Rhizobium* sp. AN70	Control	GCA_029909835.1
AN73	*Rhizobium* sp. AN73	Control	JARWME000000000
AN78	*Ochrobactrum* sp. AN78	Control	GCA_029909885.1
AN80A	*Rhizobium* sp. AN80A	Warm	GCA_030053015.1
AN83	*Rhizobium* sp. AN83	Warm	JARWMH000000000
AN88	*Rhizobium* sp. AN88	Control	JARWMD000000000
AN95	*Rhizobium* sp. AN95	Warm	JARWMF000000000
GAS191	*Rhizobiales* sp. GAS191	Control	GCA_900105365.1
GAS231	*Afipia* sp. GAS231	Control	GCA_900103365.1
GAS462	*Beijerinkia* sp. GAS462	Control	GCA_029909845.1
GAS524	*Bradyrhizobium ottawaense* GAS524	Control	GCA_900099825.1
MT12	*Bradyrhizobium erythrophlei* MT12	Control	GCA_900105845.1

operating procedures (23). Long-read ONT libraries were prepared with the Ligation Sequencing Kit SQK-LSK-109 and samples were multiplexed using the Native Barcoding Expansion Kit EXP-NBD104 (Oxford Nanopore Technologies, UK). The Oxford Nanopore Native Barcoding Protocol (Oxford Nanopore Technologies) was followed, and 6–8 strains were multiplexed together in a run. Genomes were resequenced until at least 100X coverage was reached. The Covaris g-TUBE shearing step was skipped to target long fragment DNA. Starting with 1 µg of DNA per strain, samples were repaired and end-prepped using the NEBNext FFPE DNA Repair Mix and NEBNext Ultra II End Repair/dA-Tailing kits (New England Biolabs, USA). DNA was cleaned using Ampure XP Beads (Beckman Coulter, USA). Samples were ligated to individual barcodes, and then 150 ng of each sample pooled together, for a final library of 700–1,000 ng. Adapters were ligated to the sample with Blunt/TA ligase (New England Biolabs). The long fragment buffer provided in the sequencing kit was used in an extended 10 min incubation at 37˚C to enrich for high-molecular weight DNA. The flow cell was primed using the Flow Cell Priming Kit (Oxford Nanopore Technologies), and 15 fmol of the library was mixed with the sequencing buffer and loading beads and then loaded through the Spot-On port of the flow cell (24).

For genomes sequenced using ONT, sequence runs were initially basecalled using the high-accuracy base calling (HAC) algorithm with Guppy (25). These fast5 files were concatenated into one file, then reads were subsampled based on read quality using Filtlong (26). The filtered reads were *de novo* assembled using Flye (27). A consensus assembly was generated using Racon (28), and final polishing was performed with Medaka (29). These assemblies were checked for quality using Quast (30) and CheckM (31).

Quantifying microbial growth in liquid culture

We measured bacterial growth over time using absorbance measurements for liquid cultures spanning temperatures from 22°C to 37°C along 2–3°C increments. Because

organisms tend to live at or below their optimal growth temperature (32), we chose 22–37°C to capture the temperature growth range of mesophiles, the temperature range during the growing season at the Harvard Forest (33), and to accommodate instrumental limitations. Absorbance was measured in 96-well plates by optical density at 600 nm (OD_{600} nm) as a measure of cell abundance. Each well was filled with 240 µL of 10% Tryptic Soy Broth. Colonies grown on petri plates were resuspended in 500 µL of phosphate-buffered saline and 10 µL resuspension was inoculated into each well. For temperatures at or above 30°C, we pipetted 2–3 mL of a 0.05% solution of Triton X-100 in 20% ethanol on the plate lid to prevent condensation. Each plate accommodated eight isolates with 11 replicates each and eight negative controls according to a randomized plate format. Bacterial growth was measured using a SpectraMax M2 plate reader (Molecular Devices, CA, USA) at OD_{600} nm. Growth curves lasted 72–99 h or until microbes entered death phase.

Model fitting

We fitted the Gompertz growth curve on data for OD_{600} nm over time to calculate growth rate using the R package Growthcurver (34) (Fig. 1A). A Gompertz growth curve is an established time course model that parametrizes bacterial growth over time as a sigmoidal function (17). We estimated the intrinsic growth rate from the fitted Gompertz model. This was repeated for each replicate at each temperature.

We fitted temperature response curves to estimate the microbial growth traits. Temperature sensitivity of growth is an estimate of how growth rate changes with increasing temperature, but it can be estimated using different parameters depending on the model applied (Table 1). Optimum growth temperature is the temperature at which growth rate is the greatest. Maximum growth temperature is the estimated highest temperature at which microbial growth occurs. We modeled the relationship between growth rate and temperature for each isolate using the Ratkowsky 1983 model (Fig. 1B):

$$r = [b(T - T_{\min})]^2 \times \{1 - exp[c(T - T_{\max})]\} \quad (1)$$

$T_{\min}$ is the minimum permissible temperature for growth (°C), $T_{\max}$ is the maximum permissible growth temperature (°C), and c is an empirical parameter required to model data above the optimum temperature ($°C^{-1}$). Temperature sensitivity of growth was

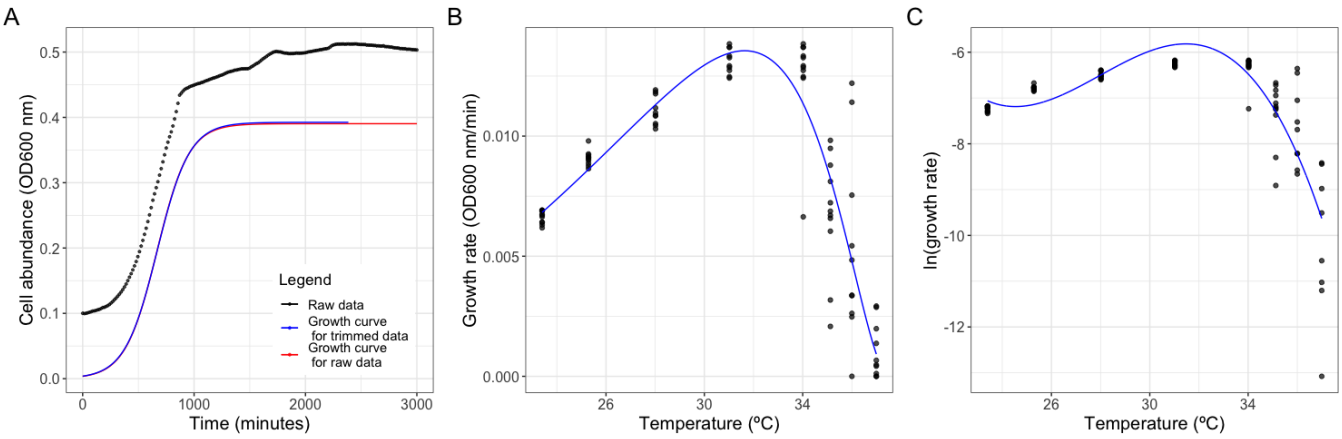

FIG 1 Example data sets for measuring growth parameters and estimating temperature sensitivity of growth using two different models. (A) Cell abundance over time was measured for liquid growth curves between 22°C and 37°C in 2–3°C increments. A Gompertz growth curve was fit on data of cell abundance over time. Intrinsic growth rate was extracted from the fitted model. (B) The Ratkowsky 1983 model was fitted to growth rate over temperature for each isolate. Temperature sensitivity of growth, optimum growth temperature, and maximum growth temperature were estimated from the fitted model. (C) A modified version Macromolecular Rate Theory was fitted to natural log-transformed data of growth rate over temperature for each isolate. Values of zero were removed from analysis. Temperature inflection point and optimum growth temperature were calculated from the fitted model. Data for (A–C) are of the same isolate.

quantified by the Ratkowsky parameter *b* (OD_{600}nm*$min^{-0.5}$/°C), which is the regression coefficient for square root of growth rate on temperature (18, 35). Maximum growth T_{max} (°C) was extracted as a parameter from the fitted model. Optimum growth *Topt* (°C) was estimated from the fitted model (36). Model fitting was performed using the R package *rTPC* (36).

The temperature optima (*Topt*) and inflection point (*Tinf*) of each bacterial growth curve were estimated using a modified version of MMRT (Fig. 1C):

$$ln(k) = ln\left(\frac{k_B T}{h}\right) - \frac{\Delta H^{\ddagger}_{T0}}{RT} - \frac{\Delta C^{\ddagger}_{P}(T - T_0)}{RT} + \frac{\Delta S^{\ddagger}_{T0}}{R} + \frac{\Delta C^{\ddagger}_{P}(lnT - lnT_0)}{R} \quad (2)$$

where *k* is the bacterial growth rate (OD_{600}nm), k_B is Boltzmann's constant, *T* is the temperature (K), *h* is Planck's constant, *R* is the universal gas constant, $\Delta H^{\ddagger}_{T0}$ (superscript denotes transition state) is the change in enthalpy (J mol^{-1}), $\Delta S^{\ddagger}_{T0}$ is the change in entropy (J mol^{-1} K^{-1}), $\Delta C^{\ddagger}_{P}$ is the change in heat capacity (J mol^{-1} K^{-1}), and T_0 is the reference temperature (set to 296.1K) (37). Temperature inflection point is the temperature at which the greatest change in growth rate occurs. The MMRT equation was modified to allow $\Delta C^{\ddagger}_{P}$ to vary linearly with temperature:

$$\Delta C^{\ddagger}_{P} = A(T - T_0) + B \quad (3)$$

where *A* is the slope and *B* is the value of $\Delta C^{\ddagger}_{P}$ at T_0 (20). We used a non-linear least-squares regression in R version 4.2.1 to fit MMRT and calculated the *Topt* and *Tinf* numerically using the first and second derivatives, respectively. We chose to use the modified version of MMRT to better capture the *Topt* due to asymmetries observed in the temperature response data and because $\Delta C^{\ddagger}_{P}$ varies over a wide temperature range (38–40). We calculated residual standard errors to determine whether the modified MMRT or original MMRT more adequately fit our data (Table S2).

To evaluate the fit of the models, we calculated the residual standard error (RSE). The Ratkowsky parameter (*b*), *Topt* estimated by both Ratkowsky 1983 and MMRT, T_{max} estimated by Ratkowsky 1983, and *Tinf* estimated by MMRT were used as traits for the phylogenetic group comparison. Outliers, potentially due to irregularities in the replicate, were excluded from analysis if they were not within the same order of magnitude as the remaining points in the data set. No more than six to seven outliers were removed. Outliers were typically due to experimental noise at higher temperatures, which was determined by assessing replicate-specific growth curves.

Phylogenetic group comparison

We conducted a phylogenetic group comparison of traits (11–13) to test our hypotheses that Alphaproteobacteria from warmed plots have (i) less temperature-sensitive growth rates; (ii) higher optimum growth temperatures; and (iii) higher maximum growth temperatures compared to isolates from control plots. We used the R package *nlme* to conduct PGLS test (41, 42). A phylogenetic group comparison accounts for the lack of independence in phylogenetic hierarchical species data. A Lilliefors test for normality was used to determine whether residuals of PGLS tests were normally distributed, and Q-Q plots were made. Trait data were transformed if the Lilliefors test failed. We removed outlier data points, which were three to four orders of magnitude greater than the remaining points in the data set.

A genome-based phylogeny was constructed using the United States Department of Energy's Systems Biology Knowledgebase (KBase) (43) (Fig. 2). Genomes were annotated by Prokka (44). The phylogeny was constructed using Insert Genome into SpeciesTree v2.2.0 (45), which creates a multiple sequence alignment based on universal genes defined by COG (Clusters of Orthologous Groups) gene families. We set the nearest public genome count to one and removed the public node in R using the package *ape*

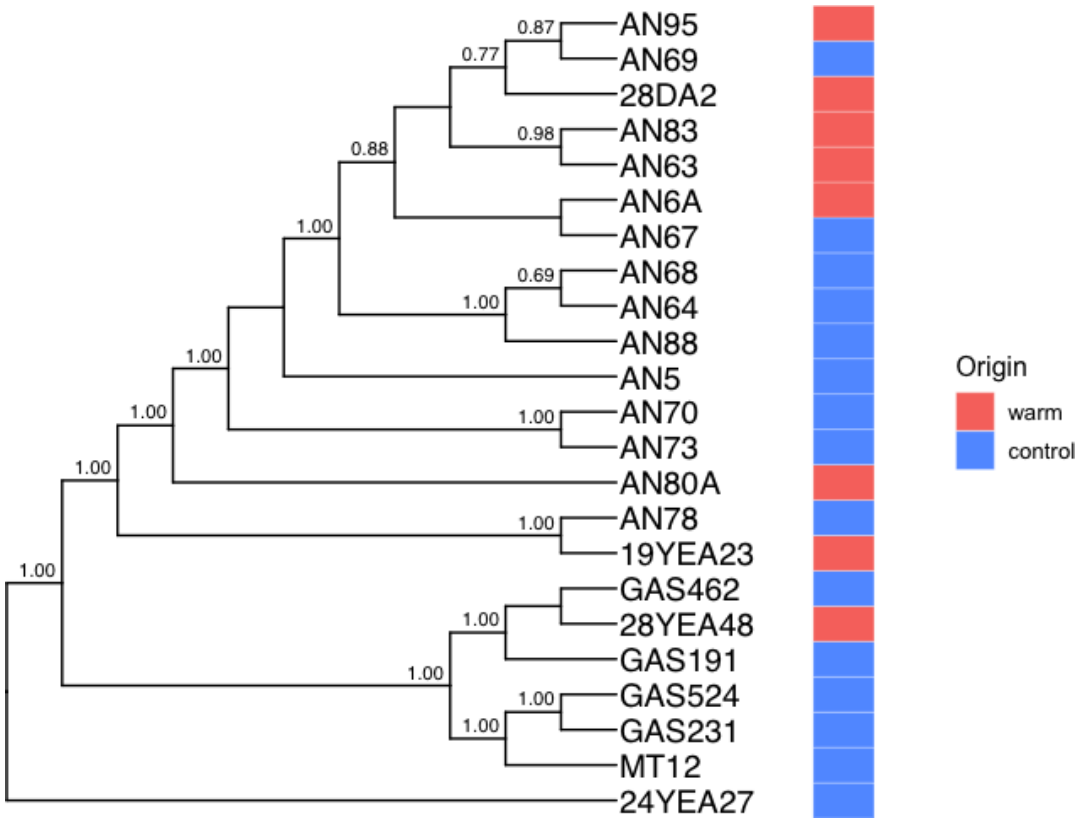

FIG 2 Genome-based phylogeny was constructed using multiple sequence alignment of universal genes found in core orthologous group genes. Nodes are annotated with bootstrapped values.

(46). Temperature sensitivity of growth, optimum growth temperature, and maximum growth temperature were mapped as traits on the phylogeny.

We also calculated phylogenetic signal using Pagel's λ to quantify the tendency of the isolates to closely resemble each other based on their phylogenetic distribution of traits. Pagel's λ is a measure of correlation between species under Brownian Motion (47) and was estimated using the R package *phytools* (48). *P* values and group means were calculated for each microbial growth trait from warmed and control soils. Soil microbial isolates were the experimental unit, and all *P* values less than 0.05 were considered statistically significant relationships. All statistical analyses were performed in R using RStudio (49).

RESULTS

Optimum growth temperature quantified by Ratkowsky 1983 model shows evidence of adaptation

There was a significant difference between optimum growth temperature (*Topt*) of isolates from heated versus control plots quantified by the Ratkowsky 1983 model ($t(23) = 2.84$, $P = 0.01$, $n_{warm} = 8$, $n_{control} = 15$) (Table S3). Optimum growth temperature for isolates from warmed plots ($M = 30.59$, $SD = 1.65$) was greater than those of control plots ($M = 29.75$, $SD = 1.88$) (Table 2). Residuals were normally distributed according to the Lilliefors test ($P = 0.41$, $n_{warm} = 8$, $n_{control} = 15$). Pagel's λ showed that *Topt* was not distributed according to Brownian motion (λ = 6.61E−05, $P = 1.00$) (Fig. 3A).

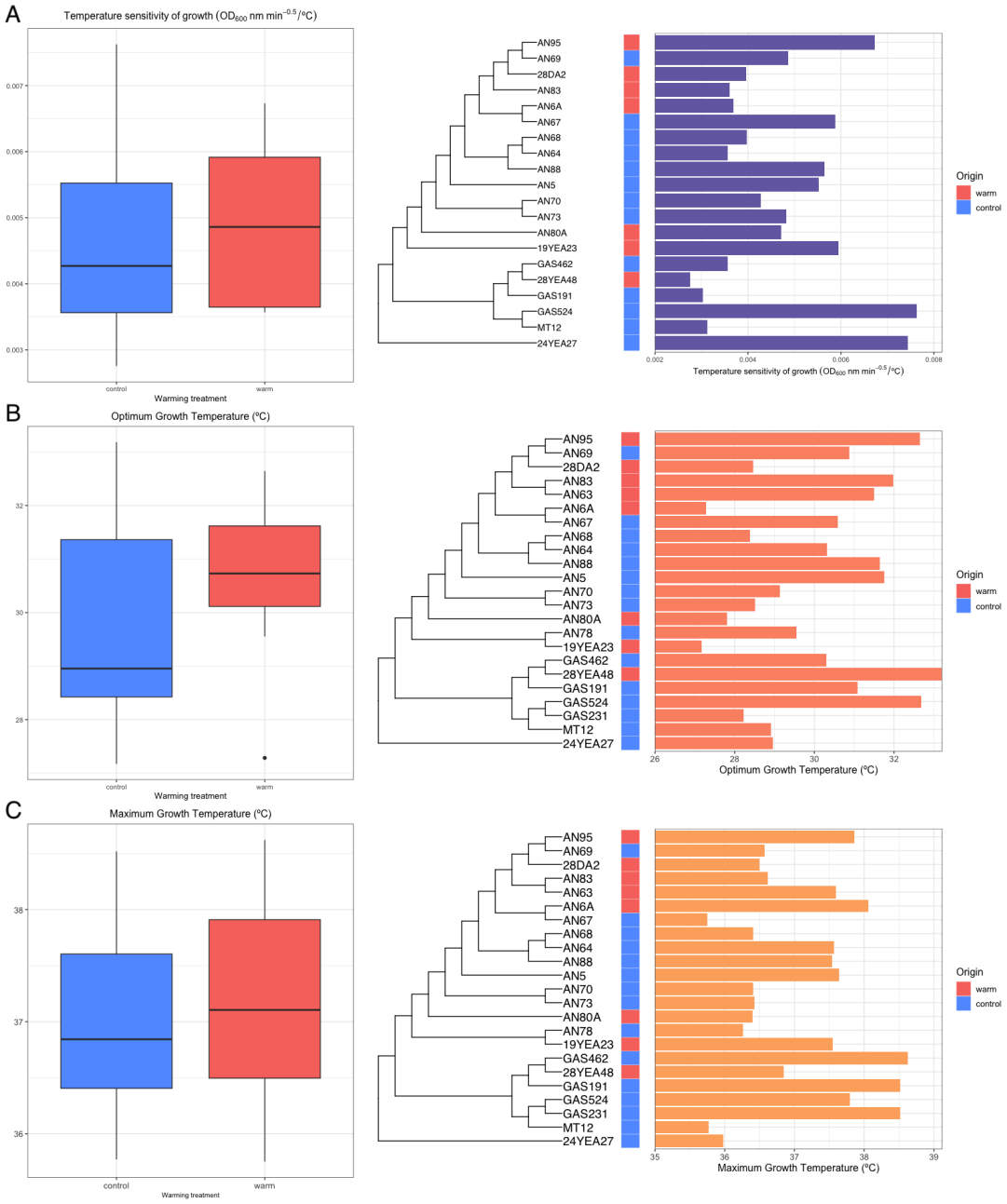

FIG 3 Optimum growth temperature (A), temperature sensitivity of growth (B), and maximum growth temperature (B) were quantified by fitting the Ratkowsky 1983 model on data for growth rate over temperature for each isolate. A multiple sequence alignment of universal genes found in core orthologous group genes was used to construct the phylogenetic tree. Phylogenetic generalized least squares test was used to test for difference in optimum growth temperature between isolates from heated and control soil plots.

No evidence of adaptation of temperature sensitivity of growth quantified by Ratkowsky 1983

Our results showed no significant difference in temperature sensitivity of growth quantified by the Ratkowsky parameter ($t(20) = 1.79$, $P = 0.09$, $n_{warm} = 7$, $n_{control} = 13$) (Table S3). Temperature sensitivity of isolates from warmed and control plots were 0.0049 ($SD = 0.001$) and 0.0047 ($SD = 0.002$) respectively (Table 2). Residuals were normally distributed according to the Lilliefors test ($P > 0.05$). Pagel's λ showed that temperature

sensitivity of growth was not distributed according to Brownian motion, suggesting a random distribution (λ = 6.61E−05, *P* = 1.00) (Fig. 3B).

No evidence of adaptation of maximum growth temperature quantified by Ratkowsky 1983

Results of the phylogenetic least squares test for maximum growth temperature quantified by the Ratkowsky 1983 model showed no significant difference between isolates from heated and control plots ($t(23) = -0.35$, $P > 0.05$, $n_{warm} = 8$, $n_{control} = 15$) (Table S3). Maximum growth temperature for isolates from the warmed and control plots were 37.17°C (*SD* = 1.00) and 37.06°C (*SD* = 0.88), respectively (Table 2). Residuals were normally distributed according to the Lilliefors test (*P* = 0.45). Pagel's λ showed that maximum growth temperature was not distributed according to Brownian motion (λ = 6.61E−05, *P* = 1.00) (Fig. 3C).

Temperature sensitivity inferred by Macromolecular Rate Theory does not show evidence of adaptation

Results of the phylogenetic generalized least squares test for optimum growth temperature quantified by MMRT showed no significant difference between isolates from the heated and control plots ($t(23) = 1.60$, $P = 0.12$, $n_{warm} = 8$, $n_{control} = 15$) (Table S4). Optimum growth temperature for isolates from warmed plots (*M* = 30.26, *SD* = 1.82) was not significantly different than those of control plots (*M* = 30.74, *SD* = 1.72) (Table 2). Residuals were normally distributed according to the Lilliefors test (*P* > 0.05). Pagel's λ showed that *Topt* was not distributed according to Brownian motion (λ = 0.45, *P* > 0.05) (Fig. 4). Residual standard errors indicated that the Ratkowsky 1983 model more adequately fit our data in comparison to the MMRT model (Table S2).

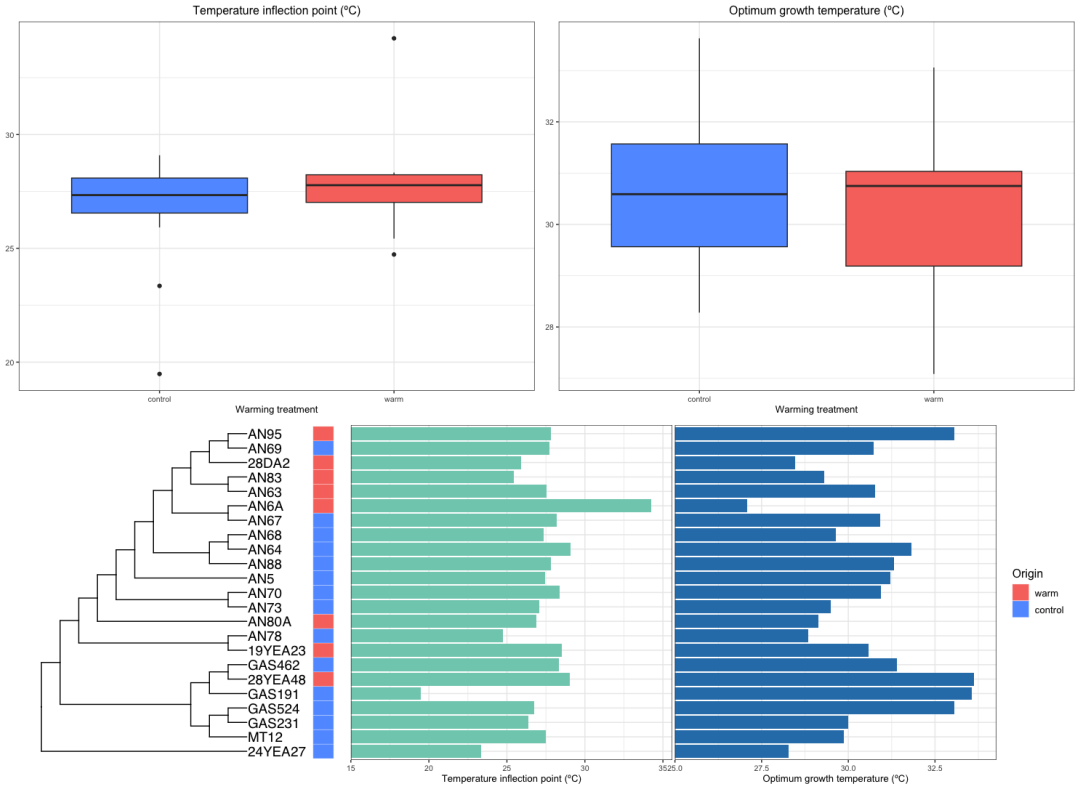

FIG 4 Temperature inflection point (*Tinf*) and optimum growth temperature (*Topt*) were quantified through fitting the Macromolecular Rate Theory model on data for growth rate over temperature for each isolate. A multiple sequence alignment of universal genes found in core orthologous group genes was used to construct the phylogenetic tree. Phylogenetic generalized least squares test was used to test for difference in *Tinf* and *Topt* between isolates from heated and control soil plots.

TABLE 2 Microbial growth traits were estimated by Ratkowsky 1983 and Macromolecular Rate Theory (MMRT) model parameters[a]

Model parameter	Model fit	Warmed mean	Warmed standard deviation	Control mean	Control standard deviation
Optimum growth temperature, *Topt* (°C)	Ratkowsky 1983	30.59	1.65	29.75	1.88
Temperature sensitivity of growth, *b* (OD_{600} nm × $min^{-0.5}$/°C)	Ratkowsky 1983	0.0049	0.001	0.0047	0.002
Maximum growth temperature, T_{max} (°C)	Ratkowsky 1983	37.17	1.00	37.06	0.88
Optimum growth temperature, *Topt* (°C)	MMRT	30.26	1.82	30.74	1.72
Temperature inflection point, *Tinf* (°C)	MMRT	28.00	2.84	26.73	2.45

[a]The mean and standard deviation of model parameters for isolates from the warmed and control plots were also calculated.

There was no significant difference in temperature inflection point (*Tinf*) between isolates from the heated and control soils ($t(23) = 1.04$, $P > 0.05$, $n_{warm} = 8$, $n_{control} = 15$) (Table S4). Residuals of PGLS on untransformed *Tinf* data failed the Lilliefors test for normality ($P < 0.05$). Average temperature inflection point for isolates from warmed plots was 28.00°C ($SD = 2.84$). Average temperature inflection point for isolates from control plots was 26.73°C ($SD = 2.45$) (Table 2). Residuals failed the Lilliefors test for normality following log, square root, and lambda (Box-Cox test) transformations ($P < 0.05$). Pagel's λ showed that *Tinf* was not distributed according to Brownian motion (λ = 6.61E−05, $P > 0.05$) (Fig. 4). Since the residuals failed the Lilliefors test for normality, we transformed *Tinf* (i.e., log transformation, square root, and box-cox). However, all transformations also failed the test for normality, and suggest that results of PGLS for *Tinf* estimated by MMRT should be interpreted with caution.

DISCUSSION

We expected Alphaproteobacteria isolated from warmed plots would have (i) lower temperature sensitivities of growth; (ii) higher optimum growth temperatures; and (iii) higher maximum growth temperatures compared to isolates from control plots. Our results showed evidence of adaptation of optimum growth temperature quantified by the Ratkowsky 1983 model, but not for other measured traits. Evidence of adaptation of *Topt* estimated by the Ratkowsky 1983 model affirm observations from previous studies, where increased optimum growth temperature is associated with warmer soils (50, 51). However, the lack of differences observed in other microbial growth traits estimated by both the Ratkowsky 1983 and MMRT models may be due to the shape of the temperature response curve, model fitting, or the magnitude and duration of warming, for example. Evidence for this conclusion lies in the observation that the Ratkowsky 1983 model fit was better than the MMRT model for optimum growth temperature.

The difference in evidence of adaptation for optimum growth temperature quantified by the Ratkowsky 1983 and modified MMRT models may be due to a difference in fits. The residual standard errors for the Ratkowsky 1983 fitted model on each isolate are two to three orders of magnitude lower than those of the MMRT fitted models (Table S2). Adequate fitting of MMRT requires a data set to at least capture the optimum growth temperature. Although our data set includes *Topt*, it is considerably limited at lower temperatures and lacks growth rate data at the temperature minima. This limitation may be associated with the less accurate MMRT fits, resulting in inaccurate estimations of *Topt*. Alster et al. (52) suggested increasing the number of temperature points for adequate MMRT model fitting, which could be applied in future studies. The difference in evidence of adaptation when *Topt* was estimated by the Ratkowsky 1983 and MMRT models suggests that microbial trait estimations may depend on model fits.

There are several differences between the Ratkowsky 1983 model and Macromolecular Rate Theory. Ratkowsky 1983 is an empirically determined model of growth rate over temperature for each isolate (18). MMRT is based on thermodynamic theory and is not empirically determined. It accounts for changes in the temperature response in

the absence of enzyme denaturation at temperatures above the optimum temperature through changes in heat capacity. The residual standard errors indicate that Ratkowsky 1983 is a more appropriate fit for our data of growth rate over temperature compared to MMRT. However, we are particularly interested in MMRT due to its underlying thermodynamic theory, as well as its application in soil ecosystems (19, 20, 52).

The lack of evidence of adaptation of other microbial growth traits demonstrates the limitations of inferring microbial growth traits based on a single temperature point. Traits such as temperature sensitivity of growth are more nuanced and may be impacted by thermal niche breadth. Thermal niche breadth is the range of temperatures that permits microbial growth. Previous studies observed that changes in the range between minimum and maximum growth temperatures depended on soil incubation temperatures (53, 54). This suggests that the relationship between growth rate and temperature may also vary between minimum and maximum growth temperatures. The rate at which growth rate changes across temperatures, or the steepness of the temperature response curve, may be impacted by the environment, thus altering thermal niche breadth. Challenges in quantifying change in microbial growth rate over temperature may result if such environmental factors are not fully accounted for. This concept of a thermal niche breadth may have an associated fitness cost, as seen with other microorganisms (55). Therefore, it may be challenging to identify microbial growth trait adaptation without also considering changing thermal niche breadths.

Pagel's λ was intermediate ($0 < \lambda < 1$) for all microbial growth traits, which suggests that the distribution of traits was not as expected under Brownian Motion. There are multiple explanations for such results. One explanation is that climate warming may be associated with selection of intermediate phenotypes (i.e., stabilizing selection) instead of extremes. This may have resulted in constrained trait evolution. Additionally, changes in evolutionary rate over time may have also resulted in non-Brownian Motion distribution of traits (51). It is possible that discontinuous substrate availability over the decades of experimental warming could have caused a difference in growth rate, and possibly evolutionary rate, over time (14). Phylogenetic signal is also often quantified by Blomberg's *K*, which is a variance ratio and has the advantage of being able to be greater than one. However, our data were not suitable for Blomberg's *K* estimations as it resulted in a singular matrix.

Soil microbial growth tends to be limited by substrate availability, so evidence of adaptation from PGLS tests may have been occluded by high levels of nutrient availability in the laboratory growth conditions of these experiments. Kamble et al. (56) observed that bacterial and fungal growth in soils was carbon limited (56). In a community-level experiment in a boreal forest, Ekblad et al. (57) observed that soil microbial biomass was limited by carbon but not nitrogen availability (57). Although these experiments were conducted in soils on the community level, it is possible that the carbon and nutrient-rich media used in this study may obscure the effect of nutrient availability and substrate-specific growth dynamics of microbes in warming soils. Studying microbial growth under lower nutrient conditions may provide a different perspective on how warming impacts microbial growth traits.

Thermal adaptation of increasing growth with temperature has been observed for other organisms in response to climate warming. Among other microorganisms, growth rate of pathogenic fungi, *Mycosphaerella graminicola* was observed to be associated with increasing temperatures (58). Globally distributed plant pathogens were also found to locally adapt to their environments, resulting in significantly different optimum growth temperatures (59). Thermal adaptation is also often investigated more broadly among other ectotherms. Villeneuve et al. (60) observed that growth of *Urosalpinx cinerea* (Atlantic oyster drill) was positively associated with spawning temperature (60). Studying thermal adaptation is highly relevant as the effects of the climate crisis increase. However, doing so is challenging among organisms with longer generation times, which highlights the importance of utilizing techniques beyond lab and field-based experiments and suggests a benefit to studying adaptation among organisms with

short generation times and large populations like microbes. It is also possible that the organismic adaptation to temperature appears overly significant due to the difficulty in publishing negative or non-significant results.

Change in microbial growth traits is just one example of how warming may impact soil microbes. Increasing temperatures are also associated with evolutionary selection of organisms with smaller genome sizes, as seen in fire-affected soils (61). Evidence of adaptation for other metabolic processes, such as respiration, has also been observed (62, 63). Differences in microbial growth traits between isolates from warmed and control soils may be due to reasons other than adaptation. Such differences may be due to depletion of labile carbon (14, 64), changing microbial community structure (14, 65), microbial physiology (66), and species sorting and functional diversity (51).

While thermal adaptation of microbial traits has been observed in other studies, our results demonstrate that measuring growth potential may be impacted by additional factors. We used laboratory settings to quantify microbial growth traits, which may be an inaccurate representation of field conditions. Under these conditions, results of our study suggest that warming has not resulted in adaptation of temperature sensitivity of growth and maximum growth temperature quantified by the Ratkowsky 1983 model and temperature inflection point and optimum growth temperature quantified by MMRT. However, optimum growth temperature estimated by Ratkowsky 1983 showed some evidence of adaptation. As temperatures increase, changes in soil microbial growth rate may affect rates of atmospheric carbon cycling. Future exploration of whether growth strategies explain microbial adaptation to warming will help predict changes in microbial community and ecosystem function and allow us to better understand soil microbial responses to warming.

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DATA AVAILABILITY

The whole genome assemblies have been deposited at GenBank under the accession numbers listed in Table S1, along with the raw data deposited in the Sequence Read Archive, and associated BioProject and BioSamples. Microbial growth data can be found in the Harvard Forest Data archive HF438. All code used for model fitting and data analysis are available at Github.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Fig. S1 (AEM00825-23-s0001.pdf). Ratkowsky 1983 fits for all isolates.
Fig. S2 (AEM00825-23-s0002.pdf). Macromolecular Rate Theory fits for all isolates.
Tables S1 to S4 (AEM00825-23-s0003.xlsx). Table S1 contains genome metadata. n.d. denotes that data is no longer available for raw reads. Table S2 includes residual standard error (RSE) data for Ratkowsky 1983 and Macromolecular Rate Theory (MMRT) for all isolates. Tables S3 and S4 include estimates of microbial traits for both Ratkowsky 1983 and MMRT models, respectively.

REFERENCES

1. Masson-Delmotte V, Zhai P, Pirani A, Connors SL, Péan C, Berger S, Caud N, Chen Y, Goldfarb L. 2021. Climate change 2021: the physical science basis. In Gomis MI (ed), Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change. Cambridge University Press.
2. Davidson EA, Janssens IA. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165–173. https://doi.org/10.1038/nature04514
3. Bradford MA. 2013. Thermal adaptation of decomposer communities in warming soils. Front Microbiol 4:333. https://doi.org/10.3389/fmicb.2013.00333
4. Wallenstein MD, Hall EK. 2012. A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. Biogeochemistry 109:35–47. https://doi.org/10.1007/s10533-011-9641-8
5. Falkowski PG, Fenchel T, Delong EF. 2008. The microbial engines that drive earth's biogeochemical cycles. Science 320:1034–1039. https://doi.org/10.1126/science.1153213
6. Pold G, Billings AF, Blanchard JL, Burkhardt DB, Frey SD, Melillo JM, Schnabel J, van Diepen LTA, DeAngelis KM. 2016. Long-term warming alters carbohydrate degradation potential in temperate forest soils. Appl Environ Microbiol 82:6518–6530. https://doi.org/10.1128/AEM.02012-16
7. Schoenholtz SH, Miegroet HV, Burger JA. 2000. A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. For Ecol Manag 138:335–356. https://doi.org/10.1016/S0378-1127(00)00423-0
8. Whalen ED, Grandy AS, Sokol NW, Keiluweit M, Ernakovich J, Smith RG, Frey SD. 2022. Clarifying the evidence for microbial- and plant-derived soil organic matter, and the path toward a more quantitative understanding. Glob Chang Biol 28:7167–7185. https://doi.org/10.1111/gcb.16413
9. Stockmann U, Adams MA, Crawford JW, Field DJ, Henakaarchchi N, Jenkins M, Minasny B, McBratney AB, Courcelles V de R de, Singh K, Wheeler I, Abbott L, Angers DA, Baldock J, Bird M, Brookes PC, Chenu C, Jastrow JD, Lal R, Lehmann J, O'Donnell AG, Parton WJ, Whitehead D, Zimmermann M. 2013. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. Agric Ecosyst Environ 164:80–99. https://doi.org/10.1016/j.agee.2012.10.001
10. Malik AA, Martiny JBH, Brodie EL, Martiny AC, Treseder KK, Allison SD. 2020. Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. ISME J 14:1–9. https://doi.org/10.1038/s41396-019-0510-0
11. Felsenstein J. 1985. Phylogenies and the comparative method. Am Nat 125:1–15. https://doi.org/10.1086/284325
12. Washburne AD, Morton JT, Sanders J, McDonald D, Zhu Q, Oliverio AM, Knight R. 2018. Methods for phylogenetic analysis of microbiome data. Nat Microbiol 3:652–661. https://doi.org/10.1038/s41564-018-0156-0
13. Yang Z, Rannala B. 2012. Molecular phylogenetics: principles and practice. Nat Rev Genet 13:303–314. https://doi.org/10.1038/nrg3186
14. Melillo JM, Frey SD, DeAngelis KM, Werner WJ, Bernard MJ, Bowles FP, Pold G, Knorr MA, Grandy AS. 2017. Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. Science 358:101–105. https://doi.org/10.1126/science.aan2874
15. DeAngelis KM, Pold G, Topçuoğlu BD, van Diepen LTA, Varney RM, Blanchard JL, Melillo J, Frey SD. 2015. Long-term forest soil warming

alters microbial communities in temperate forest soils. Front Microbiol 6:104. https://doi.org/10.3389/fmicb.2015.00104
16. Rousk J, Frey SD, Bååth E. 2012. Temperature adaptation of bacterial communities in experimentally warmed forest soils. Glob Chang Biol 18:3252–3258. https://doi.org/10.1111/j.1365-2486.2012.02764.x
17. Zwietering MH, Jongenburger I, Rombouts FM, van 't Riet K. 1990. Modeling of the bacterial growth curve. Appl Environ Microbiol 56:1875–1881. https://doi.org/10.1128/aem.56.6.1875-1881.1990
18. Ratkowsky DA, Lowry RK, McMeekin TA, Stokes AN, Chandler RE. 1983. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. J Bacteriol 154:1222–1226. https://doi.org/10.1128/jb.154.3.1222-1226.1983
19. Alster CJ, Robinson JM, Arcus VL, Schipper LA. 2022. Assessing thermal acclimation of soil microbial respiration using macromolecular rate theory. Biogeochemistry 158:131–141. https://doi.org/10.1007/s10533-021-00885-6
20. Alster CJ, van de Laar A, Goodrich JP, Arcus VL, Deslippe JR, Marshall AJ, Schipper LA. 2023. Quantifying thermal adaptation of soil microbial respiration. Nat Commun 14:5459. https://doi.org/10.1038/s41467-023-41096-x
21. Peterjohn WT, Melillo JM, Steudler PA, Newkirk KM, Bowles FP, Aber JD. 1994. Responses of trace gas fluxes and N availability to experimentally elevated soil temperatures. Ecol Appl 4:617–625. https://doi.org/10.2307/1941962
22. Ioerger TR, Feng Y, Ganesula K, Chen X, Dobos KM, Fortune S, Jacobs WR, Mizrahi V, Parish T, Rubin E, Sassetti C, Sacchettini JC. 2010. Variation among genome sequences of H37Rv strains of *Mycobacterium tuberculosis* from multiple laboratories. J Bacteriol 192:3645–3653. https://doi.org/10.1128/JB.00166-10
23. Tarver A, Fern A, Diego MS, Kennedy M, Zane M, Daum C, Hack C, Tang E, Deshpande S, Cheng J-F, Roberts S, Alexandre M, Miranda H-S, Lucas S. 2010. Illumina production sequencing at the DOE joint genome institute - workflow and optimizations. Lawrence Berkeley National Lab, Berkeley, CA (United States). https://www.osti.gov/biblio/985905.
24. Choudoir MJ, Narayanan A, Rodriguez-Ramos D, Simoes R, Efroni A, Sondrini A, DeAngelis KM. 2023. Pangenomes reveal genomic signatures of microbial adaptation to experimental soil warming. Microbiology. https://doi.org/10.1101/2023.03.16.532972
25. Wick RR, Judd LM, Holt KE. 2019. Performance of neural network basecalling tools for Oxford Nanopore sequencing. Genome Biol 20:129. https://doi.org/10.1186/s13059-019-1727-y
26. Wick R, Menzel P. 2019. Filtlong: quality filtering tool for long reads. Available from: https://github.com/rrwick/Filtlong
27. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads using repeat graphs. Nat Biotechnol 37:540–546. https://doi.org/10.1038/s41587-019-0072-8
28. Vaser R, Sović I, Nagarajan N, Šikić M. 2017. Fast and accurate *de novo* genome assembly from long uncorrected reads. Genome Res 27:737–746. https://doi.org/10.1101/gr.214270.116
29. Github. 2023. Medaka. Available from: https://github.com/nanopore-tech/medaka
30. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086
31. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. https://doi.org/10.1101/gr.186072.114
32. Willey JM, Sherwood L, Woolverton CJ, Prescott PLM. 2008. Harley, and Klein's microbiology. McGraw-Hill Higher Education, New York. http://catdir.loc.gov/catdir/toc/ecip0619/2006027152.html.
33. Harvard Forest. 2023 1. Temperature & humidity. Available from: https://harvardforest.fas.harvard.edu/real-time-data-graphs/1-temperature-humidity
34. Sprouffske K, Wagner A. 2016. Growthcurver: an R package for obtaining interpretable metrics from microbial growth curves. BMC Bioinformatics 17:172. https://doi.org/10.1186/s12859-016-1016-7
35. Zwietering MH, de Koos JT, Hasenack BE, de Witt JC, van't Riet K. 1991. Modeling of bacterial growth as a function of temperature. Appl Environ Microbiol 57:1094–1101. https://doi.org/10.1128/aem.57.4.1094-1101.1991
36. Padfield D, O'Sullivan H, Pawar S. 2021. *rTPC* and nls.multstart: a new pipeline to fit thermal performance curves in r. Methods Ecol Evol 12:1138–1143. https://doi.org/10.1111/2041-210X.13585
37. Hobbs JK, Jiao W, Easter AD, Parker EJ, Schipper LA, Arcus VL. 2013. Change in heat capacity for enzyme catalysis determines temperature dependence of enzyme catalyzed rates. ACS Chem Biol 8:2388–2393. https://doi.org/10.1021/cb4005029
38. Darros - Barbosa R, Balaban MO, Teixeira AA. 2003. Temperature and concentration dependence of heat capacity of model aqueous solutions. Int J Food Prop 6:239–258. https://doi.org/10.1081/JFP-120017845
39. Ghosh A, McSween Jr HY. 1999. Temperature dependence of specific heat capacity and its effect on asteroid thermal models. Meteorit Planet Sci 34:121–127. https://doi.org/10.1111/j.1945-5100.1999.tb01737.x
40. Prentice EJ, Hicks J, Ballerstedt H, Blank LM, Liáng LNL, Schipper LA, Arcus VL. 2020. The inflection point hypothesis: the relationship between the temperature dependence of enzyme-catalyzed reaction rates and microbial growth rates. Biochemistry 59:3562–3569. https://doi.org/10.1021/acs.biochem.0c00530
41. Garland T, Dickerman AW, Janis CM, Jones JA. 1993. Phylogenetic analysis of covariance by computer simulation. Syst Biol 42:265–292. https://doi.org/10.1093/sysbio/42.3.265
42. Lindstrom ML, Bates DM. 1990. Nonlinear mixed effects models for repeated measures data. Biometrics 46:673–687. https://doi.org/10.2307/2532087
43. Arkin AP, Cottingham RW, Henry CS, Harris NL, Stevens RL, Maslov S, Dehal P, Ware D, Perez F, Canon S, Sneddon MW, Henderson ML, Riehl WJ, Murphy-Olson D, Chan SY, Kamimura RT, Kumari S, Drake MM, Brettin TS, Glass EM, Chivian D, Gunter D, Weston DJ, Allen BH, Baumohl J, Best AA, Bowen B, Brenner SE, Bun CC, Chandonia J-M, Chia J-M, Colasanti R, Conrad N, Davis JJ, Davison BH, DeJongh M, Devoid S, Dietrich E, Dubchak I, Edirisinghe JN, Fang G, Faria JP, Frybarger PM, Gerlach W, Gerstein M, Greiner A, Gurtowski J, Haun HL, He F, Jain R, Joachimiak MP, Keegan KP, Kondo S, Kumar V, Land ML, Meyer F, Mills M, Novichkov PS, Oh T, Olsen GJ, Olson R, Parrello B, Pasternak S, Pearson E, Poon SS, Price GA, Ramakrishnan S, Ranjan P, Ronald PC, Schatz MC, Seaver SMD, Shukla M, Sutormin RA, Syed MH, Thomason J, Tintle NL, Wang D, Xia F, Yoo H, Yoo S, Yu D. 2018. KBase: the United States department of energy systems biology knowledgebase. Nat Biotechnol 36:566–569. https://doi.org/10.1038/nbt.4163
44. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153
45. Price MN, Dehal PS, Arkin AP. 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol Biol Evol 26:1641–1650. https://doi.org/10.1093/molbev/msp077
46. Paradis E, Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35:526–528. https://doi.org/10.1093/bioinformatics/bty633
47. Pagel M. 1999. Inferring the historical patterns of biological evolution. Nature 401:877–884. https://doi.org/10.1038/44766
48. Revell LJ. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol Evol 3:217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x
49. RStudio. 2022. Rstudio: integrated development environment for R. Available from: http://www.rstudio.com/
50. Donhauser J, Niklaus PA, Rousk J, Larose C, Frey B. 2020. Temperatures beyond the community optimum promote the dominance of heat-adapted, fast growing and stress resistant bacteria in alpine soils. Soil Biol Biochem 148:107873. https://doi.org/10.1016/j.soilbio.2020.107873
51. Smith TP, Mombrikotb S, Ransome E, Kontopoulos D-G, Pawar S, Bell T. 2022. Latent functional diversity may accelerate microbial community responses to temperature fluctuations. Elife 11:e80867. https://doi.org/10.7554/eLife.80867
52. Alster CJ, von Fischer JC, Allison SD, Treseder KK. 2020. Embracing a new paradigm for temperature sensitivity of soil microbes. Glob Chang Biol 26:3221–3229. https://doi.org/10.1111/gcb.15053
53. Rijkers R, Rousk J, Aerts R, Sigurdsson BD, Weedon JT. 2022. Optimal growth temperature of arctic soil bacterial communities increases under experimental warming. Glob Chang Biol 28:6050–6064. https://doi.org/10.1111/gcb.16342

54. van Gestel NC, Reischke S, Bååth E. 2013. Temperature sensitivity of bacterial growth in a hot desert soil with large temperature fluctuations. Soil Biol Biochem 65:180–185. https://doi.org/10.1016/j.soilbio.2013.05.016
55. Herren CM, Baym M. 2022. Decreased thermal niche breadth as a trade-off of antibiotic resistance. ISME J 16:1843–1852. https://doi.org/10.1038/s41396-022-01235-6
56. Kamble PN, Bååth E. 2018. Carbon and nitrogen amendments lead to differential growth of bacterial and fungal communities in a high-pH soil. Pedosphere 28:255–260. https://doi.org/10.1016/S1002-0160(18)60014-1
57. Ekblad A, Nordgren A. 2002. Is growth of soil microorganisms in boreal forests limited by carbon or nitrogen availability?Plant and Soil 242:115–122. https://doi.org/10.1023/A:1019698108838
58. Zhan J, McDonald BA. 2011. Thermal adaptation in the fungal pathogen *Mycosphaerella graminicola*. Mol Ecol 20:1689–1701. https://doi.org/10.1111/j.1365-294X.2011.05023.x
59. Boixel A-L, Chelle M, Suffert F. 2022. Patterns of thermal adaptation in a globally distributed plant pathogen: local diversity and plasticity reveal two-tier dynamics. Ecol Evol 12:e8515. https://doi.org/10.1002/ece3.8515
60. Villeneuve AR, Komoroske LM, Cheng BS. 2021. Environment and phenology shape local adaptation in thermal performance. Proc Biol Sci 288:20212554. https://doi.org/10.1098/rspb.2021.2554
61. Sorensen JW, Dunivin TK, Tobin TC, Shade A. 2019. Ecological selection for small microbial genomes along a temperate-to-thermal soil gradient. Nat Microbiol 4:55–61. https://doi.org/10.1038/s41564-018-0276-6
62. Nottingham AT, Scott JJ, Saltonstall K, Broders K, Montero-Sanchez M, Püspök J, Bååth E, Meir P. 2022. Microbial diversity declines in warmed tropical soil and respiration rise exceed predictions as communities adapt. Nat Microbiol 7:1650–1660. https://doi.org/10.1038/s41564-022-01200-1
63. Tian W, Sun H, Zhang Y, Xu J, Yao J, Li J, Li B, Nie M. 2022. Thermal adaptation occurs in the respiration and growth of widely distributed bacteria. Glob Chang Biol 28:2820–2829. https://doi.org/10.1111/gcb.16102
64. Bradford MA, Davies CA, Frey SD, Maddox TR, Melillo JM, Mohan JE, Reynolds JF, Treseder KK, Wallenstein MD. 2008. Thermal adaptation of soil microbial respiration to elevated temperature. Ecol Lett 11:1316–1327. https://doi.org/10.1111/j.1461-0248.2008.01251.x
65. Frey SD, Drijber R, Smith H, Melillo JM. 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biol Biochem 40:2904–2907. https://doi.org/10.1016/j.soilbio.2008.07.020
66. Allison SD, Wallenstein MD, Bradford MA. 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geosci 3:336–340. https://doi.org/10.1038/ngeo846