Experimental Warming Effects on Microbial Growth Evolution

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Abstract

Climate change poses critical challenges to ecosystems worldwide, and understanding the implications of prolonged warming on microbial communities is crucial to predict its broader impacts. Microbial communities, which are integral to organic matter decomposition, nutrient cycling, and carbon storage, play a key role in ecosystem functioning. This study looks at the relationship between changing temperatures, microbial evolution, and their effects on ecosystem processes. This research was conducted at the Harvard Forest Long-Term Ecological Research (LTER) site in Petersham, Massachusetts, where experimental soils have been undergoing a warming effect of 5ºC since 1991 through buried electrical cables. While increasing research supports the claim that microbial communities are vital in climate change mitigation, there is still a gap in our understanding of how long-term warming influences microbial communities. Using quantitative stable isotope probing (qSIP) this study quantifies isotopic enrichment (18O-) to observe microbial responses to warming and by extension, climate change. We hypothesize that taxa in heated plots will exhibit differential growth rates compared to disturbance control plots, with slower growth attributed to limited microbial substrate availability at higher temperatures. Additionally, we explore the temperature sensitivity of microbial growth, to understand whether adaptation to long-term warming is present in these communities. Insights gained from this study are crucial for predicting future ecosystem soil processes in the context of climate change, offering a glimpse into the potentially irreversible alterations of microbes, and the implications for the environment. Eventually this research will enhance our ability to manage the cascading effects of climate change on ecosystems.

Keywords: Soil Carbon Dynamics, Anthropogenic and Natural Disturbance, Microbial response to Climate Change, qSIP, Growth Rates, Phylogenetic Conservation of Traits, Adaptation

Section 1. Introduction

Climate change is the most pressing environmental challenge, with its vast and detrimental implications: increasing frequency and intensity of droughts, storms, heat waves, rising sea levels, melting glaciers, and warming oceans. These implications can directly harm plants and animals, destroy habitat, and alter ecosystem communities. Ecological communities are made of many species, who each have separate ecological and evolutionary purposes, with some species playing key roles in maintaining the functioning of ecosystems, and contributing dynamics needed for ecosystem functioning (Levin & Pacala, 2003). There are many examples of these dynamics, such as keystone predation which keep populations in check. Another important dynamic is that contributed by soil microbial communities acting as a carbon sink and regulating carbon from easily entering our atmosphere. The amount of carbon found stored in soil is far greater than that found in plant biomass and atmospheric carbon, thus soil carbon stability can impact global climate change (Purcell et al., 2022).

Microbial communities contribute to ecosystem functioning in many ways including organic matter decomposition, nutrient mineralization, and carbon cycling. Soil microbes both reduce and contribute to carbon stores and stability through production of by-products and biomass, with healthy soils absorbing more carbon than releasing. In this way, soil can mitigate climate change by reducing the amount of carbon in the atmosphere. Microbial processes which control carbon fluxes are temperature sensitive (Davidson & Janssens, 2006). While it is widely acknowledged that microbes are ecologically significant, there is no uniform answer to how long-term warming will shape microbial communities (Purcell et al., 2022; Propster et al., 2023). To accurately understand the role of microbes in climate change, it is important we understand how climate change drives microbial evolution.

Rising temperatures, influenced by both anthropogenic and natural disturbances, can impact the evolution of organisms by altering hybridization patterns, population size, and gene flow. Understanding how microbes are evolving in a warming climate can determine what the sustained effects of warming will be, further identifying how individual bacterial taxa and phylogenetic groups are affected by heat will help us to predict the future of ecosystem soil processes which will have an influence on the feedback loops of climate change. Additionally, the interplay between changing temperatures and microbial diversity underscores a critical link in the functioning of ecosystems (Delgado-Baquerizo et al., 2016). As the climate changes, understanding the role of microbial diversity in ecosystem function will enhance our ability to predict the repercussions of warming on soil processes.

Microbial communities are either acclimating or adapting to warmer temperatures (Bradford, 2013). Acclimation, as we define it, is the short-term process in which an individual organism adjusts to a change in its environment through a regulation of gene expression. On the other hand, adaptation is a genetic based change that results in the evolution of an organism. When microbes adapt, they acquire new traits that change the way microbes respond to changes in the environment. Microbial adaptation to climate change may alter ecosystem functioning by impacting carbon soil cycling. The possibility of these communities adapting to climate change has been speculated to lead to a loss in soil carbon (Cavicchioli et al., 2019). It is not yet known whether microbial communities are acclimating or adapting to rising temperatures. This research aims to contribute to our understanding of microbial responses to long-term warming and their role in ecosystem processes, providing insights into the potential impacts of climate change.

We hypothesize that (1) there is a difference in growth rates between taxa in heated and disturbance control plots, where at 15ºC and 25ºC taxa will grow more slowly in heated plots

despite the temperature increase because of the lack of microbial substrate availability. This is based on Propster et al. 2023 which found a decreased growth rate in heated plots. We use two separate incubation temperatures to measure additional growth rates (15ºC and 25ºC) to estimate how growth rates change with temperatures. We further hypothesize that (2) the temperature sensitivity of growth rate (calculated as Q_{10} based on growth at 25ºC and 15ºC) is phylogenetically conserved, and greater in heated plots than disturbance control. This is based on Wang et al. 2021 where it was previously shown that growth and temperature sensitivity were phylogenetically conserved. Lastly, we hypothesize that (3) the differences in growth rate and Q_{10} between taxa in heated and disturbance control plots show evidence of adaptation to long-term warming.

To study the impact of long-term warming on soil microbial communities we worked out of the Harvard Forest Long-Term Ecological Research (LTER) site in Petersham, Massachusetts. Here, soils have been heated at 5ºC above ambient temperature since 1991, by buried electrical cables. We used soils coming from the heated and disturbance control plots to perform a quantitative stable isotope probing (qSIP) analysis. To understand how microbes are responding to changes in an environment, qSIP is used to make quantitative measurements of isotope enrichment and therefore measurements of microbial activity (Hungate et al., 2015). qSIP is a technique used in microbial ecology to measure the incorporation of isotopically labeled substrates (such as oxygen via water or carbon via metabolites) by specific microbial taxa in an environment. qSIP can further be used in determining how specific microbial rates are influenced by changing climates and can help clarify the role of microbes in regulating ecosystem processes (Koch et al., 2018). To understand how microbes regulate ecosystem processes (such as carbon storage and sequestration) specific microbial rates are needed to see how microbes evolve to

their environment (Nemergut et al., 2013). Rates are crucial in discovering what the long-term impact of warming will be on the important ecosystem functions provided by microbes. Other studies look at how both the short and long-term (Propster et al., 2023; Purcell et al., 2022) warming of soil has affected growth rates of bacterial communities, but they haven't studied if microbes have adapted or acclimated to rising temperature. Both Purcell et al. 2022 and Propster et al. 2023 calculated microbial growth rates; they test if warming has a significant effect on the mean growth rates of all taxa. We calculate the warming effect on growth rates per taxa. Further, these studies and studies such as Wang et al. 2023, which use qSIP to look at temperature sensitivity haven't attributed their calculations to microbial adaptation versus acclimation. We are analyzing qSIP data in such a way that allows us to distinguish microbial adaptation from acclimation.

qSIP allows us to identify active microbial taxa through the utilization of isotopically enriched substrates, quantify microbial growth rates per taxa, and investigate microbial responses to climate change. We will use this qSIP data to calculate excess atom fraction (EAF), which is used in the calculation of microbial growth rates and the calculation of temperature sensitivity of growth. From our calculations of these traits, we will be able to distinguish whether microbes are acclimated to or adapted to warmer conditions.

Section 2. Review of literature

2.1 Ecological implications of climate change

Climate change is the long-term alteration of temperature and weather patterns (Ibáñez et al., 2023). Anthropogenic activities, primarily the emissions of greenhouse gases, have caused global warming (Calvin et al., 2023). The global surface temperature has risen by 1.1ºC above the pre-industrial levels of 1850-1900 during 2011-2020 (Calvin et al., 2023). The global annual

temperature has increased by 0.08ºC on average between 1880 and 1981 (NOAA, 2022). This rise in temperatures has caused sea level to increase by 0.20m between 1901 and 2018, expediting the average rate of sea level rise from 1.3 $mm\ yr^{-1}$ to 1.9 $mm\ yr^{-1}$ (Calvin et al., 2023). Climate change has caused substantial damages and losses to all ecosystems.

Soil erosion, which is expected to increase in response to climate change, will not only affect human well-being but also ecosystem services (Eekhout & De Vente, 2022). This erosion mostly affects the fertile topsoil layer, which is an essential part in the productivity of ecosystems and is fundamental for food security (Eekhout & De Vente, 2022). Soil erosion also interacts with climate change itself by affecting biogeochemical cycles. Micro-organisms in soil play critical roles in these soil cycles through nutrient cycling such as the carbon and nitrogen cycles. Microbes can directly affect climate change not only because of their involvement in cycles which lead to greenhouse gas (CO_2, CH_4 , and N_2O) (Tiedje et al., 2022) synthesis and consumption, but also because they can mitigate climate change (Ibáñez et al., 2023). Microbes play an important role in carbon sequestration; certain microbes which have faster metabolic rates and can sequester carbon faster than others (Ibáñez et al., 2023). Erosion increases microbial loss from soil, impacting the way in which they interact with climate change through their biogeochemical cycles (Berhe et al., 2014).

There is evidence that temperature shifts can both increase and decrease microbial biodiversity. To begin, temperature variations can increase microbial biodiversity in various ways. First, increasing temperature can lead to higher metabolism and growth rates, and population doubling times and rates of ecological and evolutionary processes (Zhou et al., 2016). Second, in terrestrial ecosystems higher temperatures are associated with higher rates of ecosystem productivity meaning more species can be supported (Zhou et al., 2016). Third, higher

temperatures can allow for more plant species, and more plant diversity can provide more substrates for micro-organisms, causing more microbial diversity. Higher microbial diversity can potentially enhance processes such as decomposition, nutrient cycling, and carbon sequestration (Ibáñez et al., 2023). On the other hand, increased temperature can also decrease microbial diversity through other processes. Temperature can be deterministic, and select upon more adapted micro-organisms and limit the dispersal of species leading to a decrease in random dispersal of microbial species thus slowing the rate at which microbial communities change over time (Ibáñez et al., 2023).

2.2 Microbial biodiversity and ecosystem functioning

Biodiversity is achieved when different species that hold unique adaptations and traits, enabling them to perform distinct ecological roles, affect different ecosystem processes (Isbell et al. 2017; Loreau et al., 2001). Ecosystems with more niches can support more biodiversity, as a niche is the range of conditions necessary for a species to survive. Species richness and evenness generally show a positive effect on ecosystem productivity, while functional, phylogenetic, and genetic diversity seem to have an effect on mixed ecosystem processes (Correia & Lopes, 2023).

Different microbial species have direct and indirect effects on the ecosystems in which they are found. Directly, soil microbes form symbiotic associations with plants and can supply limited nutrients to the plants to support plant productivity. Nitrogen-fixing bacteria are important in this productivity because plants cannot fix atmospheric nitrogen, and nitrogen along with phosphorus and potassium is necessary in plant biomass production (Chapin, 1980). Mycorrhixal fungi and nitrogen fixing bacteria contribute 20% nitrogen annually acquired by vegetation in grasslands and savannahs (Cleveland et al., 1999; Van der Heriden et al. 2006).

Microbes contribute 80% of nitrogen in temperate and boreal forests (Van der heriden et al. 2008). They also are responsible for up to 75% of phosphorus acquired by plants annually (Van der heriden et al., 2008). Fungi increase plant diversity in European grasslands by as much as 30% (Grime et al., 1987) by promoting seedling establishment and enhancing subordinate plants' ability to compete with dominant species (Grime et al., 1987). Soil microbes contribute to spatial and temporal diversity in plant communities (Van Der Putten 2003).

Microbes indirectly influence ecosystems, primarily through plant productivity, as free-living microbes alter the supply of nutrients. Free-living microbes influence plant nutrient availability by breaking down soluble and insoluble organic materials, converting them into plant available forms (Schimel and Bennett 2004). Free-living nitrogen fixing bacteria also contribute to the available nitrogen in terrestrial ecosystems (Cleveland et al. 1999). Free-living microbes contribute to plant diversity through their control on availability of organic and inorganic nitrogen in soil (Van der heriden et al. 2008).

Microbial diversity in many ecosystems has declined as a consequence of human interference (Torsvik et al., 1996). Humans have reduced microbial diversity by increasing landuse intensification, nitrogen deposition, and chemical contamination (Torsvik et al., 1996). While many studies have played with the diversity of microbes to see how this affects plant productivity, diversity and nutrient acquisition, there are no uniform findings. Some have found that increased microbial diversity leads to increased decomposition and nitrogen leaching (Bonkowski and Roy, 2005). Others have found that diversity effects are most prevalent in environments where there is a lack of microbial diversity to begin with (Wertz et al. 2006). Enhanced decomposition by microbes provides nutrients utilized for plant productivity.

2.3 Accounting for phylogeny in order to study trait evolution

Microbes are genetically interrelated with each other, and this relation makes it so that in microbial ecology, observations are not independent of one another, and this lack of independence can be accounted for by phylogeny. Phylogenetics is the study of the evolutionary relatedness among groups of organisms, using genetic sequence data to infer these relationships (Ziemert & Jensen, 2012). Microbes have lots of variation in their metabolic properties, cellular structures, and lifestyles. Their evolution can be explained by internal molecular mechanisms such as point mutations which lead to the modification, inactivation, gene elongation, loss, duplication and/or fusion. Evolution can also be looked at through external mechanisms such as cell fusion and horizontal gene transfer (Del Duca et al., 2022). We can investigate these molecular mechanisms through genomics, and the data from genomic and evolutionary studies give rise to phylogenetics and phylogeny.

Traits are the phenotypic manifestation of many parts of an organism's genome interacting with itself, and also the environment (Hill and Mackay, 2004). This environmental interaction with genotypes means that an organism's realized phenotype may differ from its potential phenotype (Martiny et al. 2015). Gene loss, rapid evolution, and horizontal gene transfer among micro-organisms can shadow phylogenetic signal, however most traits are conserved among bacteria and archaea taxa at high ranks (Philippot et al., 2010), and in microbial eukaryotes (Treseder et al., 2014). In observing microbes which reside in humans, traits related to growth in the human gut seem to be phylogenetically conserved at the genus level, thus it is crucial to consider that micro-organism traits may be correlated with each other or across different organisms (Martiny et al. 2015). There seems to be a hierarchy of phylogenetic conservatism among traits. Traits in marine cyanobacterium *Synechococcus,* such

as responses to pH and salinity, show deep conservation (Martiny et al. 2015). On the other hand, temperature trait conservation is shallow, suggesting that adaptation to temperature changes involve simpler traits that evolve quickly (Bennett et al., 1992).

Microbial growth and growth rates are traits which are measured through a calculation of EAF, which is the increase above the natural abundance isotope composition which has been incorporated into microbial DNA (Hungate et al., 2015). Growth rates of closely related organisms have similar temperature sensitivities, indicating that it is evolutionarily conserved (Wang et al., 2021). Additionally, it has been shown that growth rate of soil micro-organisms are influenced more by evolutionary history than by climate (Morrissey et al., 2019). Phylogenetic relationships can explain an average of 14% of the variation of microbial growth rates across different ecosystems (Walkup et al. 2023). Therefore, in calculating microbial growth rates it is important to account for phylogeny as traits may be phylogenetically conserved, meaning that a trait is nonrandomly distributed across a phylogenetic tree, and the "complexity of a trait indicates the level at which they were phylogenetically conserved" (Propster et al. 2023). Regardless of differences in community composition across ecosystems, shared nodes in the phylogeny of shared ancestors allow predictions to be made across ecosystems. Phylogenetic relationships can explain an average of 14% of the variation of microbial growth rates across different ecosystems (Walkup et al. 2023).

2.4 How long-term soil warming experiments achieve experimental warming

Many studies have simulated soil warming using both passive and active methods to measure microbial response. Warming was achieved in the grassland ecosystem at the JRGCE site in the San Francisco Bay area using an array of overhead infrared heaters, suspended 1.5m

above the ground in warmed plots (Gao et al., 2021). The infrared heaters resulted in a warming of 0.8-1.0ºC at the soil surface from 1998 to 2008-2009, and then 1.5-2.0ºC at the soil surface from 2009-2012 (Gao et al., 2021). After 14 years of warming in the Californian grassland, functional gene contrasts between heated and controlled plots were found (Gao et al., 2021). Gao et al. 2021, found that functional genes associated with labile carbon degradation increased in abundance in the warming treatment, and genes associated with recalcitrant carbon degradation decreased. Functional genes associated with nitrogen cycling decreased (Gao et al., 2021). Another example of active heating was done at Harvard Forest, using buried electrical cables throughout the years since 1991(Melillo et al., 2017). Melillo et al. 2017 had three treatments which were either (1) heating the plot in which the average soil temperature is elevated by 5ºC above ambient temperature via buried cables (2) disturbance control plots, which have an identical set up to the heated plots but never heated and (3) control plots which are left undisturbed. They found an oscillating pattern between four defined phases (Phase 1= resp. rate greater in heated, Phase 2= equal to or less resp. in heated than control, Phase 3 =resp. rate greater in heated, Phase 4=equal or less resp.), indicating thermal acclimation of soil carbon loss.

Warming of soil has also been done utilizing passive methods. The 18-year warming and drought experiment performed by Seaton et al. 2021, in North Wales on a peaty podzol, manipulated climate by implementing retractable roof systems. Plots were heated passively by having roofs cover plots overnight to keep in the heat, resulting in a 0.2ºC increase in mean annual temperature (Seaton et al., 2022). Changes in microbial communities in response to sustained warming were found (Seaton et al., 2022), with subsoil communities becoming similar to communities from topsoil. In the Toolik Lake Research Station, Fairbanks, AK, passive warming has been done using greenhouses of transparent plastic since 1989 to achieve a long-

term warming effect of an 1.5ºC increase in temperature (Propster et al. 2023). This method achieved short-term warming transplanted soils from control plots to an adjacent greenhouse for 3 months (Propster et al. 2023). Propster et al. 2023 found that long-term warming increased growth rates of all microbial taxa. Open-top chambers made of fiberglass material can also be used; these chambers increase temperatures in high latitude ecosystems by trapping solar energy (Purcell et al., 2023). This method was implemented near the Marr Ice Piedmont Glacier terminus and increased soil temperature by 2.0± 0.47ºC (Purcell et al., 2023). This study found that growth rates of bacterial communities increased with warming. Transplant mesocosms along elevation gradients can also simulate warming. Just north of Flagstaff Arizona. mesocosms were implemented along the C. Hart Merriam elevation gradient to simulate warming (Purcell et al., 2022). Heating was achieved by transplanting soil from a high elevation site in a mixed conifer meadow (control) to a lower elevation ponderosa pine meadow (warmed). The control was at an elevation of 2620 m with a mean annual temperature of 6.7 ± 2.3ºC, the heated mesocosm were at an elevation of 2344 m with a mean annual temperature of 10.2 ± 2.6ºC (Purcell et al., 2022). Purcell et al. 2022 found that long-term warming reduced growth rates of soil microbes, with the magnitude of this reduction varying among microbial taxa.

2.5 Background in Stable Isotope Probing (SIP) and Quantitative Stable Isotope Probing (qSIP)

Stable isotope probing (SIP) is a method used in microbial ecology which provides ways in which groups of organisms which incorporate specific substrates to be identified. These substrates are used in the form of isotopically labeled carbon (C^{13}), nitrogen(N^{15}), or oxygen (O^{18}), which are incorporated into microbial biomass (Neufeld et al., 2007). In SIP, actively growing micro-organisms which acquire the isotopically labeled "heavy" substrates build heavier

DNA, which can be separated into fractions for sequencing (Nuccio et al., 2022). Incorporation of these labeled substrates into microbial DNA and RNA shows phylogenetic and functional information about the micro-organism responsible for the incorporation of the particular substrate (Neufeld et al., 2007).

Quantitative Stable Isotope Probing (qSIP), a modification of the traditional SIP method, enables the isotopic composition of DNA from individual microbial taxa, after exposure to labeled isotopes, to be quantified (Hungate et al., 2015). In qSIP, after DNA fractions are made via centrifugation, each fraction separately undergoes sequencing. Taxon-specific density curves are made of both the labeled and unlabeled treatments, which is used in calculating taxon response to isotopic labeling from the measured density shift (Hungate et al., 2015). This shift in density is used to quantify the amount of isotope incorporated into DNA.

The quantification of isotopic incorporation into DNA and RNA enables the determination of taxon-specific growth rates (Purcell et al., 2020 and Koch et al., 2018). Understanding the growth dynamics of different microbial taxa in response to labeled substrates, will allow us to understand whether growth and temperature sensitivity traits are phylogenetically conserved. This can help in bettering our predictions for how changes in microbial communities may impact broader ecosystems. It will also allow us to understand if microbial organisms are adapting or acclimating to warmer environments.

Section 3. Materials and Methods

Study system

Harvard Forest Long Term Ecological Research (LTER) program is one of many long-term ecological research sites. Located in Petersham, which is in north-central Massachusetts (42.5°N Latitude; 72°W Longitude). This second growth, closed-canopy forest is the result of

prior land use by indigenous people followed by extensive agricultural clearing and logging in the mid-1800s (Harvard Forest Website: Physical and Biological Characteristics of the Harvard Forest). This land is now part of a 3,000-acre experimental forest owned and managed by Harvard University.

Varying from 220 m to 410 m above sea level, this cool, moist temperate forest is dominated by eastern hemlock and northern hardwood species, such as Red oak (*Quercus rubra*), Red maple *(Acer rubrum*), Black birch (*Betula lenta*), White pine (*Pinus strobus*), and Eastern hemlock (*Tsuga canadensis*). The forest has a mean temperature of 20ºC in July and -7ºC in January, with an annual mean precipitation of 110 cm. This site is composed of coarse-loamy incepistisols (Eng et al., 2023). The soil is moderately to well drained in most areas, and acidic with a depth averaging at 1m (Harvard Forest Website: Physical and Biological Characteristics of the Harvard Forest).

In this experiment, soils have been heated at 5ºC above ambient temperature throughout the year since 1991, mimicking the effects of climate change. 5ºC was chosen as an extreme scenario of soil temperature rise by 2100 (IPCC 2021). Eighteen 6 x 6 m^2 plots were randomly assigned a treatment: (i) heated 5ºC above ambient temperature throughout the year using electrical cables buried 10 cm beneath the soil; (ii) disturbance control with the same wiring as the heated plot, but never heated; (iii) undisturbed control plots (Eng et al., 2023). For our study, we use soils from the heated and disturbance control plots. In the heated plots, an oscillating pattern of soil respiration was observed, happening in four phases where respiration fluctuated between being greater than or equal to disturbance control plots (Melillo et al., 2017). These phases are the results of several factors such as depletion of carbon pool availability for

microbes, reductions in microbial biomass, shift in microbial carbon use efficiency, and changes in soil microbiome composition (Melillo et al., 2017).

Experimental design

The experimental design uses Quantitative Stable Isotope Probing (qSIP) as described by Hungate et al. 2015. Using a 5-cm soil core, samples were taken from the disturbance control and heated plots. For qSIP a total of 4.0 g mineral and 5.0 g organic horizon soil on a dry weight basis was obtained. From each plot organic horizon soil and mineral horizon soil were collected at a 10 cm depth. In the field horizon soils were separated by eye, looking for differences between color and texture of soil, and stored in whirlpac bags to be transported back to the lab. In total 2 horizon samples from 2 separate treatments were collected four replicate times making a total of 16 samples.

Once back in the lab, soils were sieved through a 2 mm mesh and measured for moisture immediately. The target soil moisture was 0.1. All soils were dried to $3.7\% \pm 2\%$. Once the soil moisture was measured to be from 0.1-0.2, 0.25 g OH soil or 0.375 g mineral, soils were placed in a 2mL tube, with each sample divided into smaller, equal parts to make three identical copies.

Once the soils were placed in triplicate tubes, the replicates were placed in a glass Hungate tube (27 mL) and adjusted to 60% water holding capacity (WHC) using either 18O- or 16O- (natural abundance) water. We decided to reach a 60% WHC as it is seen as a condition optimal for microbial growth. 60% WHC was based on values of 2.7 g water per g dry organic horizon soil, and 0.97 g water per g dry mineral soil; this is based on Domeignoz-Horta et al, unpublished NSF study from soils collected in 2019. Once a water holding capacity of 60% was

achieved the tubes were incubated for seven days at either 15ºC or 25ºC. These incubations will be used in the calculation of taxa growth rates and temperature sensitivity.

After soils were incubated, the samples were frozen at -80 °C until DNA extraction. DNA was then extracted from each sample using the Qiagen DNeasy Powerlyzer PowerSoil Kit (Hilden, Germany), and quantified using a Qubit assay kit and the Qubit fluorometer (Invitrogen, Eugene, OR, USA). The extracted DNA samples were then separated by density. To separate DNA 5.0 µg of DNA was added with 3.5 mL of CsCl, 200 mM Tris, 200 mM KCL, 20 mM EDTA to an OptiSeal Tube (Hungate et al., 2015), creating a final solution density of 1.69 gg cm^{-3}. Each sample was spun in a Beckman ultracentrifuge with TLN-110 rotor at 55000 rpm for 120 h at 20 °C (Engelhardt et al., 2018). Once centrifugation was done the density gradient was divided into fractions of 200 µL (Beckman Coulter, Palo Alto, CA, USA). The density of each fraction was measured using a Reichert AR200 digital refractometer (Reichert Analytical, Depew, NY, USA). DNA was separated from CsCl using isopropanol precipitation and DNA from each fraction was quantified using a Qubit assay.

Microbial biomass Carbon(MBC)

MBC was measured using the chloroform fumigation extraction (CFE) method at the beginning and end of the seven-day incubation. In CFE, soils are exposed to vapor chloroform for 24 hours making the microbial cells undergo lysis. 5.0 g of preincubated soil was extracted with 10 ml of 0.5 M K_2SO_4. This solution was shaken for 1 hour and filtered with Whatman #42 filter paper. Using a Shimadzu Total Organic Carbon (TOC) analyzer the filtrate was analyzed. Using the same procedure another 5.0 g soil was fumigated with chloroform for 24 hours. MBC was then calculated as the difference

between fumigated Dissolved Organic Carbon (DOC) and unfumigated DOC with a correction factor of 0.45 (X.-J. A. Liu et al., 2020).

qPCR

Each replicate of the density fractions was analyzed in 10 µL reaction mixtures with bacterial and fungal primers.

Based on the qPCR data, conventional SIP density curves were produced by graphing total 16S rRNA gene copies as a function of density (Hungate et al., 2015). Average DNA density for each tube was calculated as a weighted average of the density of each fraction that had 16S rRNA gene copies. This average DNA density calculation is used for bootstrapping. Bootstrapping is used in assessing the reliability of sequence-based phylogenies. The bootstrap value indicates, out of 100 randomly generated phylogenetic trees, how many times the same branch is observed, with a value less than 40% being considered random.

16S rRNA and ITS gene sequencing

Using an Illumina MiSeq, the 16S rRNA gene in every fraction that contained DNA was sequenced (Genomics Res. Lab. UMass Amherst). In total our sequencing libraries included eight experimental factors (warming (control, heated), soil type (O-horizon and mineral), incubation temperature (15, 25 °C), 4 field replicates (at the plot-level), 2 isotope labels (16O- and 18O-water), for a total of 64 samples with 8 density fractions each, making a total of 512 samples. The 512 samples collected were divided into three libraries for further analysis.

192 separate barcodes in two 96-well plates were used to sequence these samples. PCRs were set up in 25 μL reaction mixtures in 96-well plate format, using Platinum Hot Start Taq. Primer concentrations were 10 uM for forward primer (515F) and reverse primer (806R) to amplify bacterial communities. We ran PCR for 35 cycles, with each cycle lasting 45 seconds at 94ºC, 60 seconds at 50ºC, and 90 seconds at 72ºC. Templates were randomized across plates to minimize bias. Amplification was confirmed using gel electrophoresis, and amplicons were quantified in using picogreen. 250 ng of each amplicon was pooled for each of the three libraries.

Data-analysis (see appendix a for reference code)

Following lab protocols with the soil samples, the sequences were further processed. Using QIIME2 (Bolyen et al., 2019) we created summaries of the demultiplexed single end reads found in the three libraries. QIIME2 is a microbiome bioinformatics platform that is free and open source (Bolyen et al., 2019). QIIME2 showed that the sequences were of high quality for both the forward and reverse reads. The high-quality single end reads were merged to create paired end reads using PEAR (Zhang et al., 2014). PEAR is a memory-efficient and accurate pair-end read merger (Zhang et al., 2014). Once the sequences were merged, they were separated by sampling location (disturbance control plots, heated plots). We separated the disturbance control from the heated samples so that we would be able to see which taxa are correlated which plots in later analysis. This separation is because the 16S rRNA gene that we are using to make the trees is not under selection and cannot detect adaptation. We want to be able to detect evidence of speciation in other parts of the genome or other traits of the organism, and to do this

we need to keep OTUs separate for heated and disturbance control. This means 'forcing' the data into a shape that keeps taxa from heated and disturbance control plots separate.

Using Fastqc, a package in USEARCH (Edgar, 2010), we looked at the quality of the merger on both heated and disturbance control sequences. USEARCH is a high-throughput sequence analysis tool, which we used to run quality control checks on our raw sequence data (Edgar, 2010). Fastqc allowed us to see that both heated and disturbance control samples begin losing quality at 200 base pair length (fig. 1; fig.2). To ensure that this is correct we used VSEARCH (Rognes et al., 2016), which checked the expected error rate of the reads merged by PEAR. VSEARCH, like USEARCH, is a sequence analysis tool (Rognes et al., 2016). VSEARCH confirmed that the reads lost accuracy after 250 base pairs in both heated and disturbance control samples, thus for these samples, we denoised both 200 and 250 base pair length using VSEARCH. After running Fastqc to check the quality of all denoising, it appeared that all of the sequences which had been denoised were of high quality for both heated and disturbance control. Since all denoising resulted in high-quality sequences we used the sequences which were denoised at 250 base pairs we ran an expected error test to double check the quality of these sequences using VSEARCH. Majority of the reads passed the quality filtering threshold (maxEE 0.50) confirming the high quality of the 250-base pair denoised sequences.

Using VSEARCH we found the abundance of unique reads of sequences truncated at 250 base pairs in all samples. From the unique reads we created OTU tables based on a 99% sequence identity. These tables were then used in assigning taxonomy to each OTU. To assign taxonomy, using QIIME2, we trained a classifier using reference sequences from silva-138-99-seqs-515-806.qza, reference taxonomy from silva-138-99-tax-515-806.qza and a forward primer of GTGCCAGCMGCCGCGGTAA and a reverse primer of GGACTACHVGGGTWTCTAAT

(Quast et al., 2013). Using the trained classifier, in QIIME2, we assigned taxonomies to all OTUs.

Next steps

Using the OTU assignments, we can create a phylogenetic tree of all OTUs (fig. 3). To do this we will begin by generating a multiple sequence alignment (MSA) using the MAFFT package in QIIME2. Once the sequences are aligned we will mask the alignment to discard alignments which are phylogenetically uninformative and/or misleading before phylogenetic analysis (Bolyen et al., 2019). We will then reference based alignments using the SILVA database (Bolyen et al., 2019). Using the SINA package in QIIME2 we will align the output .qza file from MAFFT with the reference Living Tree Project arb file (Bolyen et al., 2019) the output of this will be aligned representative sequences that we will call masked_aligned_rep_seqs.qza. The masked_aligned_rep_seqs.qza file will use the raxml-rapid-bootstrap package in QIIME2 to create a phylogeny of all OTUs (Bolyen et al., 2019).

Using the data on MBC content, soil type, warming treatment, water enrichment (18O-/16O-), 16S qPCR copy number, and density we will be able to calculate the gene copy number. To do this we will multiply the total number of 16S genes of all taxa found in a specific fraction (k) of a specific replicate (j) by the relative abundance of 16S gene copies of a specific taxon (i) by that same specific fraction (k) of a specific replicate (j) (Finley et al., 2019).

$$copies_{ijk} = rel.ab_{ijk} \times copies_{jk}$$

Using steps outlined in Finley et al. 2019, we will perform functions in R to create: wad1.obs.mean, wad2.obs.mean, wad1.boot.mean, wad1.boot.median, wad1.boot.CI.L,

wad1.boot.CI.U, wad2.boot.mean, wad2.boot.median, wad2.boot.CI.L, wad2.boot.CI.U, wad.diff.obs, wad.diff.boot.mean, wad.diff.boot.median, wad.diff.boot.CI.L, wad.diff.boot.CI.U, wad.diff.p.value, ape.obs, ape.boot.mean, ape.boot.median, ape.boot.CI.L, and ape.boot.CI.U. Which are all needed in calculating weighted average density (WAD), the difference in WAD between the labled and unlabeled treatments, Guinine and Cytosine content, molecular weight of each taxon in 18O- and 16O- treatments, theoretical maximum molecular weight of 18O- labled DNA for each taxon, and the excess aton fraction (EAF) of the DNA in the 18O- treatment (Finley et al., 2019).

We will begin by calculating weighted average density per taxon (i) using $WAD_{ij} = \frac{\sum_{k=1}^{K} (density \times rel.ab_{ijk} \times copies_{jk})}{\sum_{k=1}^{K} (rel.ab_{ijk} \times copies_{jk})}$, where: WAD_{ij}= weighted average density of taxon i in replicate j, k = fraction, K= total number of fractions within a sample, density = the density of each fraction k in replicate j, $rel.ab_{ijk}$= relative abundance of taxon i in fraction k of replicate j, $copies_{jk}$= total number of gene copies in fraction k of replicate j (Finley et al., 2019). Secondly, we will calculate the difference in weighted average density for taxon i (WAD_{DIFFi}) between the isotopically labeled treatment and the unlabeled (natural abundance) control (diff.wad.calc in R) (Finley et al., 2019) using WAD_{DIFFi}=$WAD_{LABi} - WAD_{LIGHTi}$, where WAD_{LABi}= WAD of taxon i in isotopically labeled treatment and WAD_{LIGHTi}= WAD of taxon i in control treatment (Finley et al., 2019). To calculate the guanine-cytosine content for each taxon i under control conditions: $GC_i = \frac{1}{0{:}083506}(WAD_{LIGHTi} - \quad 1{:}646057)$ (Finley et al., 2019). To calculate the average molecular weight of a single strand of DNA for taxon i under control conditions: $M_{LIGHTi} = (0.496 \times GC_i) + 307.691$ (Finley et al., 2019). To calculate the average molecular weight of DNA for taxon i under isotopically labeled conditions (M_{LABi}): $M_{LABi} = (\frac{WAD_{DIFFi}}{WAD_{LIGHTi}} + 1)M_{LIGHTi}$

(Finley et al., 2019). The theoretical maximum molecular weight of labeled DNA for taxon i: $M_{HEAVYMAXi} = 12.07747 + M_{LIGHTi}$ (Finley et al., 2019). To calculate EAF of oxygen: $EAF_{Oxygen(i)} = \frac{M_{LABi} - M_{LIGHTi}}{M_{HEAXYMAXi} - M_{LIGHTi}}(1 - 0.002000429)$, where M is the molecular weight of DNA (Finley et al., 2019). From EAF we will be able to calculate growth rate as an estimate of atom percent excess of 18O- incorporated into DNA per operational taxonomic unit (OTU) (Koch et al., 2018).

Within our disturbance control and heated data, we will seperate the taxa incubated at 15ºC and 25ºC and the calculated growth rates per taxa of 25ºC (Δr_{25}) and 15ºC (Δr_{15}) using methods described in Koch et al., 2018. We will compare the Δr_{25} and Δr_{15} through a phylogenetic ANOVA, to test if there is a significant difference between heated and disturbance control growth rates of taxa in soil incubated at 25ºC and 15ºC. The phylogenetic ANOVA allows us to account for phylogeny in our calculations of growth rates, which is important because regular ANOVA assumes that observations are independent. To account for phylogeny, on the phylogenetic tree of all OTUs Δr_{15}, and Δr_{25} of each OTU will be matched to their corresponding branch tip (Propster et al., 2023). Blomberg's K and Pagel's λ will be calculated and used to show phylogenetic signals of EAF. Blomberg's K and Pagel's λ are two quantitative measures of phylogenetic signal, or the tendency of related species to resemble each other. Pagel's λ is a scaling parameter for the correlations between species relative to correlation expected under Brownian motian. Blomberg's K is a scaled ratio of the variance among species over the contrast variance. We will look for changes in growth rate across all detected taxa and individual dominant phyla, these changes indicate adaptation.

From growth rate calculations and EAF we will find the temperature sensitivity of growth (Q_{10}) (Wang et al., 2021). Q_{10} is the factor by which the rate of a biological process, such as

microbial growth, increases for every 10ºC rise in temperature. Through our analysis of all OTUs we get four traits per taxa (EAF-H-15, EAF-C-15, EAF-H-25, EAF-C-25). From these traits we can calculate Q_{10} per taxon in heated and disturbance control using these equations: $Q_{10}(H) = (\frac{EAF(H)-25}{EAF(H)-15})^{\frac{10}{T2-T1}}$ and $Q_{10}(C) = (\frac{EAF(C)-25}{EAF(C)-15})^{\frac{10}{T2-T1}}$, where $EAF(H) - 25$ is the excess atom fraction of heated microbes incubated at 25ºC, $EAF(H) - 15$ is the excess atom fraction of heated microbes incubated at 15ºC, $EAF(C) - 25$ is the excess atom fraction of disturbance control microbes incubated at 25ºC, $EAF(C) - 15$ is the excess atom fraction of disturbance control microbes incubated at 15ºC, T2 is 25ºC and T1 is 15ºC (Wang et al., 2021). If $Q_{10}(H)$ and $Q_{10}(C)$ aren't the same we will have evidence of adaptation (Eng et al., 2023). Further, we will run a phylogenetic group comparison to compare the Q_{10} between heated and disturbance control plots we will run a phylogenetic group comparison to test if there is a significant difference between Q_{10} of taxa in disturbance control and heated plots. The goal of phylogenetic comparative methods (PCMs) is to look at the distribution of traits among related species. Phylogenetic group comparisons are important in analyzing data that are not independent of one another, so that we can detect horizontal gene transfer events as well as to predict the interaction between multigene families (Choi & Gomez, 2009).

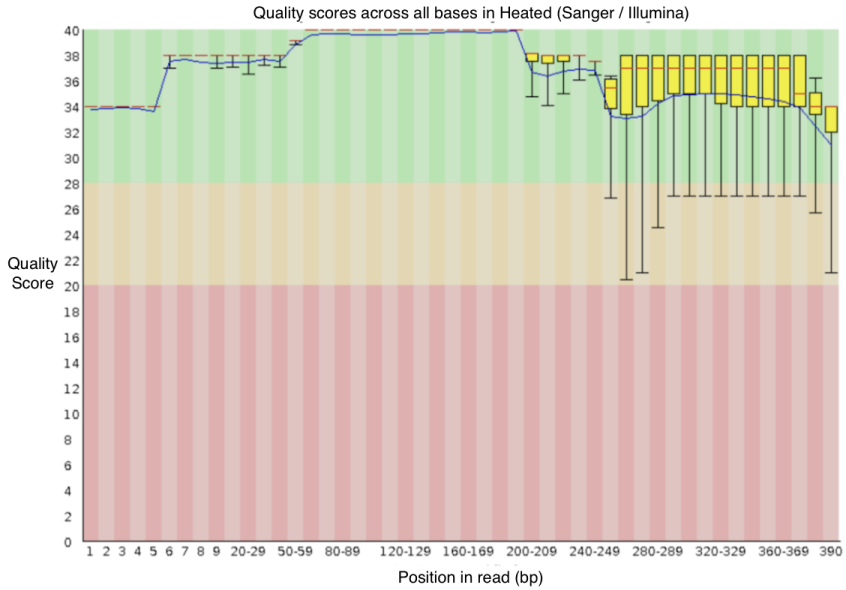

Figure 1. Fastqc of all sequences from the heated samples. Bases begin losing quality at 200 base pairs.

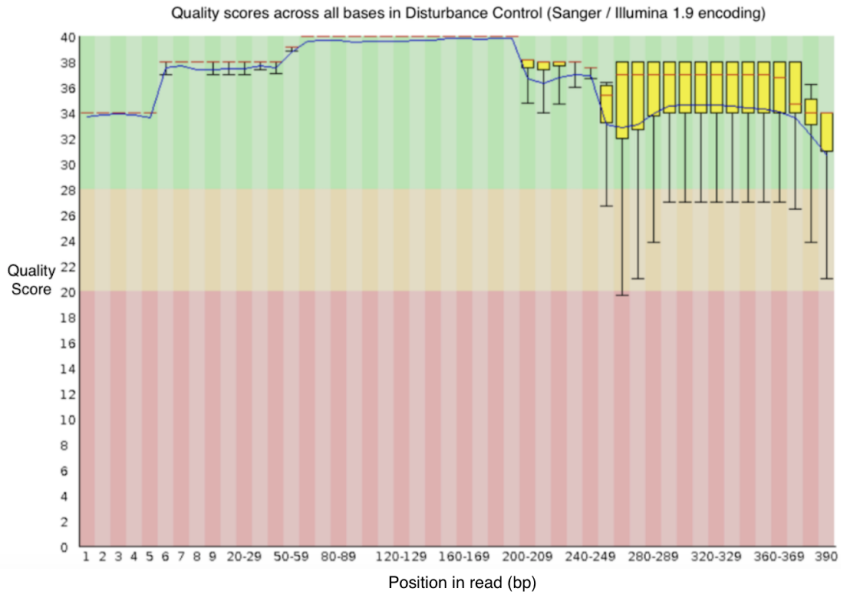

Figure 2. Fastqc of all sequences from the disturbance control samples. Bases begin losing quality at 200 base pairs.

Figure 3. First 350 OTUs assembled on a rooted phylogenetic tree. This tree was made using iTOL (Letunic & Bork, 2011), an online tool for displaying phylogenetic trees. While our actual tree cannot be vizualised in its entirety an assemblage similar to this will be used in accounting for phylogeny in our Q_{10} and our generation times.

Section 4. Expected Results and Discussion

Growth rates of taxa in heated plots are smaller than those in disturbance control

We expect to find that in our warmed plots, the growth rates per taxa will be smaller compared to those in the disturbance control plots. This is based on a qSIP study examining microbial growth over a 15-year period through passive experimental warming using transplant mesocosms along a elevation gradient to induce a warming effect of $3.4^{o}C \pm 0.89^{o}C$ (Purcell et al., 2022). The findings of this study suggest a reduction in microbial growth rates under

prolonged warming conditions. We expect that this is the result of reduced total soil and nitrogen content, with soil undergoing phases of substantial soil carbon loss which occur in rotation with phases of no loss in soil carbon (Melillo et al., 2017). Long-term warming has been shown to reduce soil carbon and nitrogen concentration (X. J. A. Liu et al., 2021), which we predict will impact microbial growth rates negatively.

However, there is no uniform finding concerning growth rates in long-term warming experiments. Studies examining how microbial growth rates respond to long-term temperature change lack consensus (Nottingham et al., 2019). This lack of consensus is partially due to the temperature sensitivity of respiration being indirectly influenced by environmental variables (Davidson & Janssens, 2006). Factors such as substrate availability and moisture also indirectly impact the measure of microbial growth in response to temperature (Davidson & Janssens, 2006). A passive soil warming effect of 1.5ºC achieved through enclosing plots with transparent plastic found that short-term warming increases the growth rates of taxa by 36% (Propster et al., 2023). This increase in growth rate was linked to previously undetected taxa from other treatments. The emergence of these new organisms resulted in a doubled diversity of microbes in the short-term warming experiment. Meanwhile in long-term warming experiments, average growth rates of all organisms increased significantly (151%), suggesting that there is a greater impact in long-term sustained warming than short-term (Propster et al., 2023). Unlike the short-term, the majority of the growth took place in organisms which were detected in the disturbance control groups, implying that some taxa adapted to long-term warming (Propster et al., 2023). Thus, it cannot be ruled out that we might find higher growth rates in heated plots compared to disturbance control.

Our anticipated findings suggest that taxa specific growth rates in heated plots will be smaller than disturbance control, with the most common soil bacteria taxa responding most negatively to warming and the rare taxa exhibiting some of the fastest growth rates (Purcell et al., 2022). The phylogenetic ANOVA we plan on running, will show that taxa growth rates are significantly different between the disturbance control and heated taxa. The decline in growth rates of heated plots has been proposed to be caused by a warming-induced decline in soil carbon availability (Conant et al., 2011; Demoling et al., 2007; Melillo et al., 2017). Reduced microbial growth rates are correlated with a decrease in carbon use efficiency as well as less carbon assimilation, explaining the positive feedback loop leading to soil carbon loss over time (Purcell et al., 2022). This decline in microbial growth rates in long-term warming experiments is speculated to impact entire community compositions (Purcell et al., 2022). Essentially the slow accumulation of microbial derived carbon, a major source of soil organic matter (SOM) (Kallenbach et al., 2016), may contribute to increased carbon loss in context of lower carbon accumulation and continued warming.

Temperature sensitivity decreases with increasing temperature

We expect Q_{10} per taxa will be greater in heated plots than disturbance control plots. Q_{10} has been seen to increase with increasing mean annual temperature (MAT) at plot level (Rinnan et al., 2009). When calculating Q_{10} for temperatures between 10ºC and 20ºC, Q_{10} increased from 2.4 at MAT of 6ºC to 3.5 at MAT of 26ºC (Nottingham et al., 2019). Therefore, we expect that taxa from heated plots will have greater temperature sensitivity than their counterparts in disturbance control plots. We expect that our phylogenetic group comparison will reveal that this difference is significant. If this is the case in our data, microbes from the heated plots have

increased sensitivity to temperature, this sensitivity will influence rates of microbial growth and other soil activities; a high degree of temperature sensitivity implies that change in temperature will immediately cause microbial response. This means that in heated plots, microbial growth will immediately change in response to warming. Knowing how microbes will respond to climate change enables scientists to predict shifts in microbiomes and their associated ecosystem functions.

However, we cannot rule out that taxa in heated plots may have a Q_{10} smaller than disturbance control plots. Wang et al. 2021 found that mean Q_{10} of microbial growth for an entire microbial community decreased with increasing incubation temperature, and Q_{10} varied across biomes. This variance in temperature sensitivity is because comparing temperature sensitivity (Q_{10}) among different studies poses challenges as Q_{10} is not constant over a given temperature range. Q_{10} of microbial growth is higher when measured at lower temperatures (Bååth, 2018). If it is found that Q_{10} of microbial growth of taxa in heated plots is less than disturbance control, this would mean that change in temperature will not immediately cause a microbial growth response.

Temperature sensitivity is phylogenetically conserved

We expect that Q_{10} of microbial growth is phylogenetically conserved as it exhibits phylogenetic clustering (Wang et al., 2021). After a phylogenetic group comparison, to test whether microbes from heated plots have larger temperature sensitivities of growth than disturbance control, we expect that there will be a nonrandom Q_{10} of microbial growth rates, and closely related organisms within the heated plot will exhibit similar temperature sensitivities. This result is expected based on Wang et al. 2021, which found that temperature sensitivity

varied among microbial taxa within soil communities across varying temperatures and biomes, and these differences are tied to evolutionary relationships among microbial taxa. The temperature sensitivity of growth exhibited phylogenetic clustering according to Blomberg's K and Pagel's λ signal tests, meaning that Q_{10} microbial growth rates were non-randomly distributed (Wang et al., 2021). Phylogeny has been shown to predict growth rates of soil bacteria, explaining an average of 31-58% of variation within ecosystems (Walkup et al., 2023).

It has been speculated that phylogenetic relatedness may contribute to microbial growth rate responses which have been observed (Purcell et al., 2023). Knowing that variation in microbial growth rate and other processes are explained by taxonomic clustering may allow more generalized predictions of bacterial growth in soil (Propster et al., 2023). This underlying phylogenetic organization of traits may shed light on how microbial biodiversity controls ecosystem functioning (Wang et al., 2021).

Microbes have adapted to a warmer climate

If we find that the Q_{10} and growth rates of taxa from heated and disturbance control plots are significantly different, we would predict evidence of microbial adaptation (Bååth, 2018; Eng et al., 2023). Evidence of adaptation is illustrated by a change in trait distribution between traits measured for taxa in heated and control plots (fig. 4). We modeled our traits (growth of disturbance control and heated taxa at 15ºC and 25ºC, and Q10) as being correlated with an OTU from heated or disturbance control plots (fig. 4). If there is no change in the distribution of traits between heated and disturbance control treatments, microbes are possibly acclimating, and rates of microbial growth are increasing less with rising temperatures compared to the un-acclimated soils (Eng et al., 2023). Further, if microbial communities are not adapting to higher

temperatures, the resulting reaction rates and substrate depletion will reduce soil carbon loss (Walker et al., 2018). Micro-organisms which have undergone adaptation, we assume, exhibit optimal growth rates, and have a higher Q_{10} under higher temperatures. Understanding how well micro-organisms are adapted to environmental temperature is necessary in predicting future levels of CO_2 in our atmosphere. Micro-organisms that are well adapted to environmental temperature are those which are very tolerant of temperature extremes.

Nottingham et al. 2019 found that microbial temperature sensitivity of growth shows signs of adaptation, however they have examined community level adaptation, not taxon level. Highest mean annual temperature (MAT) resulted in bacterial communities to adapt to higher temperatures (Nottingham et al., 2019). Based on this study we expect a difference in traits from heated and disturbance control samples. The difference between temperature sensitivities and growth rates of disturbance control and heated plots is indicative of microbial adaptation. If microbial communities are acclimating and not adapting to increasing temperatures, this will lead to soil carbon loss (Cavicchioli et al., 2019). If carbon is released from soil at a faster rate than it is replaced, the net release of carbon to the atmosphere will contribute to global warming (Todd A. Ontl & Lisa A. Schulte, 2012).

This research will contribute to our understanding of microbial responses to long-term warming and will help in predicting how different microbial species will respond to changes in temperature. We expect to find that in our heated treatments microbes have undergone adaptation, meaning they exhibit optimal growth rates and have a higher temperature sensitivity. While our analysis focuses on how long-term warming changes distribution of traits in relation to growth rates and temperature sensitivities, future directions could encompass community composition and biodiversity in relation to distribution of traits.

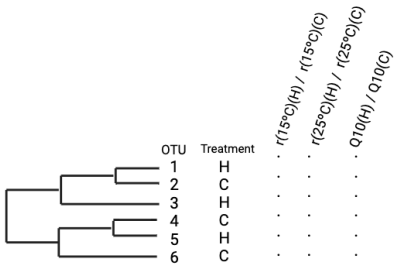

Figure 4. Model showing the traits which we calculated per taxon. r(15ºC) refers to the growth rates at 15ºC incubation for both heated and disturbance control plots. r(25ºC) refers to the growth rates at 25ºC incubation for both heated and disturbance control plots. Q10 refers to the temperature sensitivity of growth per taxon for heated and disturbance control plots.

Refrences

Asmeret Asefaw Berhe, Chelsea Arnold, Erin Stacy, Rebecca Lever, Emma McCorkle, & Samuel Negusse Araya. (2014). Soil erosion controls on biogeochemical cycling of carbon and nitrogen. *The Nature Education*.

Bååth, E. (2018). Temperature sensitivity of soil microbial activity modeled by the square root equation as a unifying model to differentiate between direct temperature effects and microbial community adaptation. *Global Change Biology*, *24*(7), 2850–2861. https://doi.org/10.1111/gcb.14285

Bennett, A. F., Lenski, R. E., & Mittler, J. E. (1992). EVOLUTIONARY ADAPTATION TO TEMPERATURE. I. FITNESS RESPONSES OF *ESCHERICHIA COLI* TO CHANGES IN ITS THERMAL ENVIRONMENT. *Evolution*, *46*(1), 16–30. https://doi.org/10.1111/j.1558-5646.1992.tb01981.x

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9

Bradford, M. A. (2013). Thermal adaptation of decomposer communities in warming soils. *Frontiers in Microbiology*, *4*. https://doi.org/10.3389/fmicb.2013.00333

Calvin, K., Dasgupta, D., Krinner, G., Mukherji, A., Thorne, P. W., Trisos, C., Romero, J., Aldunce, P., Barrett, K., Blanco, G., Cheung, W. W. L., Connors, S., Denton, F., Diongue-Niang, A., Dodman, D., Garschagen, M., Geden, O., Hayward, B., Jones, C., … Péan, C. (2023). *IPCC, 2023: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland.* (First). Intergovernmental Panel on

Climate Change (IPCC). https://doi.org/10.59327/IPCC/AR6-9789291691647

Cavicchioli, R., Ripple, W. J., Timmis, K. N., Azam, F., Bakken, L. R., Baylis, M., Behrenfeld, M. J., Boetius, A., Boyd, P. W., Classen, A. T., Crowther, T. W., Danovaro, R., Foreman, C. M., Huisman, J., Hutchins, D. A., Jansson, J. K., Karl, D. M., Koskella, B., Mark Welch, D. B., … Webster, N. S. (2019). Scientists' warning to humanity: Microorganisms and climate change. *Nature Reviews Microbiology*, *17*(9), 569–586. https://doi.org/10.1038/s41579-019-0222-5

Chapin, F. S. (1980). The Mineral Nutrition of Wild Plants. *Annual Review of Ecology and Systematics*, *11*(1), 233–260. https://doi.org/10.1146/annurev.es.11.110180.001313

Choi, K., & Gomez, S. M. (2009). Comparison of phylogenetic trees through alignment of embedded evolutionary distances. *BMC Bioinformatics*, *10*(1), 423. https://doi.org/10.1186/1471-2105-10-423

Cleveland, C. C., Townsend, A. R., Schimel, D. S., Fisher, H., Howarth, R. W., Hedin, L. O., Perakis, S. S., Latty, E. F., Von Fischer, J. C., Elseroad, A., & Wasson, M. F. (1999). Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. *Global Biogeochemical Cycles*, *13*(2), 623–645. https://doi.org/10.1029/1999GB900014

Conant, R. T., Ryan, M. G., Ågren, G. I., Birge, H. E., Davidson, E. A., Eliasson, P. E., Evans, S. E., Frey, S. D., Giardina, C. P., Hopkins, F. M., Hyvönen, R., Kirschbaum, M. U. F., Lavallee, J. M., Leifeld, J., Parton, W. J., Megan Steinweg, J., Wallenstein, M. D., Martin Wetterstedt, J. Å., & Bradford, M. A. (2011). Temperature and soil organic matter decomposition rates—Synthesis of current knowledge and a way forward. *Global Change Biology*, *17*(11), 3392–3404. https://doi.org/10.1111/j.1365-2486.2011.02496.x

Correia, A. M., & Lopes, L. F. (2023). Revisiting Biodiversity and Ecosystem Functioning through the Lens of Complex Adaptive Systems. *Diversity*, *15*(8), 895. https://doi.org/10.3390/d15080895

Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, *440*(7081), 165–173. https://doi.org/10.1038/nature04514

Del Duca, S., Vassallo, A., Mengoni, A., & Fani, R. (2022). Microbial Genetics and Evolution.

Microorganisms, *10*(7), 1274. https://doi.org/10.3390/microorganisms10071274

Delgado-Baquerizo, M., Maestre, F. T., Reich, P. B., Jeffries, T. C., Gaitan, J. J., Encinar, D., Berdugo, M., Campbell, C. D., & Singh, B. K. (2016). Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications*, *7*(1), 10541. https://doi.org/10.1038/ncomms10541

Demoling, F., Figueroa, D., & Bååth, E. (2007). Comparison of factors limiting bacterial growth in different soils. *Soil Biology and Biochemistry*, *39*(10), 2485–2495. https://doi.org/10.1016/j.soilbio.2007.05.002

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Eekhout, J. P. C., & De Vente, J. (2022). Global impact of climate change on soil erosion and potential for adaptation through soil conservation. *Earth-Science Reviews*, *226*, 103921. https://doi.org/10.1016/j.earscirev.2022.103921

Eng, A. Y., Narayanan, A., Alster, C. J., & DeAngelis, K. M. (2023). Thermal adaptation of soil microbial growth traits in response to chronic warming. *Applied and Environmental Microbiology*, *89*(11), e00825-23. https://doi.org/10.1128/aem.00825-23

Finley, B. K., Hayer, M., Mau, R. L., Purcell, A. M., Koch, B. J., Van Gestel, N. C., Schwartz, E., & Hungate, B. A. (2019). Microbial Taxon-Specific Isotope Incorporation with DNA Quantitative Stable Isotope Probing. In M. G. Dumont & M. Hernández García (Eds.), *Stable Isotope Probing* (Vol. 2046, pp. 137–149). Springer New York. https://doi.org/10.1007/978-1-4939-9721-3_11

Gao, Y., Ding, J., Yuan, M., Chiariello, N., Docherty, K., Field, C., Gao, Q., Gu, B., Gutknecht, J., Hungate, B. A., Le Roux, X., Niboyet, A., Qi, Q., Shi, Z., Zhou, J., & Yang, Y. (2021). Long-term warming in a Mediterranean-type grassland affects soil bacterial functional potential but not bacterial taxonomic composition. *Npj Biofilms and Microbiomes*, *7*(1), 17. https://doi.org/10.1038/s41522-021-00187-7

Hungate, B. A., Mau, R. L., Schwartz, E., Caporaso, J. G., Dijkstra, P., Van Gestel, N., Koch, B. J., Liu,

C. M., McHugh, T. A., Marks, J. C., Morrissey, E. M., & Price, L. B. (2015). *Quantitative microbial ecology through stable isotope probing* [Preprint]. PeerJ PrePrints. https://doi.org/10.7287/peerj.preprints.1282v1

Kallenbach, C. M., Frey, S. D., & Grandy, A. S. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications*, *7*(1), 13630. https://doi.org/10.1038/ncomms13630

Koch, B. J., McHugh, T. A., Hayer, M., Schwartz, E., Blazewicz, S. J., Dijkstra, P., Van Gestel, N., Marks, J. C., Mau, R. L., Morrissey, E. M., Pett-Ridge, J., & Hungate, B. A. (2018). Estimating taxon-specific population dynamics in diverse microbial communities. *Ecosphere*, *9*(1), e02090. https://doi.org/10.1002/ecs2.2090

Letunic, I., & Bork, P. (2011). Interactive Tree Of Life v2: Online annotation and display of phylogenetic trees made easy. *Nucleic Acids Research*, *39*(suppl), W475–W478. https://doi.org/10.1093/nar/gkr201

Levin, S. A., & Pacala, S. W. (2003). Ecosystem Dynamics. In *Handbook of Environmental Economics* (Vol. 1, pp. 61–95). Elsevier. https://doi.org/10.1016/S1574-0099(03)01007-6

Liu, X. J. A., Pold, G., Domeignoz-Horta, L. A., Geyer, K. M., Caris, H., Nicolson, H., Kemner, K. M., Frey, S. D., Melillo, J. M., & DeAngelis, K. M. (2021). Soil aggregate-mediated microbial responses to long-term warming. *Soil Biology and Biochemistry*, *152*, 108055. https://doi.org/10.1016/j.soilbio.2020.108055

Liu, X.-J. A., Finley, B. K., Mau, R. L., Schwartz, E., Dijkstra, P., Bowker, M. A., & Hungate, B. A. (2020). The soil priming effect: Consistent across ecosystems, elusive mechanisms. *Soil Biology and Biochemistry*, *140*, 107617. https://doi.org/10.1016/j.soilbio.2019.107617

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., Hooper, D. U., Huston, M. A., Raffaelli, D., Schmid, B., Tilman, D., & Wardle, D. A. (2001). Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges. *Science*, *294*(5543), 804–808. https://doi.org/10.1126/science.1064088

Melillo, J. M., Frey, S. D., DeAngelis, K. M., Werner, W. J., Bernard, M. J., Bowles, F. P., Pold, G., Knorr, M. A., & Grandy, A. S. (2017). Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. *Science*, *358*(6359), 101–105. https://doi.org/10.1126/science.aan2874

Nemergut, D. R., Schmidt, S. K., Fukami, T., O'Neill, S. P., Bilinski, T. M., Stanish, L. F., Knelman, J. E., Darcy, J. L., Lynch, R. C., Wickey, P., & Ferrenberg, S. (2013). Patterns and Processes of Microbial Community Assembly. *Microbiology and Molecular Biology Reviews*, *77*(3), 342–356. https://doi.org/10.1128/MMBR.00051-12

Neufeld, J. D., Vohra, J., Dumont, M. G., Lueders, T., Manefield, M., Friedrich, M. W., & Murrell, J. C. (2007). DNA stable-isotope probing. *Nature Protocols*, *2*(4), 860–866. https://doi.org/10.1038/nprot.2007.109

NOAA. (2022). *Annual 2022 Global Climate Report*. https://www.ncei.noaa.gov/access/monitoring/monthly-report/global/202213

Nottingham, A. T., Bååth, E., Reischke, S., Salinas, N., & Meir, P. (2019). Adaptation of soil microbial growth to temperature: Using a tropical elevation gradient to predict future changes. *Global Change Biology*, *25*(3), 827–838. https://doi.org/10.1111/gcb.14502

Nuccio, E. E., Blazewicz, S. J., Lafler, M., Campbell, A. N., Kakouridis, A., Kimbrel, J. A., Wollard, J., Vyshenska, D., Riley, R., Tomatsu, A., Hestrin, R., Malmstrom, R. R., Firestone, M., & Pett-Ridge, J. (2022). HT-SIP: A semi-automated stable isotope probing pipeline identifies cross-kingdom interactions in the hyphosphere of arbuscular mycorrhizal fungi. *Microbiome*, *10*(1), 199. https://doi.org/10.1186/s40168-022-01391-z

Philippot, L., Andersson, S. G. E., Battin, T. J., Prosser, J. I., Schimel, J. P., Whitman, W. B., & Hallin, S. (2010). The ecological coherence of high bacterial taxonomic ranks. *Nature Reviews Microbiology*, *8*(7), 523–529. https://doi.org/10.1038/nrmicro2367

Propster, J. R., Schwartz, E., Hayer, M., Miller, S., Monsaint-Queeney, V., Koch, B. J., Morrissey, E. M., Mack, M. C., & Hungate, B. A. (2023). Distinct Growth Responses of Tundra Soil Bacteria to

Short-Term and Long-Term Warming. *Applied and Environmental Microbiology*, *89*(3), e01543-22. https://doi.org/10.1128/aem.01543-22

Purcell, A. M., Dijkstra, P., Hungate, B. A., McMillen, K., Schwartz, E., & Van Gestel, N. (2023). Rapid growth rate responses of terrestrial bacteria to field warming on the Antarctic Peninsula. *The ISME Journal*, *17*(12), 2290–2302. https://doi.org/10.1038/s41396-023-01536-4

Purcell, A. M., Hayer, M., Koch, B. J., Mau, R. L., Blazewicz, S. J., Dijkstra, P., Mack, M. C., Marks, J. C., Morrissey, E. M., Pett-Ridge, J., Rubin, R. L., Schwartz, E., Van Gestel, N. C., & Hungate, B. A. (2022). Decreased growth of wild soil microbes after 15 years of transplant-induced warming in a montane meadow. *Global Change Biology*, *28*(1), 128–139. https://doi.org/10.1111/gcb.15911

Rinnan, R., Rousk, J., Yergeau, E., Kowalchuk, G. A., & Bååth, E. (2009). Temperature adaptation of soil bacterial communities along an Antarctic climate gradient: Predicting responses to climate warming. *Global Change Biology*, *15*(11), 2615–2625. https://doi.org/10.1111/j.1365-2486.2009.01959.x

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *4*, e2584. https://doi.org/10.7717/peerj.2584

Seaton, F. M., Reinsch, S., Goodall, T., White, N., Jones, D. L., Griffiths, R. I., Creer, S., Smith, A., Emmett, B. A., & Robinson, D. A. (2022). Long-Term Drought and Warming Alter Soil Bacterial and Fungal Communities in an Upland Heathland. *Ecosystems*, *25*(6), 1279–1294. https://doi.org/10.1007/s10021-021-00715-8

Todd A. Ontl & Lisa A. Schulte. (2012). Soil Carbon Storage. *The Nature Education*. https://www-nature-com.coloradocollege.idm.oclc.org/scitable/knowledge/library/soil-carbon-storage-84223790/

Treseder, K. K., Maltz, M. R., Hawkins, B. A., Fierer, N., Stajich, J. E., & McGuire, K. L. (2014). Evolutionary histories of soil fungi are reflected in their large-scale biogeography. *Ecology Letters*, *17*(9), 1086–1093. https://doi.org/10.1111/ele.12311

Walker, T. W. N., Kaiser, C., Strasser, F., Herbold, C. W., Leblans, N. I. W., Woebken, D., Janssens, I. A., Sigurdsson, B. D., & Richter, A. (2018). Microbial temperature sensitivity and biomass change explain soil carbon loss with warming. *Nature Climate Change*, *8*(10), 885–889. https://doi.org/10.1038/s41558-018-0259-x

Walkup, J., Dang, C., Mau, R. L., Hayer, M., Schwartz, E., Stone, B. W., Hofmockel, K. S., Koch, B. J., Purcell, A. M., Pett-Ridge, J., Wang, C., Hungate, B. A., & Morrissey, E. M. (2023). The predictive power of phylogeny on growth rates in soil bacterial communities. *ISME Communications*, *3*(1), 73. https://doi.org/10.1038/s43705-023-00281-1

Wang, C., Morrissey, E. M., Mau, R. L., Hayer, M., Piñeiro, J., Mack, M. C., Marks, J. C., Bell, S. L., Miller, S. N., Schwartz, E., Dijkstra, P., Koch, B. J., Stone, B. W., Purcell, A. M., Blazewicz, S. J., Hofmockel, K. S., Pett-Ridge, J., & Hungate, B. A. (2021). The temperature sensitivity of soil: Microbial biodiversity, growth, and carbon mineralization. *The ISME Journal*, *15*(9), 2738–2747. https://doi.org/10.1038/s41396-021-00959-1

Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics*, *30*(5), 614–620. https://doi.org/10.1093/bioinformatics/btt593

Zhou, J., Deng, Y., Shen, L., Wen, C., Yan, Q., Ning, D., Qin, Y., Xue, K., Wu, L., He, Z., Voordeckers, J. W., Nostrand, J. D. V., Buzzard, V., Michaletz, S. T., Enquist, B. J., Weiser, M. D., Kaspari, M., Waide, R., Yang, Y., & Brown, J. H. (2016). Temperature mediates continental-scale diversity of microbes in forest soils. *Nature Communications*, *7*(1), 12083. https://doi.org/10.1038/ncomms12083

Ziemert, N., & Jensen, P. R. (2012). Phylogenetic Approaches to Natural Product Structure Prediction. In *Methods in Enzymology* (Vol. 517, pp. 161–182). Elsevier. https://doi.org/10.1016/B978-0-12-404634-4.00008-5