MASTERARBEIT | MASTER'S THESIS

Titel | Title

Microbial activity responses to long- and short-term warming in Subarctic grassland soils

verfasst von | submitted by

Thayer Lathrop Taft

angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien | Vienna, 2025

Studienkennzahl lt. Studienblatt | Degree programme code as it appears on the student record sheet:	UA 066 299
Studienrichtung lt. Studienblatt | Degree programme as it appears on the student record sheet:	Masterstudium Environmental Systems: Processes - Pollution - Solutions
Betreut von | Supervisor:	Assoz. Prof. Mag. Dr. Christina Kaiser

ACKNOWLEDGEMENTS

There are a great many people I leaned on during my studies:

Thank you to Tina, for welcoming me to TER and for always providing excellent advice that allowed me to think about the research in new ways.

Thank you to Sean, who was always up for discussions ranging from details of the ForHot site to the role of science in society, and to Ksenia, for her contributions in sampling and making sense of the data.

I would also like to thank the entirety of TER – both because many students, scientists, and technicians directly processed and measured samples presented here, but also because you make the workplace into an exciting and supportive place to be. I would especially like to thank Stefan, Lucia, Kian, Alberto, Lili, and Julia, who patiently taught various methods that were part of my master's studies. Thank you also to administrative and cleaning staff, who allow the whole TER project to function.

Thank you to my family, partner, and friends, who have supported and encouraged me in writing this thesis and in navigating life as a whole. Without their many contributions, this thesis would not have been possible.

CONTENTS

1 GENERAL INTRODUCTION

1.1 Climate change in the Arctic and Subarctic

A wide variety of human activities, including land development, deforestation, and burning fossil fuels, have rapidly altered global systems. Among these changes is surface warming through the release of greenhouse gases. Warming effects are amplified in the polar regions, with the Arctic experiencing a temperature increase nearly four times the global average (Rantanen et al., 2022). By the end of this century, the total temperature increase in the Arctic is predicted to be between 2.2 and 8.3°C (Collins et al., 2013). Multiple mechanisms contribute to the polar amplification of warming, the most prominent driver being the surface albedo feedback. As snow and ice cover melt away, they reflect less solar radiation (Rantanen et al., 2022). Furthermore, ocean heat transport and atmospheric moisture transport increase with warming (Beer et al., 2020; Fernández-Alvarez et al., 2023). Other processes implicated in rapid warming at the poles include changes in cloud cover and the lapse-rate feedback (Stuecker et al., 2018). Both air and soil warming are occurring faster at the poles, but models predict that soil warming will lag behind air warming in polar regions. This lag results from snow and ice cover impairing heat transfer from air to soil (Soong et al., 2020).

Warming in Arctic and Subarctic regions will have wide-reaching effects. Locally, the impacts can already be observed, including widespread greening, shrubification, and browning (Arndt et al., 2019; "Polar Regions," 2023). Melting ice sheets lead to sea level rise and flooding in coastal regions around the world. Subarctic soils are also important carbon stores, with the Arctic and Subarctic estimated to contain about 40% of the world's near-surface labile soil carbon (McGuire et al., 2009). Release of this carbon could exacerbate the greenhouse gas effect and act as a positive feedback to warming. However, there is great uncertainty about the net effect of various Arctic feedbacks. For one, regional impacts can vary widely due to both natural and anthropogenic influences, such as water availability, temperature, and land use. Furthermore, some effects, such as greening and changes to the tree line, can increase the amount of carbon that is assimilated from the atmosphere ("Polar Regions," 2023).

Historically, these soils have functioned as carbon sinks. Low temperatures reduce microbial activity and the amount of carbon which is returned to the atmosphere (Lundin et al., 2016). Air temperatures are often higher than soil temperatures, providing an advantage to the producers, which carry out photosynthesis in this warmer above-ground environment (Swanson et al., 2000). However, recent studies suggest that at least some Subarctic soils have

begun to function as carbon sources due to warming (Lundin et al., 2016). Arctic soils also contain large stores of organic carbon frozen in permafrost, which might be released as methane, a more powerful high greenhouse gas than CO_2 (Zimov et al., 2006).

1.2 The ForHot site

This study uses soils collected from the ForHot site, a site in Iceland containing geothermal hotspots. The site is divided into three areas: "Grassland New" (GN), "Forest New" (FN), and "Grassland Old." The GN and FN sites have been warmed since 2008, when a major earthquake shifted geothermal systems and opened new geothermal bedrock channels below previously unwarmed areas. However, the GO site, which is the focus of this research, has been heated for at least decades. A geothermal survey completed in 1963-1965 recorded hotspots at the site. Historical evidence suggests that the site has been geothermally active for much longer: the GO site is in Grændalur (green valley), a name that refers to the green patches which persist over the warmest hotspots during winter, and which first appears in historical records in 1708. Since the appearance of hotspots, activity may have fluctuated due to seismic activity, as observed in other parts of the site after the 2008 earthquake. Permanent study transects are established along temperature gradients at the geothermally active sites, with temperature monitoring at six points along each gradient (Sigurdsson et al., 2016).

This site offers many advantages for studying warming. These hotspots have already been warmed for at least 60 years, much longer than most experimental warming setups. The hottest parts of the temperature gradient are much warmer than can be achieved with artificial warming methods, which include heat-resistance cables, infrared lamps, and open-top chambers (Aronson & McNulty, 2009). Thus, the gradient spans a wide range of future Subarctic temperature scenarios, and even far exceeds the level of warming predicted by 2100. Although geothermally warmed water can be enriched in many minerals, including boron and arsenic (Bundschuh et al., 2013; Guo et al., 2008; Navarro et al., 2011; Wetang'ula & Snorrason, 2005), which could have ecotoxic effects and confound interpretations of geothermal warming, studies of soil water chemistry at the ForHot site have revealed no such contamination. Furthermore, the warmed plots undergo seasonal variations in temperature, as they would under future global warming (Sigurdsson et al., 2016). Taken together, the ForHot site realistically mimics soil warming due to climate change.

However, there are some differences between the ForHot site and likely climate change scenarios. Geothermal warming occurs suddenly following seismic activity, unlike the gradual warming as greenhouse gases are continuously released into the atmosphere. This difference

can have meaningful impacts, illustrated by long-term studies that fail to replicate more extreme, short-lived changes induced by stepwise changes(De Boeck et al., 2015); notably, this is also a problem for experimental manipulation studies, and may be mitigated by the long period of warming at this site. Furthermore, a geothermal warming gradient does not involve changes in precipitation patterns which will occur under climate change. There is no CO_2 fertilization at the site, although under actual future climate scenarios, CO_2 level rise might increase plants' carbon inputs to soil (Rogers et al., 1994). The lack of precipitation and CO_2 changes might limit the site's realism as a simulation of a future climate, but the isolation of warming and warming-caused effects can also be useful in a research context, allowing us to consider the influence of a few factors without many competing explanations.

Aside from precipitation, climate change can increase soil drying through evaporation and transpiration (Romero-Olivares et al., 2017). The geothermally warmed soils should be subject to increased evaporation, but the effect of transpiration may be lower than in an actual future climate scenario, where higher air temperatures might lead to greater water loss from aboveground vegetation. In addition, the effects of warming on vegetation present at the ForHot site may be somewhat constrained in comparison to other Subarctic areas because Iceland is an island with a smaller species pool (Meynzer et al., 2016). While diverse flora and fauna have been expanding to higher elevations and latitudes response to warming (Chen et al., 2011; Hickling et al., 2006), Iceland is isolated from many species which might otherwise move poleward (Meynzer et al., 2016).

1.3 Soils under warming

Soil is the largest terrestrial carbon store, exceeding vegetation and atmospheric carbon combined (Schlesinger & Andrews, 2000). As such, the exchange of carbon between soil and atmosphere is a major control for the greenhouse gas effect (Figure 1.1). As soil experiences global changes such as warming and rainfall increases, this balance may shift. Yet both the size and direction of this carbon feedback are uncertain. Previously, most modelling studies assumed that warming would lead to large-scale soil carbon losses as soil respiration (R_s) increases following warming. However, empirical studies have found that this increase in R_s is typically only short-term, with R_s eventually returning to pre-warming levels (Luo et al., 2001; Melillo et al., 2002; Oechel et al., 2000; Romero-Olivares et al., 2019; Walker et al., 2018). Two main hypotheses explain the short-term nature of thermal respiration increases. One is that the supply of labile carbon or other nutrients drops with increased microbial activity and becomes a limiting factor (Eliasson et al., 2005; Kirschbaum, 2004; Knorr et al., 2005;

Schindlbacher et al., 2011; Walker et al., 2018). The other hypothesis is thermal adaptation, with the physiology of microbes in soil changing to improve fitness under the new environmental conditions. Climate models can produce varying results depending on which mechanism modelers use (Bradford, 2013). The current body of literature is inconclusive, with evidence in support of both hypotheses.

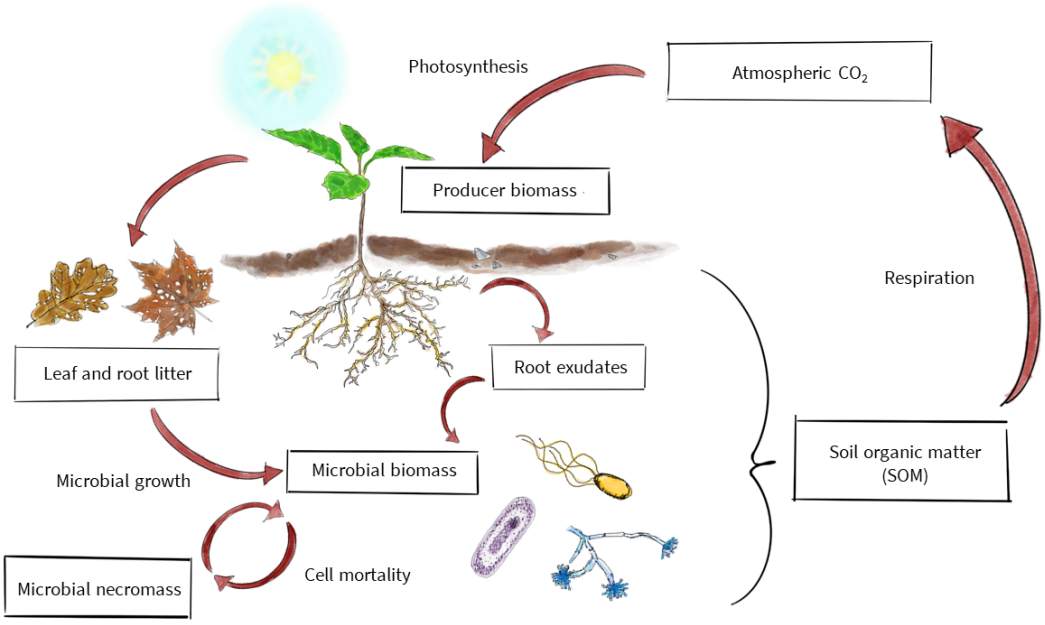

Figure 1.1 Key processes controlling the fate of terrestrial carbon.

Enzymes are intrinsically temperature sensitive. At lower temperatures, molecules undergo fewer conformational changes. Enzymes which are more flexible have an advantage, being more likely to assume binding conformations and to change shape to release the product. At higher temperatures, however, this flexibility can be a disadvantage, with the molecule changing shape more rapidly and spending less time in binding conformations (C. Liu et al., 2022; Wallenstein et al., 2010). This creates an evolutionary tradeoff between flexibility and stability, with selection pressure for a particular microbe to produce enzymes well-suited to the temperatures it experiences. The temperature at which an enzyme functions most rapidly is referred to as the temperature optimum, T_{opt}. In assays of catalytic rate at different temperatures, T_{opt} is visible as the point where catalytic rate peaks.

Temperature sensitivity can be assayed by measurements of a microbial process during incubation at different temperatures. It is most often described with the metric Q_{10}, the factor of difference in the rate of a microbial process accompanying a 10°C change in temperature (Kirschbaum, 1995).

Different isoenzymes – enzymes with different structures that perform the same function – can vary in optimum temperature. Soil may contain a wide variety of isoenzymes, produced by the same or different microbes. The combined action of the isoenzymes present produces a soil-wide T_{opt} . Donhauser et al. (2020) propose that a soil's T_{opt} for bacterial growth is typically at or even 5-15°C above the local maximum annual temperature.

1.3.1 Thermal adaptation

As soils experience warming, one might expect the exact enzymes produced by soil microbes to shift, so that the temperature optimum moves upward along with the mean and maximum annual temperature. Such a shift is described in the literature as thermal acclimation or thermal adaptation. The two terms are often used interchangeably, although this work will use them as distinct terms as laid out by Bradford (2013). Thermal acclimation refers to physiological changes within organisms on a timescale of days to weeks following warming. A change in gene expression, for example, would be a form of thermal acclimation.

Thermal acclimation is one form of adaptation. However, thermal adaptation also encompasses a wider variety of changes to soil microbes' physiology. These changes may occur over many generations, as the alteration in soil temperature and the indirect effects of warming create different selection pressures. This can include selection between pre-existing variation within a species, selection for de novo mutations, and selection for entirely different species and functional groups of microbes (Barrett & Schluter, 2008). This sort of genetic turnover would likely be an important form of adaptation in the case of long-term warming (Bradford, 2013).

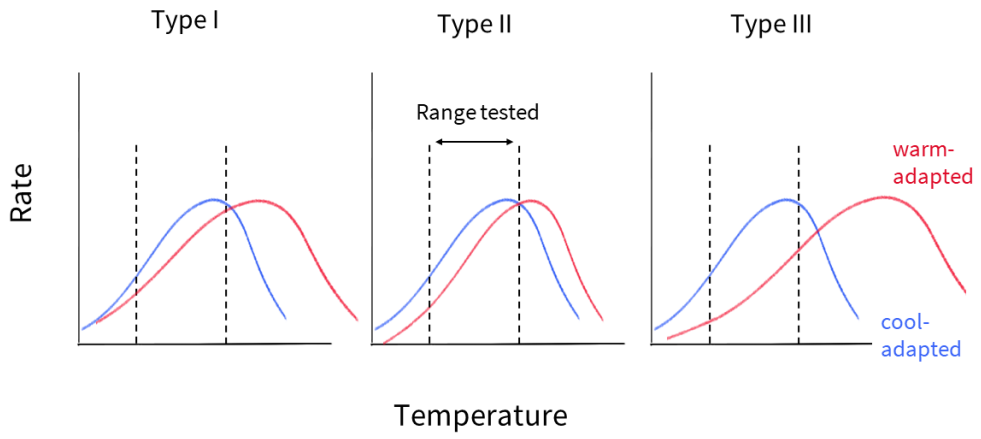

Figure 1.2 The theoretical forms of adaptation in temperature response outlined by Bradford (2013) describe changes along the entire temperature response curve; the exact range tested by a particular experiment influences the appearance of adaptation.

Thermal adaptation can be divided into different categories based on the changes observed in temperature sensitivity, optimum temperature, and baseline catalytic rate (Figure 1.2). In Type I adaptation, temperature sensitivity decreases such that the T_{opt} increases. In Type II adaptation, the entire temperature response curve is shifted rightward without a change in temperature sensitivity (Bradford, 2013). Type III describes a combination of both, such that the shape of the temperature response curve can appear very different in the range of temperatures assayed (Bradford et al., 2008). All three of these theoretical forms of adaptation should be detectable as a reduction in activity when the rate is measured at an intermediate temperature, although measurement at multiple temperatures is needed to calculate a sensitivity across a certain range.

1.3.2 Substrate limitation and drying

In addition to the intrinsic temperature sensitivity of enzymes, warming has a variety of other effects which can indirectly impact microbial activity in soils. If soil enzymes' temperature sensitivity drives a short-term increase in respiration, this burst of activity may deplete carbon stocks and other nutrients in soil. Substrate availability might then begin to limit microbial activity, even if the microbial community composition and the temperature sensitivity and T_{opt} of the enzymes present remains the same.

Substrate depletion can create the appearance of thermal adaptation without actual adaptation or acclimation. To clarify whether microbial physiology has changed, we can calculate a mass-specific respiration rate, R_{mass} , and other mass-specific microbial activity rates (Bradford, 2013; Walker et al., 2018). Rather than evaluating respiration per unit of dry soil, respiration is calculated per unit of microbial biomass carbon, $\mathrm{C_{mic}}$. If some microbes have died following substrate depletion while the remaining organisms have continued to produce enzymes with an unchanged temperature response, then mass-specific measurements of activity will remain the same even as activity per unit of dry soil drops.

Soil warming tends to be joined by another physical change that often inhibits soil microbes: drying. Drought can inhibit microbial activity, partially offsetting the effect of warming on CO_2 efflux (Muhr et al., 2010; Schindlbacher et al., 2012; Z. Zhang et al., 2022). Drought's negative effect on microbial activity is mediated not just by osmotic stress and lack of water per se, but by reduced diffusion of carbon and other nutrients, as well as the microbes' own extracellular enzymes, throughout the soil (Muhr et al., 2010; Schimel et al., 2007). Because soil warming and drying go hand-in-hand, teasing apart their effects can prevent a challenge in some experimental setups. Bao et al. (2016), for example, report a decrease in soil respiration in

warmed soils which they attribute to soil moisture effects rather than substrate depletion or adapted temperature response.

While drying per se might reduce heterotrophic respiration, this does not show us the full effect of drying on soil carbon stocks. A related phenomenon, the Birch effect, can lead to soil C depletion when the drying-rewetting cycle intensifies (Bailey et al., 2019). The Birch effect describes a pulse of elevated respiration when dried soil is rewetted (Birch, 1958). In some systems, Birch effect pulses may account for as much as two-thirds of total annual respiration (Fan et al., 2015). The Birch effect results from a combination of micro-scale biological and physical factors induced by drought, although the relative contribution of each factor is the subject of some debate (Evans et al., 2016; Schimel, 2018; Singh et al., 2023). During drought, resource diffusion throughout the soil is limited. Microbes, although inhibited, may continue to produce extracellular enzymes. Microbes also experience drying stress, possibly leading to cell death. During rewetting, water destabilizes soil aggregates and transports the unequally distributed enzymes and substrates (Schaeffer et al., 2017; Warren, 2016); there may be a spike in available organic matter from drought-induced cell death. Altogether, these micro-scale responses to drought and precipitation lead to a burst of respiration with significant implications for the global carbon balance (Bailey et al., 2019; Evans et al., 2016).

Rewetting events are likely to increase because of global warming. In high latitudes, drying is exacerbated by changes in snow cover. Earlier summer snow melt allows for more intense heating of the dark soil surface and greater evaporation (Callaghan et al., 2011). Furthermore, global warming causes changes in precipitation patterns. In the Arctic, precipitation has increased (McBean et al., 2005). However, the Arctic has also undergone soil drying (Oechel et al., 2000). Increased precipitation does not necessarily result in consistently higher moisture levels. Rather, global warming has led to an intensification of the water cycle, with more frequent periods of drought followed by more extreme precipitation (Dore, 2005). This in turn might increase carbon losses.

1.3.3 Plant-microbe interactions

Although warming can indirectly cause substrate depletion, it also might have the opposite effect. Elevated atmospheric CO_2 concentrations, extended growing seasons, and increased precipitation might allow for an increase in primary production, including in models of temperate grasslands (Hunt et al., 1991). This increase in growth might be diminished by nitrogen limitation. However, human activities can also have an impact on nitrogen availability. CO_2 fertilization itself can lead to greater carbon allocation to N-fixing root symbionts and

thereby stimulate N fixation in legume/bacterial root nodules (Rogers et al., 1994). Furthermore, fuel combustion has led to substantially increased N deposition, albeit mostly limited to developed areas and away from highly productive, N-limited forests (Schlesinger & Andrews, 2000).

Plant productivity and microbial activity are linked, with both roots and aboveground litter exerting an influence on microbes (Legay et al., 2014; Paterson, 2003; Y. Zhang et al., 2020). Plants are estimated to allocate between 11 and 17% of net fixed C to rhizodeposition (F. A. Dijkstra et al., 2013), and roots have important effects on microbial community and carbon storage. Rhizodeposition includes both release of soluble exudates and cell sloughing from abrasion and root death. Both the scale of root deposition by plants and the effects of this deposition vary widely, and are affected by plant species and age as well as abiotic factors. Most often, it results in a positive rhizosphere priming effect (RPE), where planted soil has higher respiration than unplanted soil (F. A. Dijkstra et al., 2013). A positive priming effect likely results from a release of extracellular enzymes that remain active and increase the rate of SOM decomposition even after the rhizodeposited substrate has been exhausted (Kuzyakov, 2010). As a result, even an increase in primary production could stimulate SOM decomposition and act as a positive feedback for carbon loss (Hopkins et al., 2014). Rhizosphere priming has been found to increase the temperature sensitivity of soil respiration (Zhu & Cheng, 2011). However, studies of soil respiration under warming typically do not examine the role of the RPE. Field studies make no distinction between the rhizosphere and the rest of the soil, while laboratory studies, including the one presented here, typically exclude roots and thus implicitly ignore any effects specific to the rhizosphere (J. Zhou et al., 2024).

Among the most significant of plant-microbe interactions are mycorrhizal symbioses between plant roots and fungi. Mycorrhizal symbioses are speculated to have facilitated vascular plants' colonization of terrestrial environments. These relationships vary substantially with regard to morphology, evolution, and function. Mycorrhizal relationships can be broadly grouped into two categories, ectomycorrhizal and endomycorrhizal, based on the location where carbon and nutrient exchange take place. Ectomycorrhizal (EcM) fungi are polyphyletic, and include representatives of Basidiomycota, Ascomycota, and Zygomycota. EcM fungi are estimated to colonize only 2% of plant species, but are the dominant partners for trees in boreal and temperate forests, which make up an estimated 21% of aboveground biomass (Soudzilovskaia et al., 2019). In addition to their function in nutrient acquisition, the thick sheath which EcM form around plant roots provides protection to the plant against pathogens

(Zengpu et al., 1994). EcM fungi are not particularly relevant to our research site, a grassland dominated by herbaceous species. The dominant species of plants at the long-term warmed grassland instead form associations with the ancient, monophyletic clade Glomeromycotina, also known as arbuscular mycorrhizal (AM) fungi because of their arbuscules: tree-shaped structures in the plant's root cells where nutrient exchange occurs. Around 80% of extant vascular plant species form AM associations (Wang & Qiu, 2006). Both ericaceous plants and orchids form distinctive mycorrhizal relationships with a variety of fungal partners (Perotto et al., 2002; Yukawa et al., 2009). Orchid mycorrhizae demonstrate another function of mycorrhizal fungi. These mycorrhizae are characterized by the transfer of C from fungus to plant, at least in young seedlings. Without the fungus' contribution, orchids' tiny seeds would contain insufficient nutrients (Jansa & Treseder, 2017). In the case of C flow to plants, it is sometimes unclear how – or if – the fungus benefits, with some authors suggesting that plants can function as a sort of storage unit protected from fungal-feeding predators (Bonfante & Anca, 2009; Lekberg et al., 2010). There are examples of complex symbioses between plants and bacteria, as well, such as the actinorhizal symbiosis, which facilitates a large portion of terrestrial N inputs (Lambers et al., 2009).

Given the significance of producers for microbial activity, the specific plants at our research site demand some attention. At the long-term warmed grassland site, the three most dominant species of vascular plants include a grass, *Agrostis capillaris*, and two dicotyledonous herbs, *Ranunculus acris* and *Galium boreale* (Sigurdsson et al., 2016). The dominant vascular plant species are consistent across the temperature gradient (Gargallo-Garriga et al., 2017). *Equisetum pratense* and *Equisetum arvense* are also dominant vascular species at the site (Van Loock, 2017). Their presence is notable from a microbiology perspective because equiseta are typically considered non-mycorrhizal, more commonly forming associations with endorhizal fungi (Giesemann et al., 2020; Hodson et al., 2009). Although the dominant species have remained the same, there is evidence of some warming-related changes. Leblans et al. (2017) found a linear increase in the growing season across plots warmed up to +10°C, while others report an earlier start to the growing season mitigated by early senescence (Meeran et al., 2023). In addition, a study of the metabolomes of *A. capillaris* and *R. acris* showed indicators of stress beyond a +5°C warming threshold (Gargallo-Garriga et al., 2017).

In addition to the vascular plant species, over 60% of the area of GO plots is covered by moss (Sigurdsson et al., 2016). In addition to making up a substantial part of plant biomass, mosses are responsible for a large portion of N fixation in the Subarctic (Permin et al., 2022).

Simulations suggest that moss coverage will increase in the Arctic (Epstein et al., 2000). Mosses tend to produce litter with carbon that is decomposed more slowly by microbes, which could have major implications for carbon cycling (Lindo et al., 2013). At our research site, the ratio of mosses to vascular plants increases with warming (Fang et al., 2023). However, studies of natural and experimental gradients indicate that mosses' responses to warming might vary considerably across species, and that many species respond negatively (Deane-Coe et al., 2015; Lang et al., 2012; Norby et al., 2019). Mosses are vulnerable to increased shading by shrubs, and when growing conditions are suitable for vascular plants, mosses may fail to compete (Norby et al., 2019). Because they lack the ability to regulate their water content, mosses are especially responsive to changes in moisture; drier mosses have reduced growth (Elmendorf et al., 2012). The directionality of the response to warming might also vary seasonally (Osaki & Nakatsubo, 2021). Many mosses' growth seasonality differs from that of vascular plants, growing primarily in the shoulder seasons, shortly before and after the growing season for vascular plants (Deane-Coe et al., 2015).

Besides changes in growth and species composition, climate change is likely to modify N fixation rates in bryophytes (Lett et al., 2024; Permin et al., 2022; Rzepczynska et al., 2022). N fixation in bryophytes is also dependent on moisture (Lett et al., 2024). In a study of two species of Subarctic feather mosses in northern Sweden, both showed a negative effect of warming, but sensitivity was different between species (Permin et al., 2022). Other studies confirm that responses are species-specific, and suggest that precipitation changes can counter a negative effect of warming on N fixation (Lett et al., 2024; Rzepczynska et al., 2022).

1.4 Studying community composition and function

Terrestrial microbial communities are complex: an estimated 59% of Earth's species inhabit the soil (Anthony et al., 2021). The actors in this system are a diverse bunch of both eukaryotes and prokaryotes, interacting with each other in various forms of competition, predation, and symbiosis. Different actors may fix or mineralize C or N, protect or attack plants, and grow or respire with varying degrees of efficiency. Accordingly, ecologists employ a wide variety of techniques to study these organisms' presence and function, methods which all present different tradeoffs.

To know exactly which organisms are present in a sample, we turn to DNA sequencing. Next-generation sequencing allows for DNA metabarcoding, in which a short DNA region present in many species is sequenced. In soil science, the most common selections are the 16S ribosomal RNA gene for prokaryotes and the fungal internal transcribed spacer (ITS)

(Bellemain et al., 2010; Lewe et al., 2021). Because these methods provide an exact sequence, they also allow researchers to reconstruct evolutionary relationships, although the genes most suited to phylogenetic studies may differ from the regions used for characterizing diversity in a soil sample. Among fungi, for example, some protein-coding genes are more conserved than the ITS region, which undergoes relatively frequent insertion and deletion mutations that pose problems for sequence alignment (Bruns & Shefferson, 2004). Studies using metabarcoding show that climate-change related disturbance is likely to alter species composition of the soil and its response to events such as drying-rewetting (Evans & Wallenstein, 2014). Furthermore, metabarcoding has shown warming-related community shifts (Bradford et al., 2010; DeAngelis et al., 2015; Donhauser et al., 2020), and demonstrated that phylogeny is poorly predictive of temperature response (Oliverio et al., 2017). Of course, metabarcoding has limitations. DNA in dead organisms can be preserved for years, and many of the taxa identified by metabarcoding are not active (Carini et al., 2016; Metze et al., 2024). Metabarcoding is subject to many possible sources of bias, such as cells that are more or less prone to lysis and amplification biases during the polymerase-chain reaction (PCR) (Lewe et al., 2021). Precise quantification of relative sequence abundances can be improved by the addition of an internal standard, known as a "spike-in," with a sequence distinct from species in the sample microbial community. Depending on when such a standard is added, it can provide information about amplification and sequencing biases or extraction efficiency (Alteio et al., 2021).

Even if relative quantification is correct, it only produces a relative abundance for a particular gene. Absolute quantification can be achieved with techniques such as real-time quantitative PCR (qPCR), digital PCR (dPCR), or droplet digital PCR (ddPCR)(Bouchez et al., 2016; Quan et al., 2018; D. Wang et al., 2022) . In the case of qPCR, target DNA is quantified using a fluorescent marker for double-stranded DNA (dsDNA) at the end of each PCR cycle. This can be used to estimate the number of copies present prior to amplification (Bouchez et al., 2016). dPCR and ddPCR, in contrast, function by dividing the PCR mixture into thousands of smaller reactions, referred to as partitions or droplets, respectively. After PCR amplification, fluorescence of the dsDNA marker provides binary information about the presence of the target sequence within each partition. The absolute copy number in the entire sample can then be calculated using a Poisson distribution (Quan et al., 2018; D. Wang et al., 2022).

Metabarcoding with 16S and ITS can provide information about the members present in a microbial community. However, other genetic data can provide information about their physiology. The taxa present can provide some information about potential ecological

functions, as sequenced genomes may contain genes with known functions in decomposition and nutrient cycling (Baldrian & López-Mondéjar, 2014). Transcriptomic data provides a clearer picture of which genes are actually being expressed, although extracting sufficiently high quantities of easily degraded messenger RNA can present a methodological challenge (Poursalavati et al., 2023; Wellington et al., 2003). Some transcriptomics studies indicate warming-induced functional changes which might allow communities to use more recalcitrant C sources (Choudoir et al., 2014; Jun Liu et al., 2024; Pold et al., 2016; Roy Chowdhury et al., 2021). Romero-Olivares et al. (2019) report greater investment in cell maintenance activities, in line with the idea that higher temperatures could increase maintenance costs and decrease carbon use efficiency (Frey et al., 2013). However, not all results of transcriptomics studies are universal; at least one study shows an increase in lignin-degrading transcripts and decrease in cellulose-degrading transcripts (Jun Liu et al., 2024), while another warming study shows responses in the opposite direction (C. Luo et al., 2014). Some differences might be related to soil fraction or timeline: mineral soil, for example, may receive more partially-degraded carbon sources following warming, while carbon in the organic layer becomes increasingly scarce (Pold et al., 2016). In addition to changes in genes expressed, warming might have impacts on how genetic information is stored, such as changes in codon usage bias (Choudoir et al., 2014) and increase in G+C content (C. Luo et al., 2014).

Not all community composition data relies on DNA or RNA. A variety of other molecules can function as less specific biomarkers for bio- and necromass of various origins. These include amino sugars (Glaser et al., 2004; Salas et al., 2024), neutral sugars (Gunina & Kuzyakov, 2015; Salas et al., 2024), and ergosterols (Montgomery et al., 2000). This study makes use of two sets of fatty acid biomarkers, phospholipid fatty acids (PLFAs) and neutral lipid fatty acids (NLFAs).

PLFAs make up the bulk of cell membranes, while NLFAs are significant as storage compounds (Gorka et al., 2023). Unlike DNA, PLFAs are rapidly degraded after cell death, so they provide a better idea of the groups currently active in a community (Y. Zhang et al., 2019). PLFAs also provide an accurate estimate of microbial biomass, which is essential for calculating R_{mass} (Caro et al., 2023). Culture studies have identified certain PLFA biomarkers for broad categories of microbes, such as fungi, gram-positive bacteria, and gram-negative bacteria; however, many species produce the same membrane lipids, which makes this technique inappropriate for studying taxonomic diversity (Frostegård et al., 2011). Both PLFAs and NLFAs can be extracted concurrently and fractionated by origin (Gorka et al., 2023). This is

advantageous as different groups of fatty acids can vary in their specificity as biomarkers. Arbuscular mycorrhizal fungi can be reliably detected using NLFA 16:1ω5 NLFA, but PLFA analysis alone is insufficient, as certain bacteria can also produce PLFA 16:1ω5 (Lekberg et al., 2022; van Aarle & Axel, 2003; Olsson & Lekberg, 2022). However, linking shifts in PLFA composition to community shifts is complicated by the fact that microbes may alter their membrane lipids in response to environmental conditions. This includes the response to temperature, which may trigger changes in fatty acid saturation or branching in order to maintain membrane fluidity (Wixon & Balser, 2013). Microbes' ability to dynamically resynthesize membrane lipids may be impacted by their nutritional status, with starved cells being less able to respond to changing environmental conditions (Petersen & Klug, 1994). We can apply different strategies to handle the interacting effects of community composition and physiological challenges. PLFAs can be assigned to categories constructed to include related molecules produced by the same organisms; gram-negative bacteria, for example, should be represented not just by PLFAs cy17:0 and cy19:0, but also by their precursors 16:1ω7 and 18:1ω7. We can also calculate physiological metrics, like degree of unsaturation, ratio of iso- to anteiso-branched fatty acids, and cis/trans ratios (Wixon & Balser, 2013). However, these metrics can still be ambiguous. Membrane unsaturation, for example, has an effect on membrane fluidity, and can thus be influenced by temperature, but has also been used as an indicator of starvation and physiological stress (Schütz et al., 2009).

A variety of study designs combine PLFA analysis with stable isotope probing. Zheng, et al. (2021), for example, use ^{13}C -labelled Escherichia coli biomass to trace the decomposition of the E. coli cells and uptake by other organisms. ^{13}C enrichment was initially highest in fungal PLFA biomarkers, demonstrating that they decomposed and utilized the bacterial biomass C source before other groups. At later time points, other microbes began to use ^{13}C derived from fungal biomass, and the relative levels of ^{13}C enrichment between groups stabilized. Kramer & Gleixner (2008), too, used ^{13}C to study carbon transformation – in this case exploiting a vegetation change from C_3 to C_4 plants and the differing isotopic signatures of the C_3 and C_4 photosynthetic pathways. Gram-negative bacteria tended to prefer plant-derived carbon, while gram-positive bacteria used more older, SOM-derived carbon. At the ForHot site, a ^{13}C pulse labeling experiment found increased uptake of plant-derived C by AM fungi and gram-positive bacteria at warmed plots (Verbrigghe et al., 2022).

In this study, we use a different stable isotope method involving the addition of deuterated water (^2H_2O). Because there is a high relative mass difference between deuterium (^2H) and ^1H_2O, biological processes discriminate heavily between the two isotopes, and ^1H_2O is

typically quite depleted in naturally occurring membrane lipids (Wegener et al., 2016). All organisms incorporate hydrogen atoms derived from water molecules into their membrane lipids; ^2H_2O is also not a source of nutrition and should not select for specific taxa (Caro et al., 2023). In our soils, collected from a site that is not vulnerable to drought, ^2H_2O was added directly. However, ^2H_2O can also be added via water vapor equilibration to avoid rewetting effects (Canarini et al., 2024).

1.5 The state of literature on adaptation and substrate depletion

Researchers have examined thermal adaptation in a variety of soil ecosystems, with conflicting results. In a study of experimentally warmed soil microcosms, Bradford et al. (2010) observed a drop in R_{mass}. In another other microcosm study, alpine soils incubated above their temperature optimum demonstrated both a shift in the temperature range for growth and an increase in heat-adapted and stress-resistant OTUs (Donhauser et al., 2020). And naturally occurring temperature gradients suggest that, at least over a long enough timespan, soil communities' growth and respiration rate adapt in a way that matches expectations. Both within drylands (Dacal et al., 2019) and across different biomes, higher mean annual temperature (MAT) is associated with lower mass-specific respiration at a given temperature (Bradford et al., 2019), as well as with higher carbon use efficiency (Ye et al., 2019).

However, other studies support substrate depletion as the limiting factor. In one study, respiration rates of intact soil cores were lower in soils that had been subject to three years of experimental +3°C in situ warming. This difference was smaller in sieved soils, possibly because sieving can make physically protected C available and alleviate substrate limitations. Similar effects were observed in shaded plots, which likely experienced a drop in primary productivity without any temperature effects (I. Hartley et al., 2007). Another research group examined respiration rates by transferring soil microbes between warmed and unwarmed soils. Warmed soils had depressed respiration rates compared to control soil, even when the soils were sterilized and inoculated with microbes from the control soil. The difference was alleviated by N supplementation, suggesting an N limitation on microbial activity in the warmed soils (He et al., 2023).

If microbial adaptation does occur, it is unclear on what sort of timeline this would take place, and evidence conflicts. While microcosm studies have demonstrated evidence of adaptation within a few months (Bradford, 2013; Donhauser et al., 2020), an in situ experimental warming study conducted in Iceland found evidence of adaptation only after 15 years (Rinnan et al., 2007, 2009). Similarly, a study of 5-, 8-, and 20-year +5°C-warmed forest soils at a forest

site showed a significant change in bacterial community only in the 20-year warmed samples (DeAngelis et al., 2015).

Both adaptation and substrate depletion could contribute to the long-term pattern of soil microbial respiration. However, in addition to evidence supporting substrate depletion, there is some evidence which explicitly refutes the adaptation hypothesis. In an experimental warming study, Schindlbacher et al. (2011) repeatedly measured respiration and microbial biomass at an alpine Austrian spruce forest. Warming during the snow-free season had begun four years prior, and measurements were taken over the course of two years. The group also assessed microbial community composition by PLFA analysis and rRNA qPCR. Biomass-specific respiration and microbial metabolic activity was elevated, but there was no significant difference in microbial community composition. Again, timeline poses an issue: perhaps four years is simply not long enough for this adaptation to take place. In this respect, the timescale of the ForHot site, with over fifty years of warming, is advantageous.

Studies thus far at the site have found mixed evidence of microbial adaptation in either the short- or long-term. Walker et al. (2018) collected ambient soils not subject to geothermal heating and incubated the samples for six weeks at +3°C or +6°C relative to ambient temperature. In addition, long-term heated soils were collected and incubated at their field temperatures. Mass-specific microbial growth and respiration remained accelerated after six weeks or decades of warming. Furthermore, the authors were able to replicate these results in a microbial-scale model using rates of microbial activity that were slightly but permanently elevated in response to warming.

In contrast to this, more recent studies of microbial community composition and temperature sensitivity at the ForHot site have indeed found evidence of adaptation – b not within the range of +6°C warming. Weedon et al. (2023) studied both forest and grassland temperature gradients at the site, investigating plots warmed as much as 40°C above the ambient temperature. They found that microbial communities varied between sites, but the threshold at which this change occurred was between +6°C and +9°C of warming, for both forest and grassland plots. Above this threshold, alpha diversity of bacterial communities decreased. Furthermore, the same threshold was observed for growth rate temperature sensitivity in soils from both vegetation types. The fact that a similar temperature threshold existed in both grassland and forest, which have vastly different starting carbon inputs, suggests that the community and temperature sensitivity shift resulted from adaptation to the temperature, rather than a response to indirect substrate effects.

1.6 Research aims

This work will shed further light on the microbial response to long-term soil warming using soils collected from across the temperature gradient at the ForHot site. First, I present mass-specific respiration measured for each soil during three separate incubation experiments. In the first of these experiments, soil at warmed sites was incubated at temperatures similar to their field temperatures. In a second experiment, soil from an unwarmed area of the ForHot site was incubated at the same elevated temperatures. In a third, soil from the warmed sites were incubated at a lower temperature similar to the unwarmed parts of the site. These data allow us to see changes in mass-specific respiration, which could be evidence of acclimation, following long-term warming ranging from 0 to +15°C. Second, I will analyze PLFA and NLFA composition, through which we can observe certain community shifts, as well as the growth rates of different microbial groups measured by deuterium incorporation into PLFAs. I will integrate these results into a broader understanding of shifts in microbial communities and traits during long-term warming.

2 MANUSCRIPT

2.1 Introduction

Soils are the largest terrestrial reservoir of carbon, containing more carbon than vegetation and the atmosphere combined (Swift, 2001). Soil microbes play a crucial role in the carbon cycle and the fate of these carbon stocks; carbon fixed by plants enters the soil through root exudates or as leaf and root litter, and bacteria, fungi and archaea use carbon for growth and release carbon dioxide into the atmosphere through respiration(Schlesinger & Andrews, 2000). However, there is a great deal of uncertainty around how temperature-sensitive biological processes will change and impact greenhouse gas fluxes as global temperatures continue to rise (Y. Liu et al., 2018). A better understanding of soil microbes' responses to long-term warming will help to predict the global implications of soil warming (Wieder et al., 2013).

Microbial respiratory enzymes function fastest at an optimal temperature, T_{opt}, which is typically higher than the maximum annual temperature experienced by a particular soil (Donhauser et al., 2020; Liu et al., 2018). Thus, short-term warming leads to an increase in respiration and carbon loss. However, rates of respiration and carbon loss do not usually remain elevated when warming continues long-term (Luo et al., 2001; Melillo et al., 2002; Oechel et al., 2000; Romero-Olivares et al., 2019; Walker et al., 2018). Throughout this paper, we refer to this reduction in soil respiration under long-term warming as apparent thermal adaptation, a term which is agnostic as to the mechanism behind this phenomenon (Bradford, 2013). In some cases, total respiration may be no different from that of unwarmed soils (Y. Luo et al., 2001; Walker et al., 2018), while in others respiration remains elevated, but with a muted temperature response in comparison to unwarmed soil (Strömgren, 2001). There are multiple mechanisms which could explain apparent thermal adaptation, and they are not mutually exclusive. One hypothesis is substrate depletion: increased respiration of soil organic matter eventually limits the available C substrate, decreasing microbial biomass and leading to a drop in respiration simply because of the smaller population of microbes (Eliasson et al., 2005; Kirschbaum, 2004; Knorr et al., 2005; Schindlbacher et al., 2011; Walker et al., 2018). Similarly, warming can lead to soil drying, which places additional stress on microbes, reduces the diffusion of nutrients, and limits microbial growth (Allison & Treseder, 2008; Bao et al., 2016; Bradford, 2013).

However, microbes may also undergo physiological changes, leading to a decrease in respiration rate independent of changes in microbial biomass. These changes might be

described as acclimation, community shifts, or adaptation. Acclimation describes changes within an individual, such as altered gene expression, in response to environmental changes (Bradford, 2013; Schimel et al., 2007). A community shift, on the other hand, would result from population changes over multiple generations, as temperature affects the fitness of entire taxa within the soil. Adaptation is a broad concept, which is used here as an umbrella term describing both acclimation and community shifts. It also includes other genetic changes, such as selection for de novo mutations within a species (Barrett & Schluter, 2008).

Adaptation and substrate depletion are not mutually exclusive, but the contribution of each is meaningful: Earth systems models that incorporate adaptation produce different predictions of how much carbon dioxide will be released from soils in the future (Wieder et al., 2013).

Because substrate depletion and adaptation both lead to changes in total respiration rates, we need a metric which distinguishes between these mechanisms. One solution is to measure a soil's microbial biomass concentration and calculate respiration per unit of microbial biomass, which is referred to as the mass-specific respiration rate (R_{mass}) (Bradford, 2013; Walker et al., 2018). R_{mass} should reflect changes in microbial physiology and not shifts which are purely the result of a larger or smaller microbial population.

Adaptation encompasses a variety of responses to temperature. We can distinguish between a "compensation response," in which microbial adaptation leads to lower mass-specific microbial activity under long-term warming, and an "enhancement response," in which adaptation leads to higher mass-specific activity (Bradford et al., 2019). A compensation response would dampen carbon losses, while an enhancement response would increase carbon losses (Alster, 2019).

There is already a substantial body of literature examining these two hypotheses with a variety of experimental methods, but the results of these studies conflict. R_{mass} has been observed to decrease following months of warming in multiple soil microcosm studies (Bradford et al., 2010; Donhauser et al., 2020), and higher mean annual temperature (MAT) is associated with higher carbon use efficiency (Ye et al., 2019) and lower temperature sensitivity of respiration (Bradford et al., 2019). While these results support compensatory adaptation, others contradict them. He et al. (2023), for example, found that inoculating 6-year warmed soils with microbes from an unwarmed control did not alleviate apparent adaptation, and Hartley et al. (2007) report apparent thermal adaptation alleviated by sieving, which can release physically protected C.

The research site presented here is a grassland in Iceland subject to at least 50 years of geothermal warming. High-latitude soils store a disproportionately large amount of carbon due to low rates of decomposition at cooler temperatures (Lundin et al., 2016; McGuire et al., 2009). However, these stores are particularly threatened by global warming. The Subarctic has experienced faster warming due to the ice-albedo feedback, ocean and moisture heat transport, and the uneven effects of the Planck feedback (Beer et al., 2020; Fernández-Alvarez et al., 2023; Rantanen et al., 2022); Stuecker et al., 2018). It is expected that Arctic and Subarctic areas will have warmed between 2.2 and 8.3°C by the year 2100 (Collins et al., 2013). The long-term warmed geothermal site is an intriguing model for these imperiled soils. Despite the source of warming, mineral-rich geothermal water has not contaminated the soil (Sigurdsson et al., 2016). The long time frame covers many generations of microbes which could have adapted to the warmed conditions, a process which some studies suggest might take years or decades (Rinnan et al., 2007, 2009).

There are some studies investigating apparent thermal adaptation at this site. Walker et al. (2018) collected unheated soils from the grassland and soils heated as much as 6°C by the geothermal hotspots. Although soil C was found to be depleted by up to 40%, respiration rates calculated per gram of dry soil were not significantly different after decades of warming. However, R_{mass} remained significantly higher in warmed soils. This was interpreted as a lack of adaptation and evidence that the apparent thermal adaptation was in fact explained by substrate depletion.

A more recent study at the site found evidence of adaptation at a threshold temperature outside the range of the previous experiment. Between 6 and 9°C of warming, there was an abrupt shift in community composition as measured by 16S sequencing and temperature sensitivity, SI, of microbial growth (Weedon et al., 2023). The same thresholds were observed in soils from a nearby spruce forest which also contains geothermal hotspots. Because there was a similar dynamic in two ecosystems with vastly different carbon inputs, the authors concluded that temperature, and not substrate effects, likely drove the shifts in microbial growth and community (Weedon et al., 2023). Notably, +6 to +9°C is in the upper range of mainstream climate models' predictions for (Sub)arctic warming this century (Collins et al., 2013); even if temperature leads to adaptation, this feedback may not occur under actual climate change conditions.

In this experiment, we pair measurements of mass-specific respiration with measurements of microbial growth measured by deuterium incorporation into phospholipid (PLFA) and

neutral fatty acids (NLFA) (Canarini et al., 2024). This method provides insight into physiological changes in soil microorganisms. PLFAs can act as biomarkers for specific taxa, such as fungi or gram-positive bacteria (Frostegård et al., 2011; Garcia et al., 2020). NLFAs exhibit similar variation (Gorka et al., 2023), including a notable biomarker for arbuscular mycorrhizal (AM) fungi (Lekberg et al., 2022; van Aarle & Axel, 2003; Olsson, 1999; Olsson & Lekberg, 2022; Sharma & Buyer, 2015) . Fatty acids provide less granular information about soil microbial community in comparison to genotypic methods, such as 16S and ITS metabarcoding. However, they do have certain advantages over DNA methods: PLFA turnover is relatively fast (Y. Zhang et al., 2019), and the lack of amplification step makes it possible to more quantitatively compare groups identified by fatty acid analysis (Ramsey et al., 2006). Fatty acid analysis ultimately provides information about phenotypes in a community, not genotype, which can make it difficult to separate acclimation from community shifts (Garcia et al., 2020). Phospholipid membrane function is affected by temperature, and microbes can respond to warming by resynthesizing membrane lipids -- such as more saturated, less fluid fatty acids at higher temperatures (Garcia et al., 2020; Wixon & Balser, 2013).Thus, not all shifts in PLFA abundances represent a shift in taxonomic groups present. However, we can measure changes in multiple PLFAs specific to particular taxonomic groups in order to infer community shifts.

The study presented here includes three combinations of soils and treatments: soils sampled along a long-term warming gradient and incubated in the laboratory at temperature close to their field temperatures (the "field warming" group); the same, long-term warmed soils incubated at a near-ambient temperature (the "intermediate acclimation" group); and samples from an unwarmed area of the site incubated across the field-like temperature range (the "short-term warming" group). We had three hypotheses related to thermal adaptation. (H1) We expected to see apparent thermal adaptation, with total soil respiration and growth lower in warmed samples than ambient temperature samples at the same temperature. (H2) We also anticipated compensatory adaptation in growth and respiration. This would be demonstrated at both field warming and an intermediate temperature by lower R_{mass} and $Growth_{mass}$ in long-term warmed samples than in ambient temperature samples incubated at the same temperature (Figure 2.1). (H3) And we expected this adaptation to occur only at a higher warming threshold; thus, mass-specific activity would be unchanged between unwarmed and ambient soils at lower levels of warming. This would account for previous findings that there is no acclimation in respiration at +6°C (Walker et al., 2018), yet changes in community composition and growth temperature sensitivity at a +6-9°C warming

threshold (Weedon et al., 2023), and is also informed by non-linear temperature responses of soil organic carbon (SOC) pools at this site (Poeplau et al., 2020). In addition to our hypotheses around growth, we wanted to examine if and what ways growth rates varied between microbial groups.

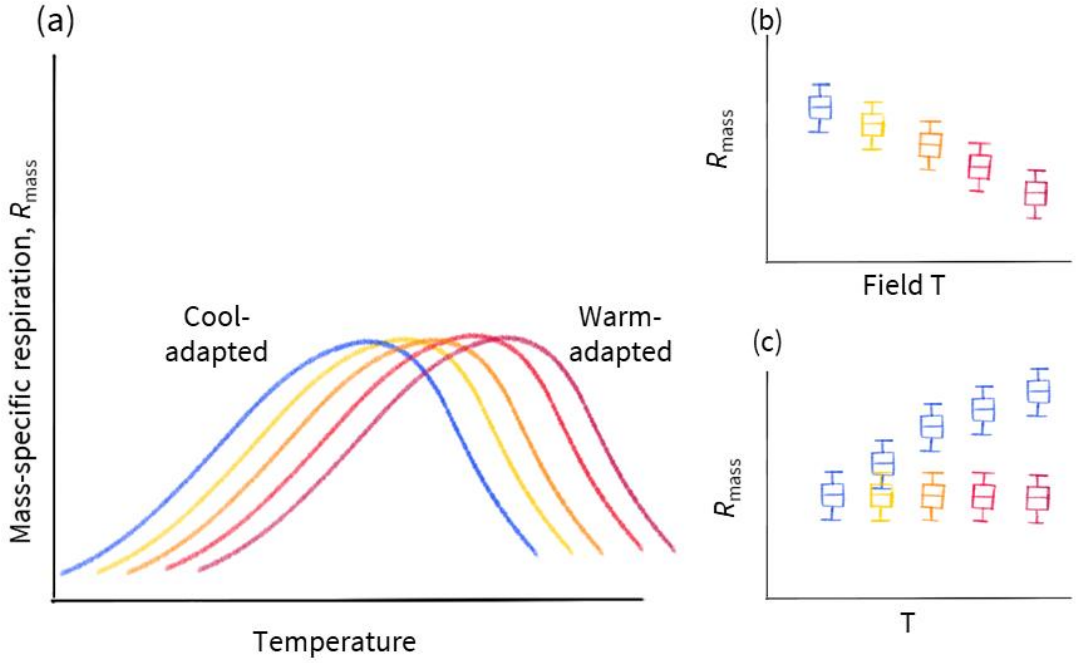

Figure 2.1 Compensatory thermal adaptation would lead to decreased mass-specific respiration and/or growth rates in warm-adapted soil. Rates could differ from unwarmed soils (a) across a wide range of temperatures. (b) Measured at a constant temperature, as in the "intermediate acclimation" experiment, this would appear as a decreasing R_{mass} with increasing levels of field warming. (c) When activity is measured at soils' warmed temperatures, as in the "field warming" experiment, compensatory adaptation would cause R_{mass} to remain constant with increasing temperature, or at least diminished in comparison to ambient temperature soils newly exposed to the same elevated temperatures.

2.2 Methods

2.2.1 Sampling

Soils were collected from plots in the grassland old (GO) section of the ForHot site in southern Iceland (64°00'01"N, 21°11'09"W) in August 2023. The GO site has been subject to natural geothermal warming for at least >50 years, and transects have been established across the warming gradient (Sigurdsson et al., 2016). Warmed soils were collected from the GO2 and GO3 transects. In total, 32 warmed soils were collected, with 16 points from each gradient. Temperature at each sampling site was measured twice daily, in morning and afternoon, for four days prior to sampling; field temperatures reported here are the mean of these eight

measurements. Temperatures ranged between 11.5°C (+0°C field warming) and 28.7°C (+17.2°C field warming). In addition, four soils were collected from an area of the site that does not experience geothermal warming.

The first 10 cm of soil were sampled after removing litter and cutting away the root layer. Soils were sieved with 2 mm sieves and placed into incubators 3 days after sampling.

2.2.2 Soil pH, C and content, and moisture

pH was measured in soil slurries prepared with 2 g of fresh soil and 10 mL of 10 mM $CaCl_2$.

Water content was determined gravimetrically after drying 5 g aliquots of fresh, sieved soil for 24 hours at 105°C.

Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were extracted from 2 g aliquots of each soil in 1M KCl and measured using a TOC/TN analyzer (TOC-V CPH E200V/TNM-122V; Shimadzu, Vienna, Austria). In addition, 2 g aliquots of each soil were fumigated in chloroform for 24 hours prior to extraction with 1M KCl and measurement. DOC and DON measurements were subtracted from measurements of chloroform-fumigation extracted soils to calculate microbial carbon (C_{mic}; mg g^{-1} dry soil weight) and microbial nitrogen (N_{mic}; mg g^{-1} dry soil weight), and divided by their respective calibration coefficients, k_{EC} and k_{EN} (Jenkinson et al., 2004).

$$C_{mic} = \frac{DOC_{fum} - DOC_{con}}{k_{EC}} \qquad k_{EC} = 0.45$$

$$N_{mic} = \frac{TDN_{fum} - TDN_{con}}{k_{EN}} \qquad k_{EN} = 0.45$$

Aliquots of dried soil were milled to homogenize. 5 mg of each homogenized soil was weighed into tin capsules and total C and N were measured by elemental analyzer (EA 1110, CE Instruments, Italy) coupled to isotope ratio mass spectrometer (IRMS; Finnigan MAT Delta Plus IRMS, Thermo Fisher Scientific, USA).

2.2.3 Incubation experiments and respiration measurements

After sieving, soils were incubated in three experiments with similar designs: field warming, short-term warming, and intermediate acclimation. For each experiment, 6 grams of wet soil were weighed and added to 50 mL Greiner tubes. Soil source and incubation temperature varied between the four experiments.

In the field warming experiment, soils were incubated at temperatures mimicking their respective temperatures at the sampling site; due to space limitations, these temperatures are not identical to the field warming, but rather each soil was placed into one of eight incubation temperature groups (12.3, 13.1, 13.8, 15.3, 16.7, 18.3, 21.2, and 27°C). In the acclimation experiment, soils from these same plots were instead incubated at a single temperature, 15.3°C. In the short-term warming experiment, each of four control soils were incubated at ten temperatures: the eight field temperatures between 12.1 and 27°C, and two additional intermediate temperatures, 19.7 and 24.2°C.

At the start of each experiment, each soil was labelled with heavy water, ^2H_2O, to achieve a target concentration of 4% ^2H content in soil water. The volume of ^2H_2O added was adjusted based on soil weight and water content, and varied between 110 and 680 µL. In addition, a duplicate set of natural deuterium abundance soils were incubated; H_2O was added to these samples instead of ^2H_2O. Vials were capped and incubated at their respective temperatures for 48 hours. Immediately after ^2H_2O addition, and again after 48 hours, the concentration CO_2 in the vial headspace was measured using an infrared gas analyzer (EGM4, PP Systems, USA). The difference between timepoints was used to calculate microbial respiration. After the incubation, sample tubes were flash-frozen and stored at -20°C prior to freeze drying and PLFA extraction.

To calculate respiration rate, CO_2 concentration measurements were converted to absolute grams of carbon within the syringe and tube headspace. The amount of carbon removed during the initial measurement was added to the final carbon amount to correct for this loss, and the amount of carbon added in the synthetic air was subtracted. Initial carbon was subtracted from final carbon and divided by time elapsed and dry weight of soil to calculate soil respiration rate (R_s; mg C g^{-1} dry soil weight h^{-1}). R_s was divided by the total mass of PLFA carbon to produce biomass-specific respiration (R_{mass}; mg C g^{-1} PLFA C h^{-1}).

2.2.4 Fatty acid extraction and analysis

All deuterium-labelled soil samples were freeze dried for 48 hours and returned to storage at -20°C. One unlabeled replicate of each treatment was also freeze dried and processed further in order to calculate deuterium enrichment in comparison to a natural abundance control.

For PLFA analysis, all glassware used was muffled at 500°C for >4 hours to avoid contamination with organic material. Lids and lid septa, which could not be muffled, were washed and rinsed with ethanol and acetone.

To extract PLFAs, 1.5 g of freeze-dried soil was weighed into 10mL glass vials. Lipids were extracted in 3.0 mL of a mixture of chloroform, methanol, and citrate buffer (CMB; v:v:v = 1:2:0.8, citrate buffer pH 0 4.0). Soil-CMB slurries were vortexed and allowed to settle overnight and then centrifuged for 10 minutes at 3000 rpm. The supernatant was collected using a Pasteur pipette. 2.6 mL of CMB was again added to each sample and vortexed, and supernatants were collected.

Following CMB extraction, 1.5 mL chloroform and 1.5 mL citrate buffer were added to each vial of CMB extracts. The mixtures were vortexed and allowed to separate overnight. The organic fractions from each vial were pipetted into flat-bottomed vials with known weights. Filled vials were weighed again to calculate yield from the organic phase. The solutions were then dried under N_2 and stored at -20°C until lipid fractionation, at most 6 weeks.

Lipid fractionation was carried out using 96-well plates with 50 mg silica per well (Phenomenex, Strata SI-1 Silica, 55 µm, 70 A), washed 3 times with 1.5 mL methanol and 3 times with 1.5 mL chloroform. 1.5 mL (1 mL and then 0.5 mL) chloroform:ethanol mixture (v:v 98:2) were added to each flat-bottomed vial, then transferred to a silica column and collected in 1.5 mL glass vials. The glycolipid fatty acid (GLFA) fraction was eluted second in 1.5 mL acetone and discarded. The third, PLFA fraction was eluted with 1.5 mL methanol-chloroform-water (v:v:v 5:5:1). Both NLFA and PLFA tubes were dried under N_2 and stored overnight at -20°C.

Prior to transesterification, 100 µL of 0.1 mg/mL methyl nonadecanoate in methanol-toluene (v:v 1:1) was added to each tube of both NLFAs and PLFAs to account for losses in the next steps. A transesterification reagent of approximately 0.07M KOH in methanol was added to each NLFA vial and placed in a 37°C chamber for 25 minutes. After 25 minutes, transesterification was stopped by addition of 0.4 mL 0.075M acetic acid. The same steps were repeated with the PLFA vials.

0.4 mL chloroform was added to each sample, samples were shaken, and 0.3 mL from the bottom phase was transferred to another 1.5 mL vial. Chloroform addition and phase separation was repeated, this time collecting 0.4 mL lower phase. Samples were dried under N_2 and stored at -20°C until gas chromatography measurements.

Dried fatty acids were resuspended in 60 or 80 µL GC-grade iso-octane and transferred to clear screw-top autosampler vials with 100 µL micro-inserts. NLFAs of field samples were identified and quantified using a gas chromatograph coupled to a time-of-flight mass

spectrometer (TOF-GCMS; GC 7890 B Agilent Technologies, USA; Pegasus BT, LECO, St. Joseph, USA). Peaks were identified in ChromaTOF (LECO) using mixtures of bacterial and fungal FAMEs (Bacterial Acid Methyl Ester CP Mixture, Matreya LLC, State College, USA; 37 Component FAME MIX; Supelco, Bellefonte, USA). NLFA areas, measured only in the field warming samples, were also quantified in ChromaTOF.

PLFAs and ^{2}H incorporation were measured for all samples using a GC-Isolink coupled to a Delta V Advantage Mass Spectrometer (ThermoFisher Scientific). Peak areas were quantified in Isodat 2.0 (ThermoFisher) and peaks were assigned manually by cross-referencing with the GC-IRMS chromatographs. Measured values of isotope ratios were corrected against a standard row with known isotopic values, prepared using mixtures of icosanoic acid methyl ester ranging from $\delta^{2}H_{VSMOW}$ -183.9‰ to 384.3% (USGS70 and USGS72, Arndt Schimmelmann, Indiana University, USA).

Absolute quantity of each fatty acid was determined by dividing peak area by the area of the internal standard in the sample and multiplying by the amount of standard (640 nmol) added prior to transesterification. These quantities were corrected for losses during the first step of extraction by dividing by the organic phase yield. The amounts of fatty acids identified in each sample's respective blanks were subtracted from amounts measured in samples.

Low-abundance fatty acids – those that made up less than 0.5% of the PLFA or NLFA carbon in a given sample -- were excluded from analysis.

The PLFAs 14:1ω5, 15:1ω5, 16:0-2-OH, 16:1ω5, 16:1ω6i, 16:1ω7, 16:1ω9, cy17:0, 17:1ω8, 18:1ω5, 18:1ω7, 18:1ω9-*trans*, cy19:0, and 19:1ω9 were used to represent gram-negative bacterial biomass. 14:0i, 15:0a, 15:0i, 16:0i, 17:0a, 17:0i, and 18:0i were taken to represent Firmicute bacterial biomass. 10Me17:0 and 10Me18:0 were taken to represent Actinobacterial biomass. Total bacterial biomass was calculated as the sum of these biomasses and of additional general bacterial PLFAs: 14:0, 15:0, 16:0Me, 17:0, and 17:0Me. The sum of PLFAs 18:1ω9-*cis*, 18:2ω6,9 and 18:3ω6,9,12 was used to represent fungal biomass. Absolute biomasses (ng PLFA C g^{-1} soil dry weight) were divided by the sum of all PLFAs to calculate a relative biomass for each taxon; taxa relative biomasses sum to less than 1 because of the inclusion of general PLFAs not assigned to any taxon in the denominator.

Total carbon atoms and chain length were added manually in Excel based on structure of the identified fatty acid.

Fatty acid saturation was calculated as the fraction of bonds in the fatty acid chains which were single bonds, such that a set of fatty acids containing no double bonds would have a value of 1:

$$\text{Saturation} = 1 - \frac{\bar{d}}{\bar{l} - 1}$$

where $\bar{d}$ is the mean number of double bonds in a sample's fatty acids, and $\bar{l}$ is the mean chain length of the sample's fatty acids.

$\bar{d}$ and $\bar{l}$ were weighted to account for the biomass contribution of each different fatty acid, and calculated as follows:

$$\bar{d} = \frac{\sum(\text{PLFA}_d \times d)}{\text{PLFA}_{\text{total}}}$$

$$\bar{l} = \frac{\sum(\text{PLFA}_l \times l)}{\text{PLFA}_{\text{total}}}$$

where d is the number of double bonds in a fatty acid, $PLFA_d$ is the moles of PLFAs containing d double bonds, l is the length of a fatty acid's main chain, PLFA_l is the moles of PLFAs of chain length l, and $\text{PLFA}_{\text{total}}$ is the total moles of PLFAs in a particular soil.

2.2.5 Growth

For each experiment (field warming, short-term warming, and acclimation) isotope ratios were measured in at least 8 unlabeled samples. The mean natural abundance of deuterium in each fatty acid in each experiment ($^2\text{H at\%}_{\text{nat ab}}$) was calculated from these samples.

The difference between the deuterium abundance in the labelled and unlabeled samples' fatty acids was divided by the maximum theoretical incorporation of deuterium into PLFAs (a_{w} ; 0.71) and the calculated deuterium abundance in the labelled sample ($^2\text{H at\%}_{\text{soil water}}$; target 0.04). This represents the fraction of the PLFA which was produced during the incubation period (Fraction $\text{C}_{\text{prod, PLFA}}$; g PLFA C produced g^{-1} PLFA C).

$$\text{Fraction C}_{\text{prod, PLFA}} = \frac{^2\text{H at\%}_{\text{lab}} - {}^2\text{H at\%}_{\text{nat ab}}}{a_{\text{w}} \times {}^2\text{H at\%}_{\text{soil water}}}$$

We divided this by the incubation time (h) to calculate a mass-specific growth rate for each PLFA ($\text{Growth}_{\text{mass, PLFA}}$; g PLFA C produced g^{-1} PLFA C h^{-1}).

$$\text{Growth}_{\text{mass, PLFA}} = \text{Fraction C}_{\text{prod, PLFA}} / \text{ time}$$

We multiplied each PLFA's $\text{Growth}_{\text{mass}}$ by an individual PLFA's biomass (C_{PLFA}; ng PLFA C g^{-1} dry soil) to calculate that PLFA's growth rate, and sum them up to calculate total soil microbial growth, Growth_s (ng PLFA C produced g^{-1} dry soil h^{-1}).

$$\text{Growth}_s = \Sigma \left(\text{Growth}_{\text{mass, PLFA}} \times C_{PLFA}\right)$$

In addition, the absolute growth of individual taxa ($\text{Growth}_{\text{taxon}}$) was calculated using only PLFAs specific to that taxon, as assigned to the groups described above.

Growth_s was divided by total PLFA biomass (ng PLFA C g^{-1} soil dry weight) to yield an overall $\text{Growth}_{\text{mass}}$; note that this step and the previous step essentially produce an average $\text{Growth}_{\text{mass, PLFA}}$ weighted by the contribution of each individual PLFA to total PLFA biomass.

$$\text{Growth}_{\text{mass}} = \frac{\text{Growth}_s}{\Sigma C_{PLFA}}$$

Mass-specific rates of growth were also calculated for each taxon. This metric is necessary because, as for total microbial respiration, we expect higher absolute growth among more abundant taxa, and need mass-specific growth in order to separate the effects of biomass changes from physiological changes.

$$\text{Growth}_{\text{mass, taxon}} = \frac{\text{Growth}_{\text{taxon}}}{\text{C taxon}}$$

Another metric, relative growth, was also calculated for each taxon. Relative growth describes the share of total microbial growth which is accounted for by a particular taxon:

$$\text{Growth}_{\text{relative, taxon}} = \frac{\text{Growth taxon}}{\text{Growth}_s}$$

Taxon-specific relative growth rates sum to less than 1 because Growth_s also includes the production of PLFAs not assigned to any taxon.

For the purpose of calculating CUE, we also calculated total microbial growth using another method, multiplying $\text{Growth}_{\text{mass}}$ by the CFE-based C_{mic} to yield CFE based Growth_s (ng C g^{-1} dry soil h^{-1}). This estimate of microbial growth, based on a more comprehensive measurement of biomass than PLFA C alone, is on a scale that is more comparable to respiration (Canarini et al., 2024).

$$\text{CFE based Growth}_s = \text{Growth}_{mass} \times C_{mic}$$

Growth and respiration for Total growth and total respiration were used to calculate a carbon use efficiency (CUE):

$$\text{CUE} = \frac{\text{CFE based Growth}_s}{\text{CFE based Growth}_s + R_s}$$

2.2.6 Statistics

Statistical analyses were carried out in R. The packages "dplyr," "ggplot2," "ggfortify," "cowplot," "viridis," and "ggrepel" were used for handling data and generating graphics.

The threshold for significance was 0.05.

Correlations between field temperature and selected soil properties (C, N, pH, moisture, PLFA biomass, AMF NLFA biomarker relative biomass) at the long-term site were tested using Pearson's product-moment correlations.

Principle component analysis (PCA) was performed using the prcomp() function, including for scaling and centering. PCA was carried out on soil properties in long-term warmed soils; on the relative biomasses of all PLFAs across all three warming experiments; and on mass-specific growth of all PLFAs across all three warming experiments.

Differences in activity rates between long- and short-term warmed soils at specific incubation temperatures were tested using Mann-Whitney *U* tests.

To test the effect of long-term warming on mass-specific growth in the short- and long-term warming experiments, a null model was constructed where Growth_{mass} was and incubation temperature was a predictor. Other models including incubation temperature as well as field warming level, or presence or absence of field warming, were also tested. Model parsimony was compared by BIC. To test the effect on respiration, the null model and all models tested used R_s as the dependent variable and additionally included total PLFA biomass as a predictor, to avoid using a calculated ratio as the dependent variable.

To evaluate potential effects of microbial community composition on respiration, linear models were built where R_s was the dependent variable and the independent variables were incubation temperature and PLFA biomass of each taxon (Fungi, Gm- bacteria, Firmicutes, and Actinobacteria), including interactions between temperature and taxa. In addition, a null

model was created where temperature and total PLFA biomass were used as independent variables, and model parsimony was compared by BIC.

2.3 Results

2.3.1 Response of soil properties to long-term warming

Long-term warmed soils show carbon, nitrogen, and biomass losses, as well as an increase in pH (Figure 2.2, Figure 2.3, Table 2.1). However, if the two highest warming groups of soils are excluded, there is no significant correlation between temperature and C or N. In addition, there is depletion in ^{13}C, indicating a change in the soil's recycling status perhaps linked to the overall losses in soil carbon.

A PCA biplot of these soil properties also shows that most of these properties are closely linked, with highly-warmed samples separated along the first principal component (Figure 2.4). However, it also highlights the separate behavior of ^{13}C, which is depleted relative to ^{12}C even at lower levels of warming.

Table 2.1 Summary of Pearson's product-moment correlations between field warming and key soil properties. Degrees of freedom = 30. Significance codes: * p < 0.05 , **p<0.005, ***p<0.001.

Metric	*r*
C	-0.658***
C_{mic}	-0.285
PLFA C	-0.618***
$\delta^{13}C$	-0.687***
N	-0.690***
pH	0.671***
soil moisture	-0.576***
relative NLFA AMF biomarker	0.344

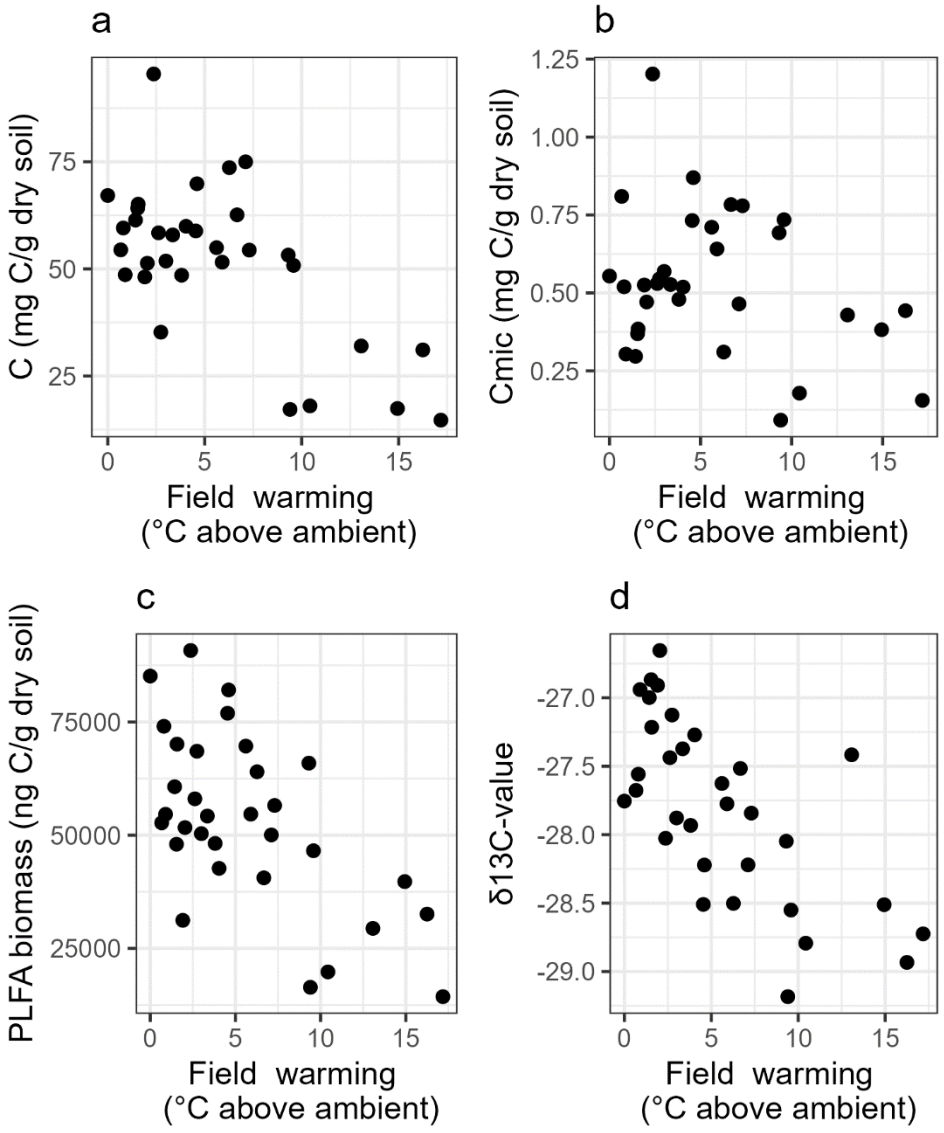

Figure 2.2 (a) C, (b) microbial C, (c) PLFA C, and (d) ^{13}C content across the field warming gradient.

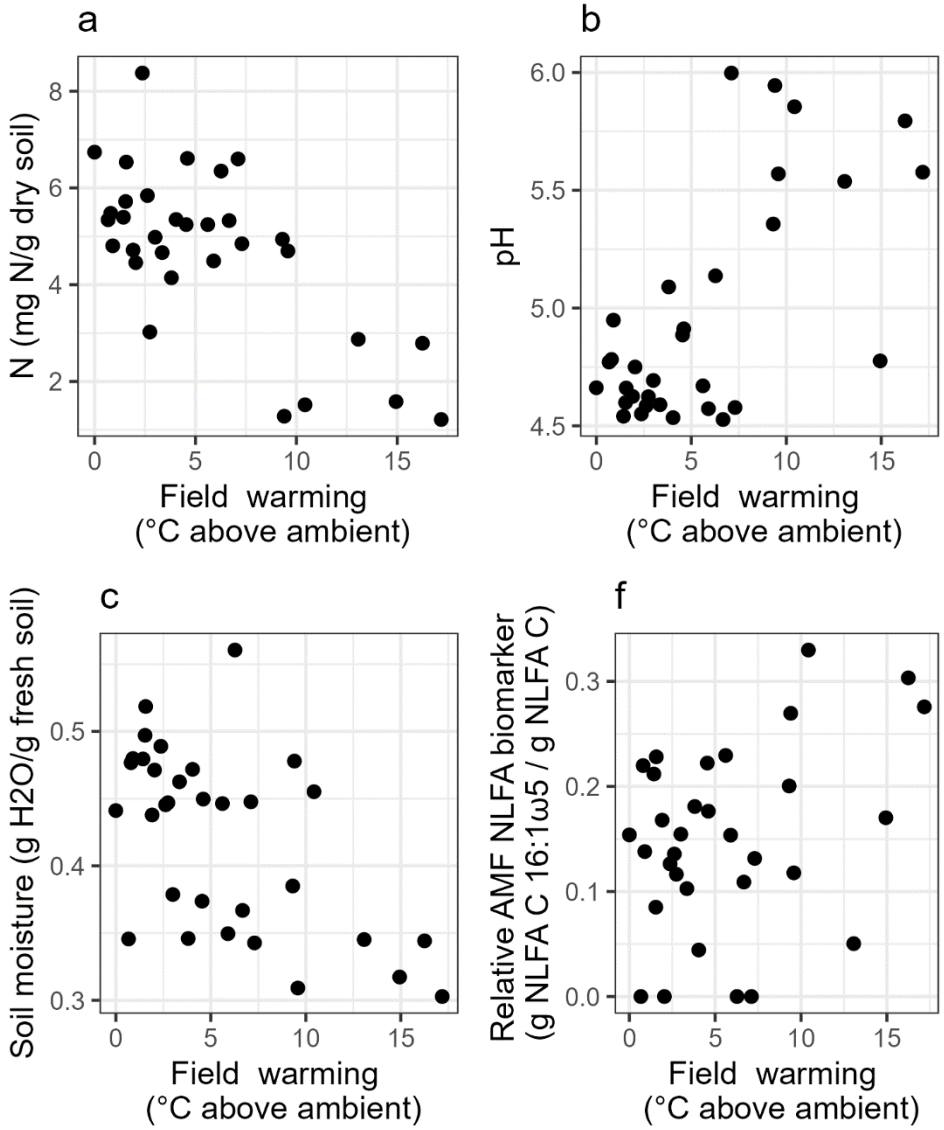

Figure 2.3 (a) N, (b) pH, (c) soil moisture, (d) and relative biomass of the AM fungal biomarker NLFA C16:1ω5 across the field warming gradient.

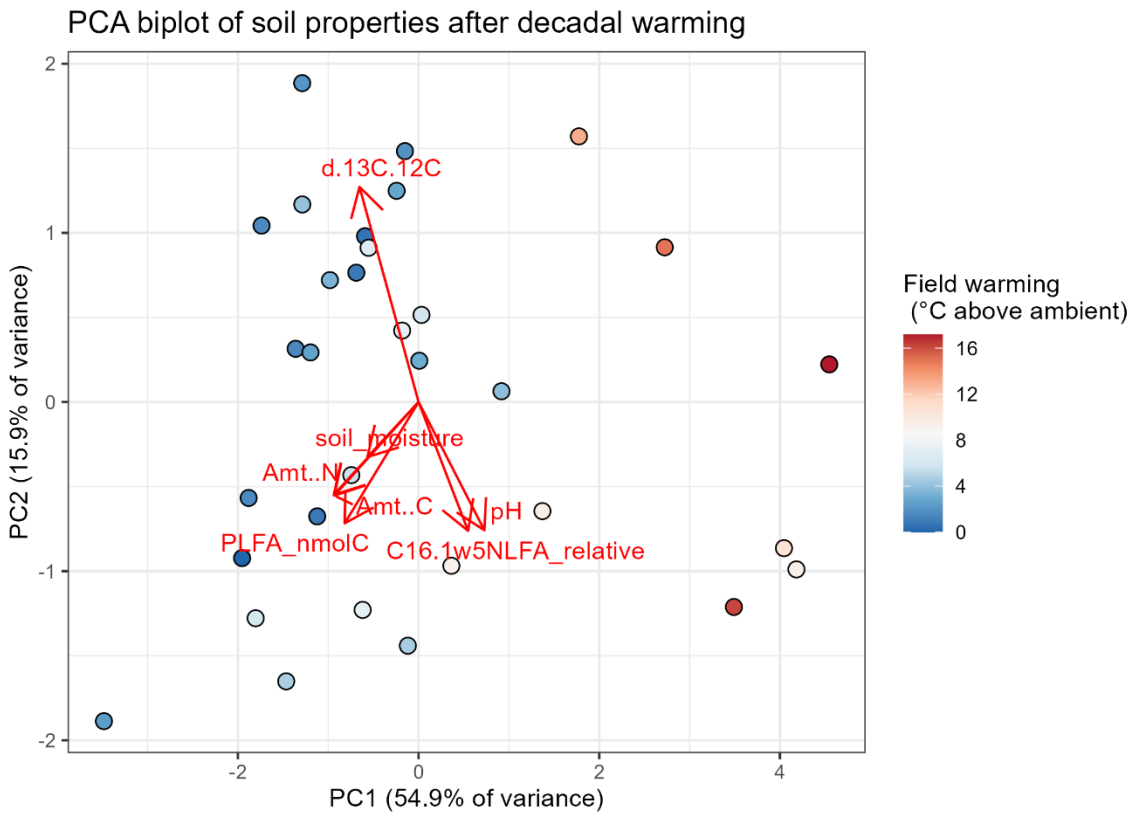

Figure 2.4 PCA of soil properties (C, N, pH, moisture, PLFA biomass, AMF NLFA biomarker relative biomass), including arrows representing loadings, in long-term warmed soils.

2.3.2 Apparent thermal adaptation in total activity rates

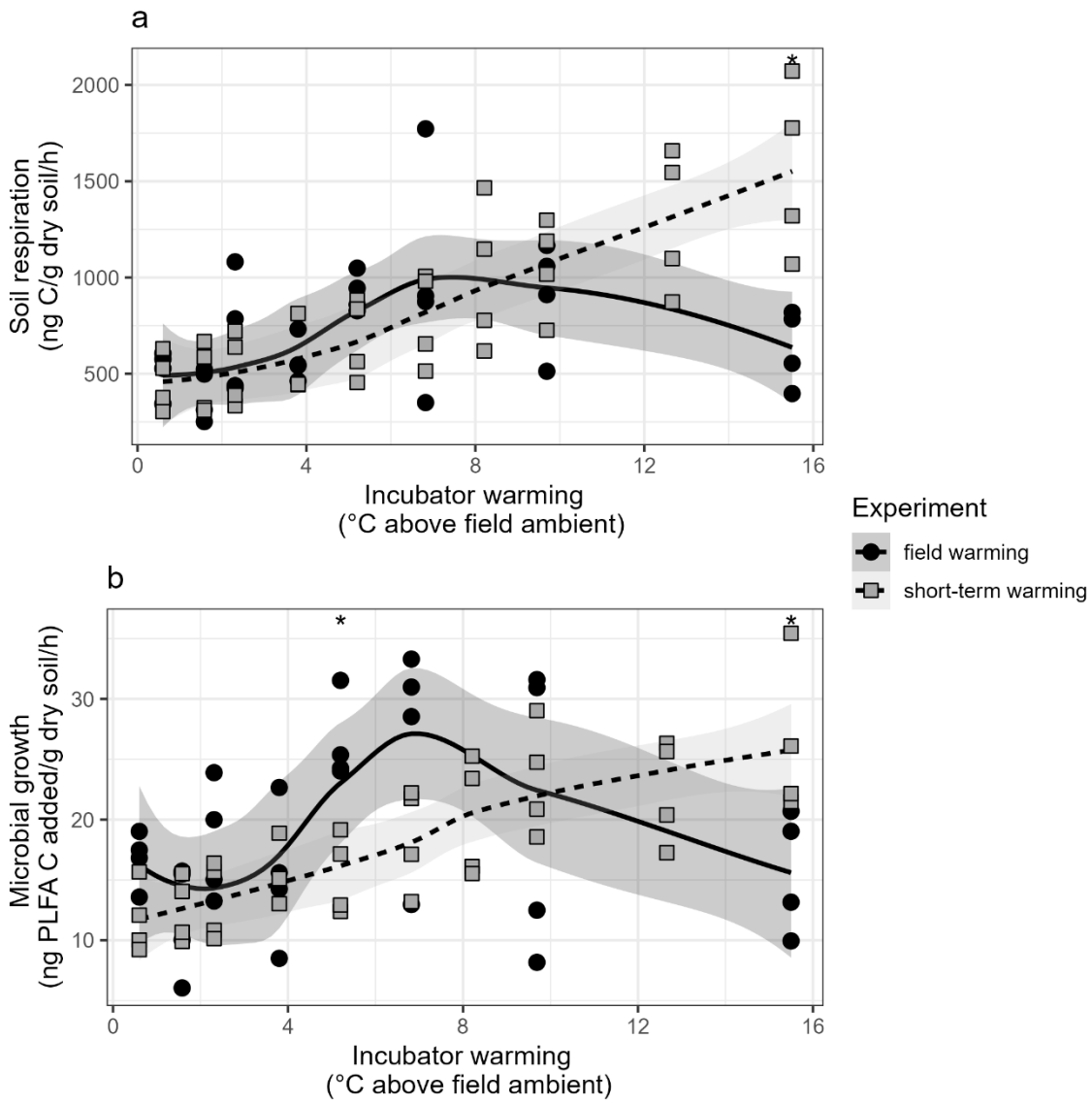

Figure 2.5 (a) Total soil respiration, R_s and (b) total growth, $Growth_s$, of long- and short-term warmed soils across a range of temperatures. Lines represent LOESS regressions with shading representing 95% confidence interval. Significance codes: * p < 0.05 , **p<0.005, ***p<0.001, Mann-Whitney *U* test between field and short-term warming groups.

Long-term warmed soils demonstrate apparent thermal adaptation, as expected, with both R_s and $Growth_s$ being significantly higher in ambient soils at the highest level of warming (H1, Figure 2.5). However, this effect is not apparent at lower levels of warming. Particularly noteworthy is that R_s is significantly positively correlated with field warming ($r(22) = 0.592$, $p = 0.0023$) and incubator temperature ($r(22) = 0.0038$) for soils at field warming levels up to +6°C, indicating long-term elevated respiration even without subsequent C losses. R_s and $Growth_s$ increase with temperature at similar rates for both short- and long-term warmed

soils. This high threshold for apparent thermal adaptation corresponds to the observed drop in carbon and biomass only at higher temperatures (Figure 2.2).

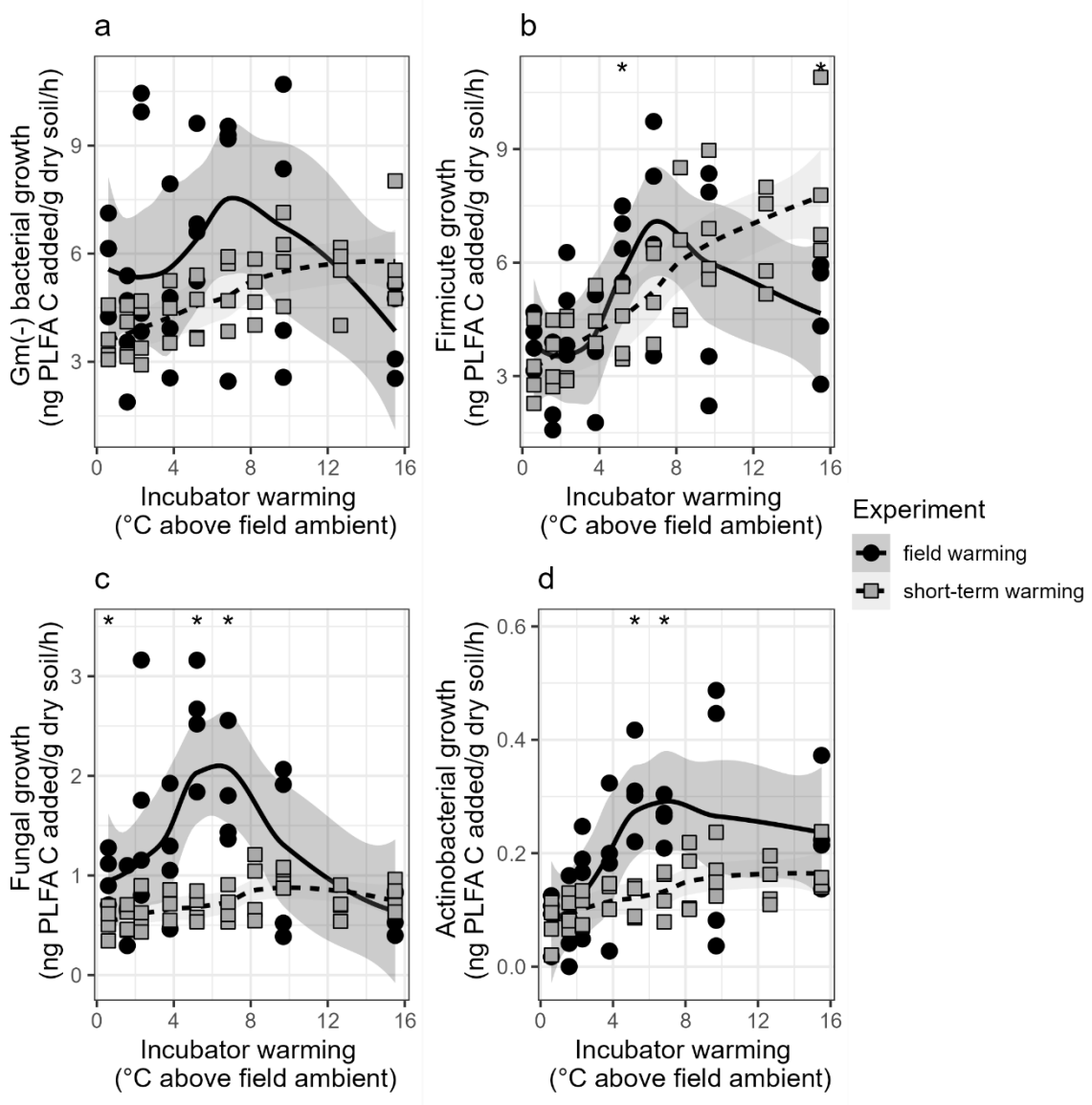

Figure 2.6 Total growth of PLFAs associated with (a) gram-positive bacteria, (b) Firmicutes, (c) fungi, and (d) Actinobacteria in long- and short-term warmed soils across a range of temperatures. Lines represent LOESS regressions with shading representing 95% confidence interval. Significance codes: * p < 0.05 , **p<0.005, ***p<0.001, Mann-Whitney *U* test between field and short-term warming groups.

The pattern is similar but less pronounced in total growth of individual taxa, with total growth increasing across the temperature range in short-term warmed soils, but highest at intermediate levels of warming in long-term warmed soils (Figure 2.6). Actinobacteria and fungi, in particular, have higher total growth rates at intermediate long-term warming than in short-term warming, where they show very little increase in growth rates with temperature.

2.3.3 Responses of mass-specific activity rates to short- and long-term warming

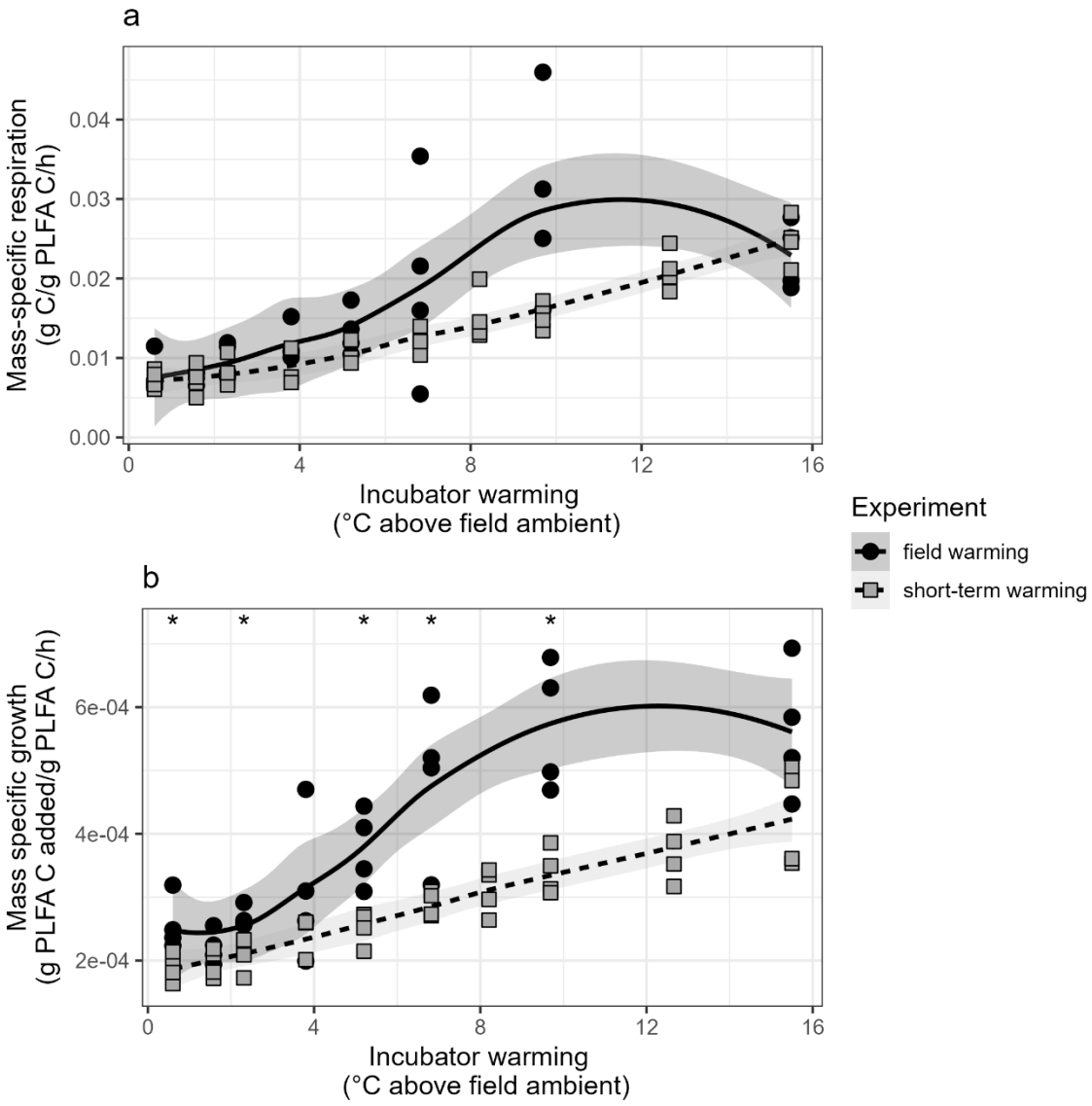

Figure 2.7 (a) Mass-specific soil respiration, R_s and (b) mass-specific microbial growth, $Growth_{mass}$, of long- and short-term warmed soils across a range of temperatures. Lines represent LOESS regressions with shading representing 95% confidence interval. Significance codes: * p < 0.05 , **p<0.005, ***p<0.001, Mann-Whitney *U* test between field and short-term warming groups.

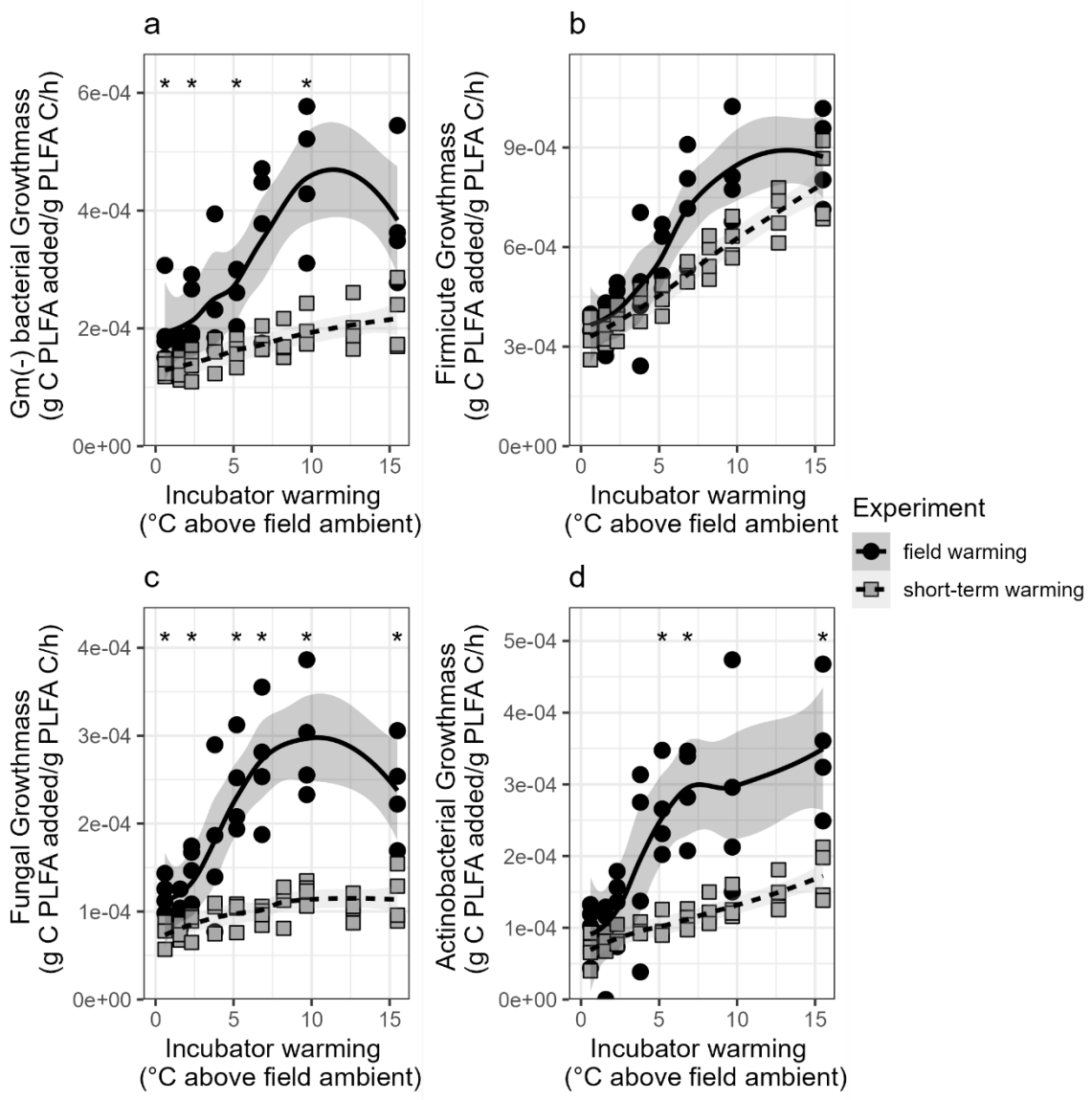

Figure 2.8 Mass-specific growth rates of PLFA biomarkers for (a) gram-negative bacteria, (b) Firmicutes, (c) Fungi, and (d) Actinobacteria in long- and short-term warmed soils incubated across a range of temperatures. Lines represent LOESS regressions, shading represents 95% confidence intervals. Significance codes: * p < 0.05 , **p<0.005, ***p<0.001, Mann-Whitney *U* test between field and short-term warming groups.

R_s showed a strong and positive response to incubation temperature (p<0.001) and PLFA biomass (p<0.001) when both included as predictors terms in a linear model (adjusted r^2 = 0.645) (Figure 2.7). However, there was no significant effect of field warming level or whether soil had been warmed long-term, and model parsimony decreased when either of these terms was included – counteracting the idea of compensatory thermal adaptation (H2). $Growth_{mass}$, in contrast, showed a significant positive response to both to incubation temperature (p<0.001) and field warming level (p<0.001), included together in a model (adjusted r^2 =

0.727); inclusion of field warming improved model parsimony. This, too, suggests a lack of compensatory thermal adaptation (H2), but it further points to an enhancement response in growth. This enhancement response also appears to be diminished at the highest incubation temperature, suggesting that the adaptation of microbial activity to temperature may vary with warming intensity (H3). It also varies between taxa, with Firmicutes notably showing a greater short-term response than other taxa and no significance differences in growth rate between short- and long-term warmed soils (Figure 2.8).

2.3.4 Activity rates of long-term warmed soils incubated at the same temperature

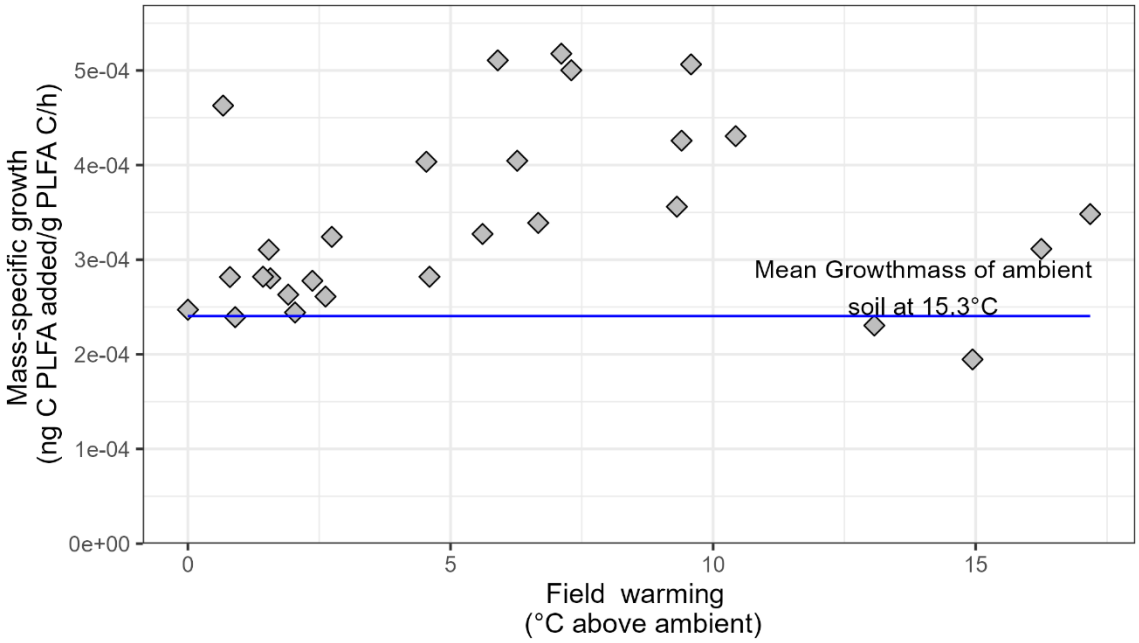

Figure 2.9 Mass-specific growth of field warmed soils incubated at 15.3°C (+3.2°C warming), plotted against field warming. For comparison, the line indicates the mean $Growth_{mass}$ of ambient soils incubated at the same temperature.

The intermediate acclimation experiment also provides no evidence of a compensation response to long-term warming. There was no significant relationship between field temperature and mass-specific growth at +3°C (Figure 2.9). Furthermore, we see that total respiration is highly correlated with C ($r(67) = 0.782$, $p<0.001$, Figure 2.10); including field warming level on its own or with C in a linear model worsens the model's fit. Thus, substrate losses, and not adaptation, appear to explain changes in total activity at an intermediate temperature (H2).

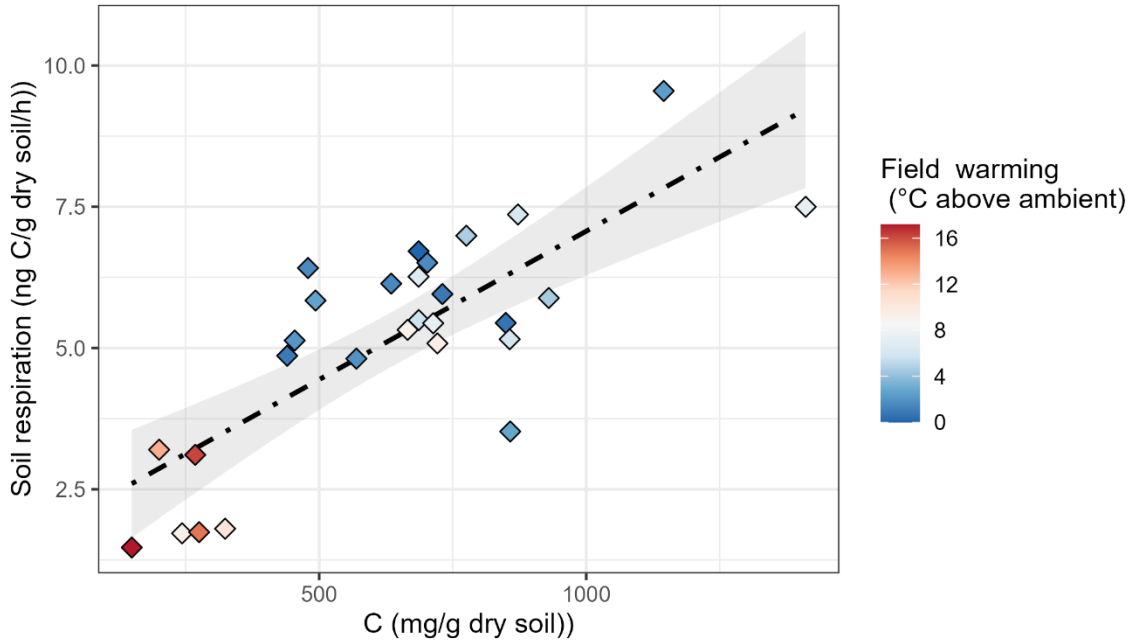

Figure 2.10 Total soil respiration plotted against soil C content for long-term warmed soils incubated at +3°C. Line represents a simple linear regression and shading represents 95% confidence interval.

2.3.5 Carbon use efficiency (CUE)

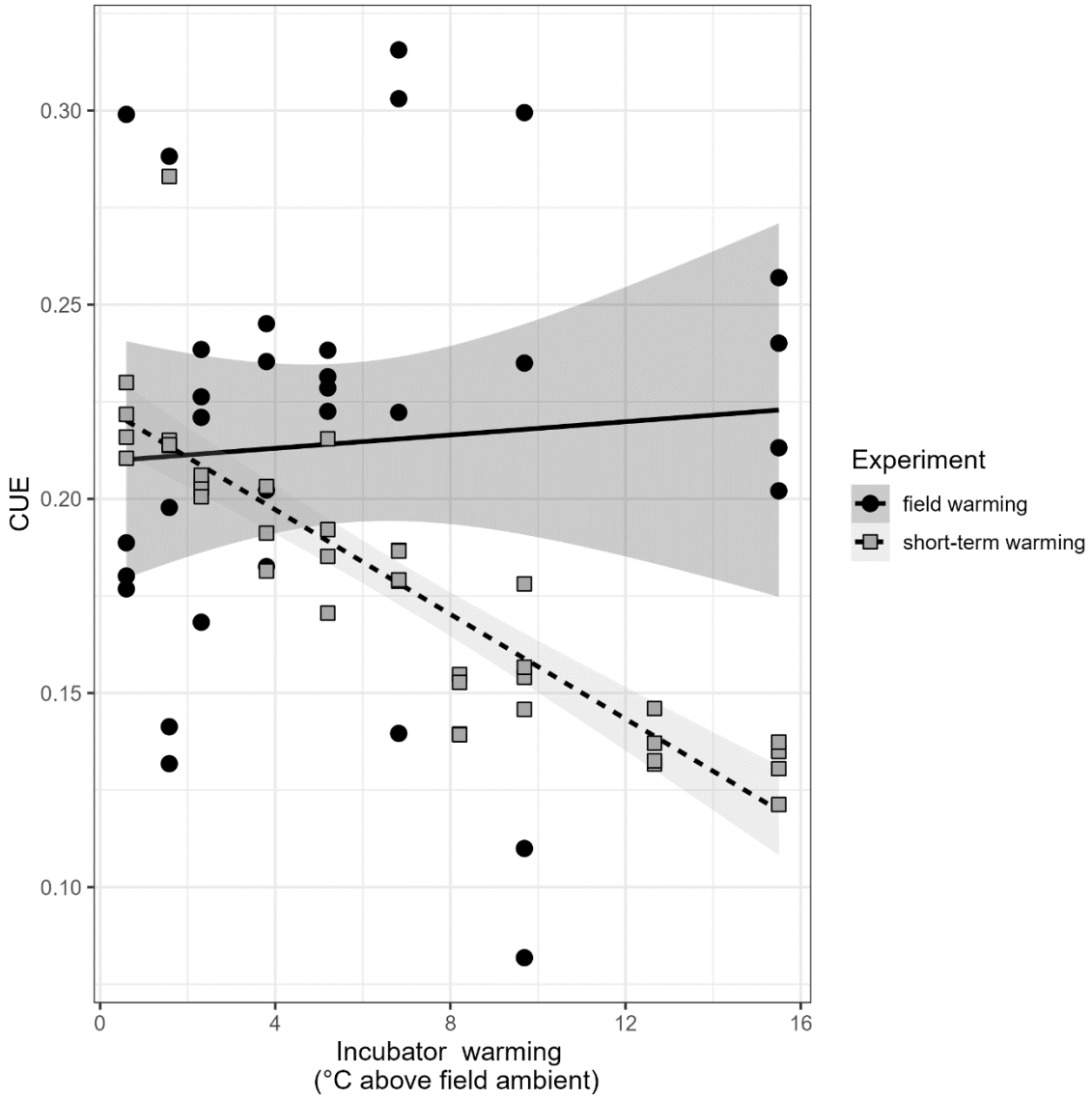

Figure 2.11 Carbon use efficiency of field and short-term warming samples across different incubation temperatures. Lines represent simple linear regressions and shading represents their 95% confidence intervals.

Among the ambient soils, carbon use efficiency can be seen to decrease in a linear fashion with increasing incubation temperature, while there is high variability and no significant relationship to temperature among long-term warmed samples.

2.3.6 Relative growth rates of microbial groups

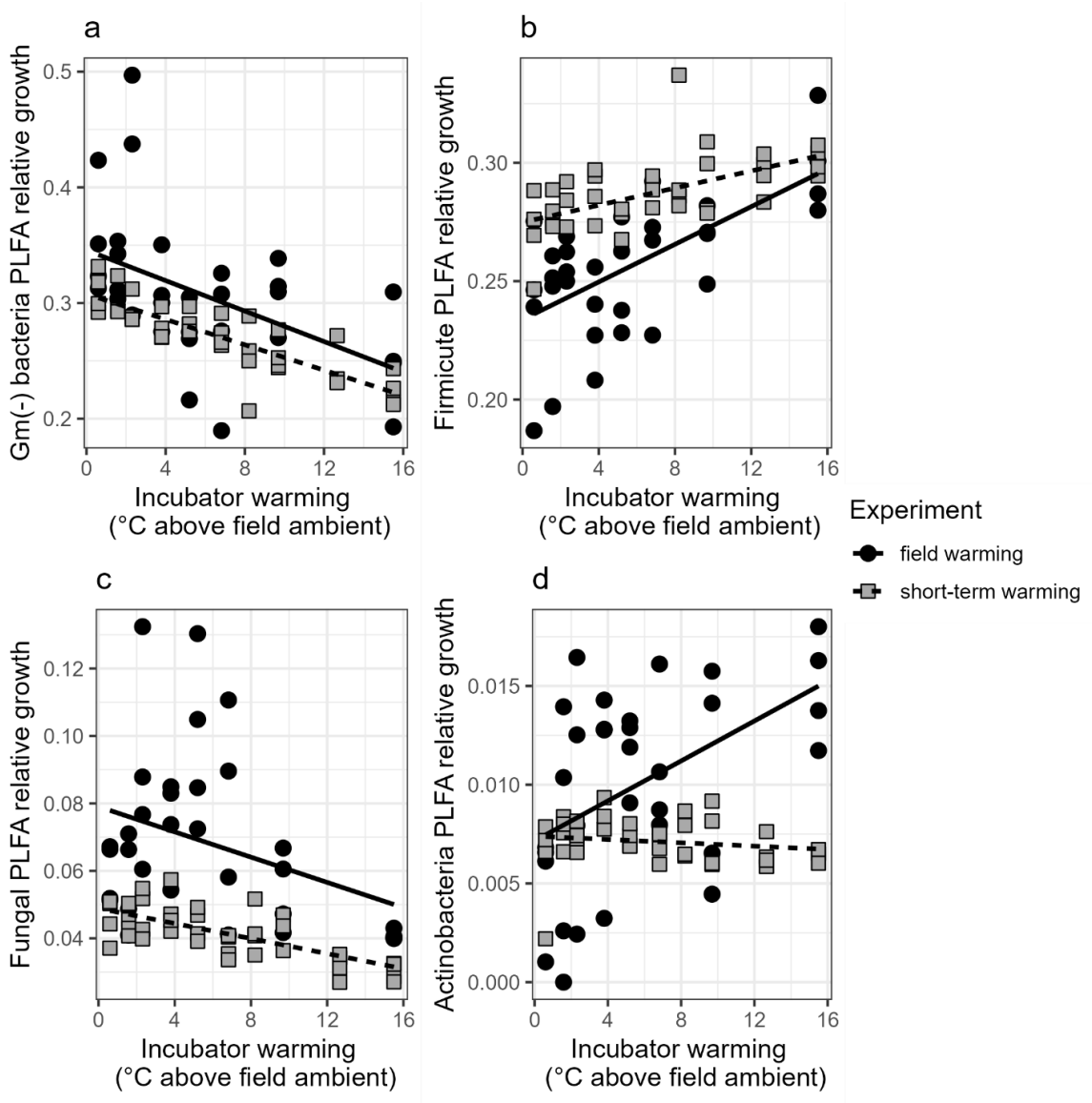

Figure 2.12 Relative growth rates of PLFA biomarkers for (a) gram-negative bacteria, (b) Firmicutes, (c) Fungi, and (d) Actinobacteria, plotted against incubation warming, for short-term and field warmed samples. Lines represent simple linear regressions.

We see that the relative growth rates of individual taxa follow similar trends under both long- and short-term warming, with Firmicutes making up a larger share of growth and both fungi and gram-positive bacteria seemingly at a disadvantage

The exception is Actinobacteria, which have higher relative growth under long-term warming, but show no change under short-term warming. In addition, these changes in relative growth rate occur along the entire temperature range, both as a result of short-term warming and

along the long-term gradient, rather than following the observed step changes in C and N (Figure 2.2, Figure 2.3).

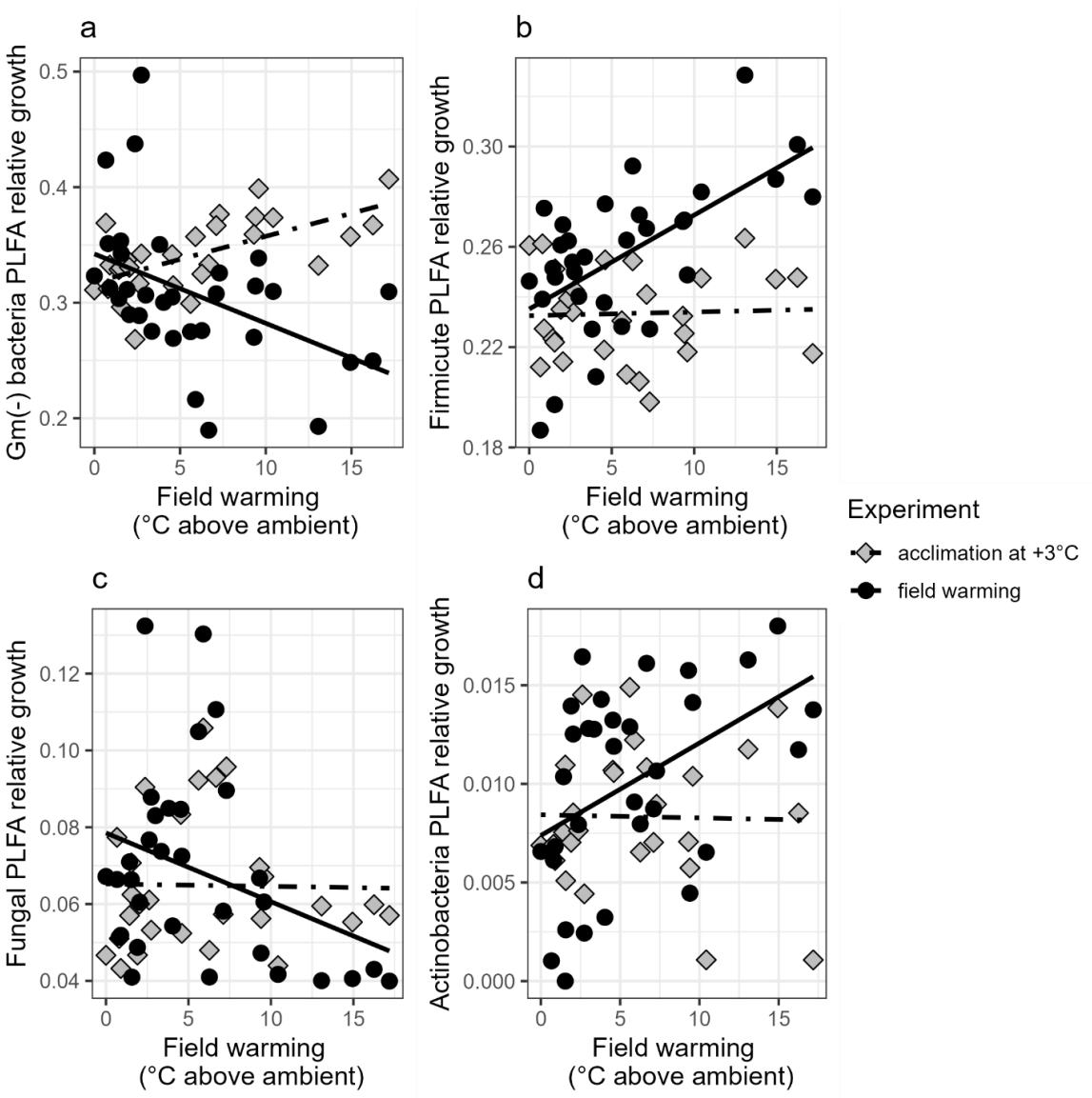

Figure 2.13 Relative growth rates of PLFA biomarkers for (a) gram-negative bacteria, (b) Firmicutes, (c) Fungi, and (d) Actinobacteria, plotted against incubation warming, during incubation of long-term warmed soils at either field-like temperatures or +3°C. Lines represent simple linear regressions.

Similarly, the relative growth rates of taxa begin to diverge when long-term warmed soils are incubated at different temperatures, most notably with an increase in the relative growth rate of gram-negative bacteria (Figure 2.13). Even after decades of warming, temperature itself can lead to different growth responses among different taxa, which might eventually shift community composition.

2.3.7 Response of microbial community composition to changes in temperature

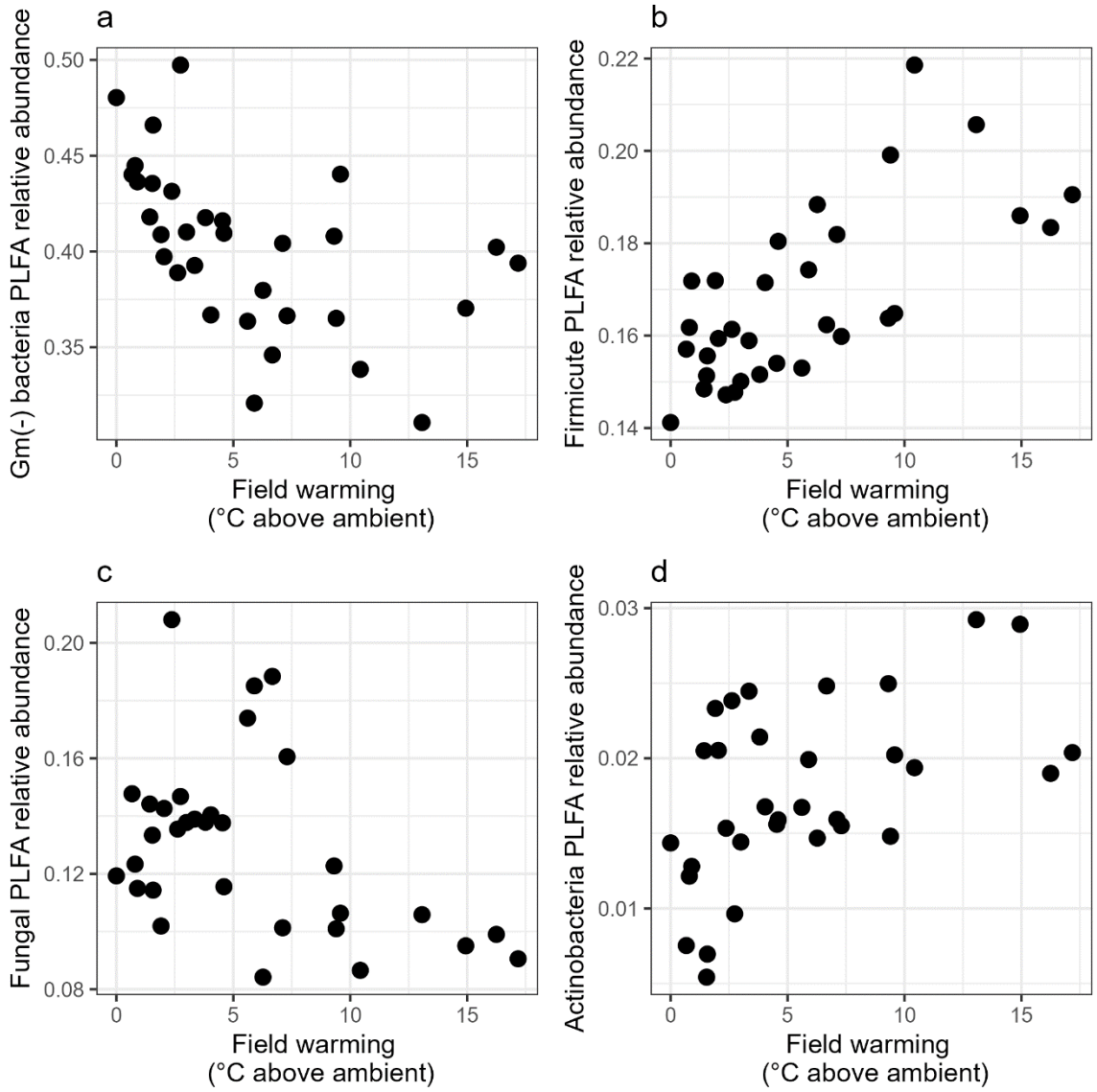

Figure 2.14 Share of PLFA biomass made up of biomarkers for (a) gram-negative bacteria, (b) Firmicutes, (c) Fungi, and (d) Actinobacteria in long-term warmed soils, plotted against field warming.

Across the long-term warming gradient, we observed not just decreasing overall biomass with temperature, but also a drop in biomass with increasing temperature in Firmicutes ($r(30) = -0.53$, $p = 0.002$), gram-negative bacteria ($r(30) = -0.65$, $p < 0.001$), and fungi ($r(30) = -0.57$, $p < 0.001$). The abundances of these taxa relative to total PLFA biomass changed across the temperature gradient: gram-negative bacteria ($r(30) = --0.52$, $p = 0.0022$) and fungi ($r(30) = -0.43$, $p = 0.0134$) made up a smaller portion of biomass with increasing long-term warming, while Firmicutes ($r(30) = 0.697$, $p < 0.001$) and Actinobacteria ($r(30) = 0.514$, $p = 0.0026$) increased in relative biomass.

Temperature was better than total carbon for predicting relative biomass of gram-positive bacteria, gram-negative bacteria, and Actinobacteria. For these groups, carbon content was not significant and produced a less parsimonious model when included alongside temperature as a dependent variable in a linear fixed-effects model. For fungi, however, there was a stronger correlation with total carbon, which was positively related to fungal relative biomass ($r(39) = 0.49$, $p = 0.0045$).

Relative biomass of specific taxa, when included in linear models of mass-specific respiration, were not significant and produced less parsimonious models.

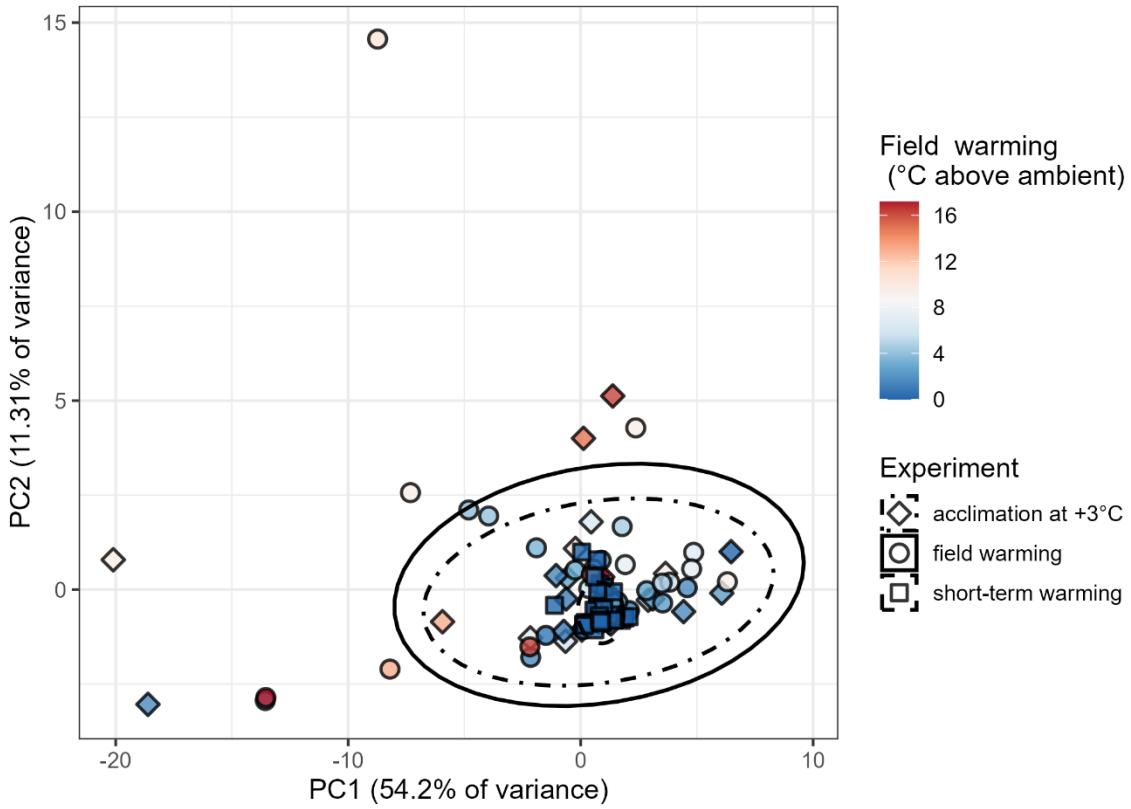

Figure 2.15 PCA of relative biomasses of all PLFAs across all three experiments. Ellipses represent 95% confidence intervals.

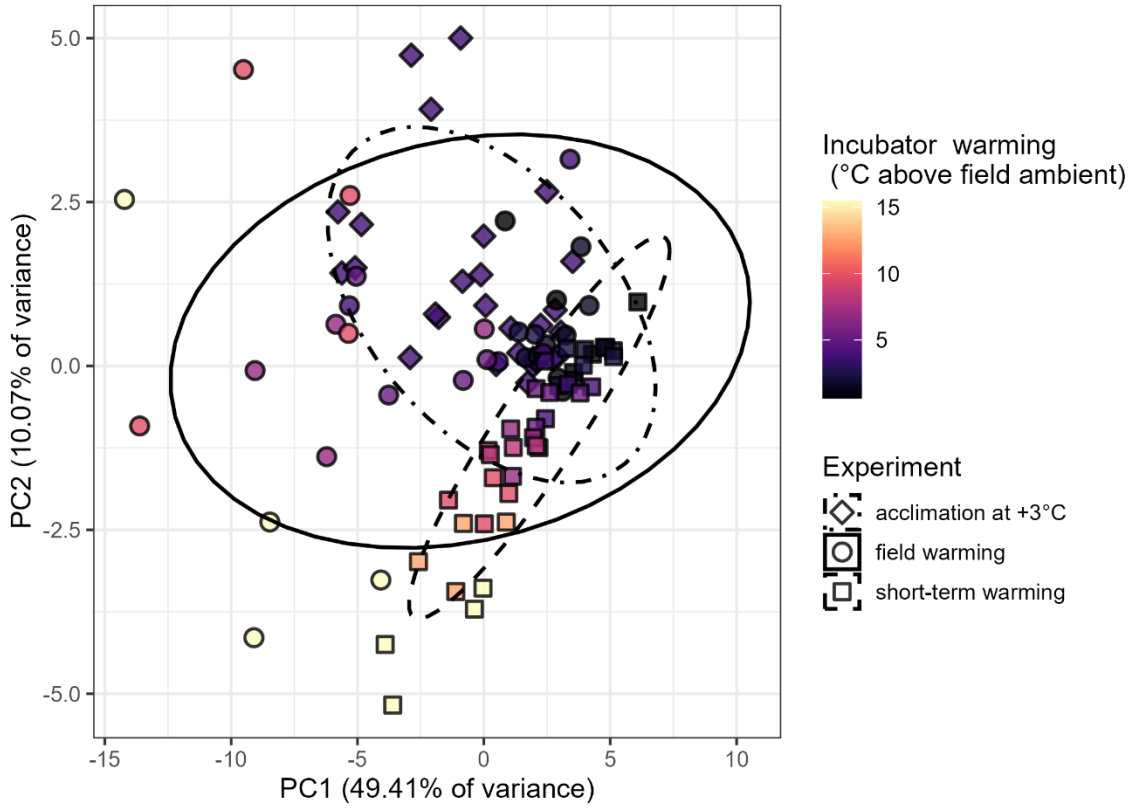

Figure 2.16 PCA of mass-specific growth rates of all PLFAs across all three experiments. Ellipses represent 95% confidence intervals.

Changes in PLFA composition appear to reflect long-term community shifts and acclimation, and not a response to warming during the 48-hour incubation. In a PCA of relative biomass of all PLFAs measured in the field warming and short-term warming samples (Figure 2.15), the first principal component is more closely correlated with C ($r(97) = 0.432$, $p<0.001$) than with temperature, suggesting substrate availability and composition are key for driving long-term changes in community composition at the gradient. The second principal component is more closely correlated with field temperature ($r(98) = 0.343$, $p<0.001$), and neither PC1 nor PC2 are significantly correlated with incubation temperature.

In contrast, the growth rates of specific PLFAs respond rapidly to the experimental temperature change. In a PCA of relative growth rates (Figure 2.16) both PC1 ($r(98) = -0.272$, $p=0.006$) and PC2 were more closely correlated with incubation temperature ($r(98) = -0.428$, $p<0.001$) than field temperature, and neither was significantly correlated with C.

2.3.8 Membrane fluidity

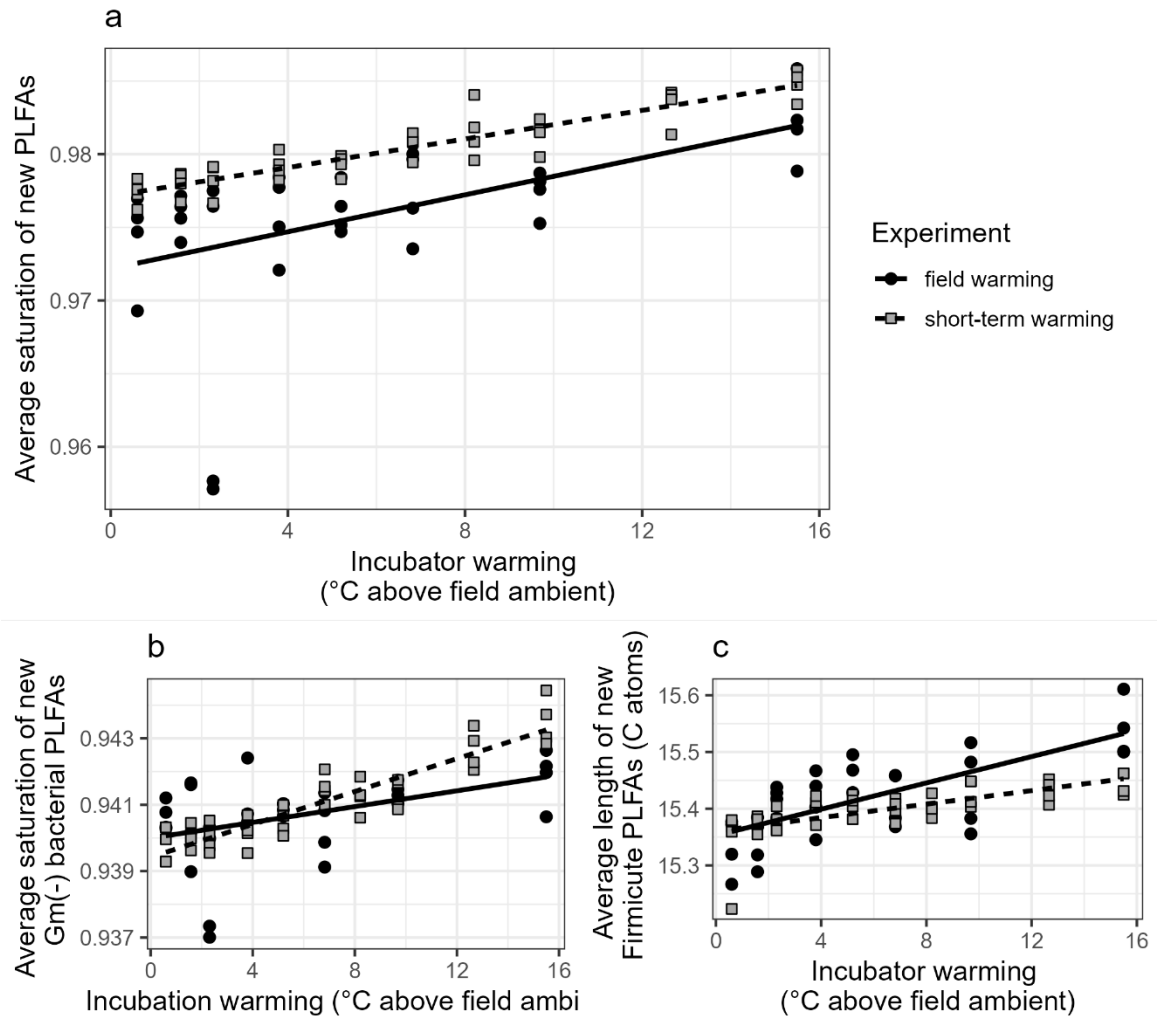

Figure 2.17 Membrane fluidity metrics such as (a) average saturation of newly produced PLFAs, (b) average saturation of new Gm- PLFAs, and (c) average chain length of new Firmicute PLFAs plotted against incubation warming. Lines represent simple linear regressions.

Samples show temperature-related changes in PLFA saturation and chain length which likely indicate lipid membrane resynthesis. Among the short-term and field warming samples, newly-produced PLFAs were more saturated as incubation temperature increased ($r(69) = 0.586$, $p < 0.001$). Average PLFA main C chain length and PLFA saturation—both associated with decreasing fluidity – were significantly negatively correlated ($r(70) = -0.637$, $p < 0.001$). Within taxon-specific marker PLFAs, there were significant relationships between incubation temperature and membrane fluidity metrics of newly-produced PLFAs, summarized in Table 2.2.

Table 2.2 Relationships between incubation temperature and the membrane fluidity metrics of newly-produced taxon-specific PLFAs.

Taxon	Warming	Metric	*r*	p
Firmicute	short-term	new PLFA average saturation	0.914	<0.001
	field	new PLFA average saturation	0.452	0.009
	short-term	new PLFA average chain length	0.705	<0.001
	field	new PLFA average chain length	0.722	<0.001
Gm- bacteria	short-term	new PLFA average chain length	0.669	<0.001
	field	new PLFA average chain length	0.390	0.027

2.4 Discussion

2.4.1 Apparent thermal adaptation explained by C and biomass losses

Both our ambient and long-term warmed soils showed strong increases in respiration rate under warming, while more more intensely warmed decadal warming soils show apparent thermal adaptation (H1). For short-term warmed samples, the temperature response appears to be linear across the entire range of incubation temperatures, 12-27°C, with no plateau or drop in temperatures beyond a certain level of warming (Figure 2.5). This suggests that the soil-wide T_{opt} of respiration is higher than our highest incubation temperature. Although our incubation temperatures do not exceed the soil-wide T_{opt} for respiration, individual taxa likely have a variety of different respiration rates (Bradford, 2013; Donhauser et al., 2020), and warming is known to affect microbial communities not just by altering the function of community members which are already active, but also by activating dormant species that may function best in other temperature ranges (Metze et al., 2024).

We expected that, at least at the highest temperatures, we would see a decrease in R_{mass} following long-term warming (H2). Across biomes, soil respiration at a given temperature tends to decrease with increasing MAT (Bradford et al., 2019). This is in line with evolutionary theory around temperature trade-offs: at higher temperatures, flexible enzymes that can function efficiently at lower temperatures become a liability, and more stable enzymes that spend more time in particular binding configurations confer an advantage (Bradford, 2013). However, we find no evidence that microbial respiration has adapted to long-term warming The apparent thermal adaptation observed at the highest warming appears to be explained

entirely by the reduction in microbial biomass and C, and we see no difference – or even a slight enhancement response – when we look at mass-specific respiration rather than total respiration (Figure 2.7).

2.4.2 Enhanced mass-specific growth following long-term warming

As with respiration, microbial growth rate does not appear to be less temperature-sensitive in long-term warmed samples (H2). In fact, long-term warmed samples have higher $Growth_{mass}$ than the ambient soils (Figure 2.7b, Figure 2.9). Similarly, carbon use efficiency decreases with increasing incubation temperature among the ambient soils, but is unrelated to temperature – albeit highly variable – among the field-warmed soils (Figure 2.11). Other studies have found CUE to increase with MAT within and across different biomes, which might indicate that higher temperatures select for higher CUE (Sinsabaugh et al., 2017; Takriti et al., 2018; Ye et al., 2019).

Temperature sensitivity of cellular machinery might enable microbes in long-term warmed soils to enhance their growth. Soil microbes exposed to long-term warming soils have been observed to downregulate energy metabolism and protein biosynthesis pathways, including a reduced number of ribosomes and lower ribosomal RNA copy number (DeAngelis et al., 2015; Söllinger et al., 2022, 2024). As warming accelerates biochemical processes, cells can invest less energy and materials in molecules that carry out those processes without a reduction in activity, and can instead allocate resources towards growth. This acceleration is also known to occur in extracellular enzymes that degrade microbes' energy sources. Crucially, temperature sensitivity varies between enzymes (J. Chen et al., 2018). Even if cellular machinery begins to work faster immediately after warming, certain processes may lag behind others, such that a newly-warmed microbe is overly invested in some tasks and can grow less efficiently.

While downregulation and reallocation of cellular machinery might explain the growth enhancement effect observed here and by Walker (2018), it is surprising that long-term warmed soils from the upper end of the gradient do not have lower $Growth_{mass}$ when incubated at a much lower level of warming, +3°C (Figure 2.9). At lower temperatures, downregulation of this machinery should impede cell function. Perhaps we do not observe such an impact because the experiment was carried out in summer, and incubation temperatures were relatively high: even the most warmed soils are likely to experience the temperature from the acclimation experiment over the course of a year (Sigurdsson et al.,

2016). It might be that our long-term warmed soils do experience a tradeoff, but their activity rates only drop below those of ambient soils at lower temperatures than those tested.

While accelerated reaction rates could provide some benefits to species, warming is coupled with C and N losses. Labile carbon losses can make catabolism less efficient (Frey et al., 2013). This could act as a counterweight to efficiency benefits from elevated temperature, and explain why the highest temperature long-term warming samples show diminished growth enhancement along with a sudden drop in C and N.

The observed drop in CUE, and its attenuation in the field-warmed samples, might also result from short-term stress and species coming out of dormancy. Both initiation of dormancy and resuscitation are energy-intensive processes, requiring microbes to break down spores and release resuscitation-promoting factors (C. Wang & Kuzyakov, 2023). Investment in these could result in a lag in growth and a lower CUE relative to the long-term warmed soils, where the pool of actively growing species should be unchanged during the incubation period. Even aside from a change in actively growing species, the sudden change in temperature might require other forms of acclimation, such as the production of different isoenzymes, which require energy but do not contribute to the cell's growth.

2.4.3 Reconciling elevated respiration with stable carbon stocks

As expected, the carbon content of the soils studied decreased with warming. However, the threshold at which carbon loss occurs is surprising. No carbon or nitrogen loss was observed in the first +6°C of warming (Figure 2.2, Figure 2.3). In contrast, Verbrigghe et al. (2022) studied both the GO site, where our samples are from, and the grassland new (GN) area at the ForHot site, which has been warmed since 2008, when an earthquake led to the development of new geothermal hotspots. They found that SOC stocks in topsoil decreased linearly with warming and stabilized within five years of the start of warming. This SOC decrease was observed even within a lower temperature range, where our data show no change in total soil carbon or biomass in our samples. They do note, however, that SOC stock estimates were highly variable, owing to both measurement errors and heterogeneity in the soil.

This difference from previous research findings might simply be a result of variability between transects, but the lack of C loss is also puzzling in combination with our respiration data, where we saw elevated respiration rates comparable to those of ambient soils under short-term warming. If temperature is elevated long-term without any change in temperature response, then we would anticipate carbon losses. However, in our first five warming bins, we

see no such thing. This suggests that there may be other simultaneous changes to carbon flux which have prevented net carbon loss at least at some plots.

These changes could include higher carbon inputs from primary production. Increases in biomass, sometimes exceeding increased ecosystem respiration, have been observed in some grassland warming experiments (Kreyling et al., 2019; Luo et al., 2009; Sheik et al., 2011; Wang et al., 2019). Other studies finding negative effects on net primary production (NPP) often link this to drought stress, which is less likely to play a role in relatively moist Iceland (De Boeck et al., 2015; Sheik et al., 2011; Wu et al., 2021). Perhaps the simplest explanation for such an increase would be an extension of the growing season, which has been observed in other cold climate grasslands (Richardson et al., 2013). However, exceptions occur, with other environmental factors, including daylight and precipitation, acting as possible constraints on the growing season (Leblans et al., 2017)

Increasingly productive plants could explain some of our data, but studies of plant biomass and growing season already conducted at the ForHot site are ambiguous. In a study measuring normalized difference vegetation index (NDVI) at plots up to +10°C, Leblans et al. (2017) found a linear increase in the growing season by 2.1 days per °C soil warming. Meeran et al. (2023) also observed an earlier start to the growing season but found that the extension of the growing season was mitigated by plants' early senescence, a phenomenon also seen in other cold climate grasslands (Khorsand Rosa et al., 2015). Early senescence was alleviated by N addition, suggesting that N limitation led to the early end of the growing season. Fang et al. (2023), reporting a decrease in aboveground biomass with warming, also point to N limitation affecting plant growth. They also found an increase in moss:vascular plant ratio, which might influence C loss: moss-derived carbon sources are typically less labile and decomposed more slowly than vascular plant litter (Fang et al., 2023; Lindo et al., 2013). However, a lack of labile carbon seems unlikely to explain our data, as microbial biomass and total remain stable within the same range, whereas we might expect microbial biomass and SOC stocks to diverge if microbes were limited by falling carbon quality.

Aside from increasing carbon inputs, a reduction in other forms of carbon loss could also lead to the lack of net carbon loss that we observe. Our study only involved sieved soil, and thus only offers information about microbial respiration, but plant respiration could also exhibit adaptation or acclimation in temperature response. However, T_{opt} of respiration appears to often be higher than that of photosynthesis, with plant respiration – like microbial respiration – likely being substrate limited, rather than exhibiting acclimation, under long-term warming

(Smith & Dukes, 2013). There are a few processes other than microbial and plant respiration which can remove carbon from terrestrial systems. These include erosion, fire, insect damage, and agricultural harvest. There is no reason to believe that any of these carbon loss mechanisms would be greater at lower temperatures and explain the lack of net carbon loss; thus, a change in input is more likely to explain our observed pattern of C loss.

While carbon stocks are stable at low warming levels, there is a clear, linear decrease in $\delta^{13}C$ with temperature, including at lower levels of warming (Figure 2.2d). Like our respiration data, this indicates temperature-sensitive changes in C cycling processes. The direction of this effect is initially surprising: older, more decomposed SOM is typically, though inconsistently, enriched in ^{13}C (P. Dijkstra et al., 2006). A study of isotope ratios in respired CO_2 and microbial biomass following C_3-C_4 vegetation change found that old C contributed only 20% of microbial biomass, but the majority of CO_2. This suggests that older C sources may be used catabolically while newer C is preferentially used for growth (Blagodatskaya et al., 2011). Thus, while microbes respire ^{12}C slightly faster (Werth & Kuzyakov, 2010), leaving behind a recalcitrant, ^{13}C-enriched SOM pool, increasing energy demands may result in microbes preferentially respiring this older pool and incorporating fresher, ^{13}C-depleted compounds into SOM. The disruption of aggregates and subsequent availability of older, more recycled carbon sources could also contribute to this effect (Poeplau et al., 2020).

In addition to microbial processes, changes in plant community composition can cause changes in $\delta^{13}C$. C_3-C_4 vegetation shifts are the focus of much study: C_3 plants produce litter with a mean $\delta^{13}C$ of -27%, while C_4 plants are ^{13}C-depleted to a mean $\delta^{13}C$ of -13% (Pessenda et al., 1996). We can rule out a C_3-C_4 vegetation shift based on simple species distribution patterns: Iceland likely has no C_4 or CAM plants, which are instead characteristic of tropical and subtropical regions (X. Luo et al., 2024; Osborne et al., 2014; Y. Wang & Wooller, 2006). In the unlikely event of C_4 invasion at the ForHot site, we would expect it to produce the opposite pattern – inherently cold-intolerant, more drought-resistant C_4 plants would have a better chance at the most warmed plots, where their isotope preferences would lead to less negative ^{13}C values (X. Luo et al., 2024). Even if a C_3-C_4 shift is implausible, other community composition changes could affect carbon isotope ratios. $\delta^{13}C$ values of plant communities and even of individual plant species have been observed to vary across ecological gradients (S. Chen et al., 2007; Cooper et al., 2023; Stewart et al., 1995; Y. C. Zhou et al., 2013). The ^{13}C content of mosses, in particular, is known to be heavily dependent on cell water content and the presence of water films, although the range of ^{13}C signatures in mosses is similar to that

of vascular C_3 plants (Bramley-Alves et al., 2015; Brooks et al., 1997; Dong et al., 2019; Skrzypek et al., 2007).

2.4.4 Chewing the fat: PLFA and NLFA insights

While most taxa identified by PLFA biomarkers decreased in absolute biomass across the warming gradient, not all groups were impacted to the same degree (Supplementary Materials, Figure 2.20). Gram-positive (Gm+) bacteria including Actinobacteria became relatively more abundant (Figure 2.13). This is in line with previous research finding the promotion of Actinobacteria and Firmicutes at elevated temperatures and decreases in many gram-negative (Gm−) phyla (Deslippe et al., 2012; Waghmode et al., 2018; Zhao et al., 2024). This shift might not be a direct response to temperature, but rather to indirect effects of carbon limitation. Gm+:Gm− ratio has been proposed as an indicator of low carbon availability, increasing down the soil profile or after plant removal (Fanin et al., 2022; Zheng et al., 2021). Gm− bacteria exhibit a preference for newer plant-derived carbon, while Gm+ bacteria are more likely to use older SOM carbon sources (Kramer & Gleixner, 2008). However, the share of biomass made up of gram-negative bacteria was significantly and negatively correlated with temperature even between 0 and +6°C warming, where there was no statistically significant effect of temperature on soil carbon stocks. If anything, these less warmed soils might be expected to have more labile carbon, as warming could increase annual plant growth and speed up carbon turnover. This suggests that the temperature itself may put the gram-negative bacteria at a relative fitness disadvantage.

Our taxon-specific growth rates, calculated from the deuterium labelling experiment, also support this conclusion. The short-term warming soils show changes in relative growth rates which are similar to changes across the long-term warming gradient, although there is no major substrate depletion during the 48-hour incubation (Figure 2.12). Conversely, the taxon-specific growth rates diverged between the long-term warming and intermediate acclimation experiments. As soon as warmed soils were incubated at a lower temperature, Gm- bacteria began to account for a larger portion of growth, even though carbon remained depleted.

Competitive differences between taxa extend beyond carbon source. For example, gram-positive bacteria are also thought to be inherently resistant to some other stresses, in particular drought and rewetting, as a result of their thick cell walls, which impose production costs that might reduce fitness in less dry conditions (Schimel et al., 2007). Geothermal warming can induce new forms of stress, but it also might alleviate other stressors; for example, long-term monitoring indicates that the grassland plots at the site warmed above

+1.7°C typically do not experiencing freezing temperatures (Sigurdsson et al., 2016); freezing can destroy cells through ice crystal formation and cell membrane freezing, as well as interfering with diffusion and nutrient availability (Schimel et al., 2007).

One fatty acid of interest is 16:1ω5 NLFA, which is a reliable biomarker for arbuscular mycorrhizal fungi. Most vascular plant species form arbuscular mycorrhizal relationships; this symbiosis is even more dominant in a grassland ecosystem, given that the second most common mycorrhizal symbiosis, the ectomycorrhizal symbiosis, is primarily formed with forest trees (Nehls et al., 2010; Read & Perez-Moreno, 2003). Most dominant vascular species at the site have AM symbioses, although *Equisetum* species, which are also present, typically do not (Giesemann et al., 2020; Hodson et al., 2009; Van Loock, 2017). In a grassland warming experiment, increases in AMF root colonization and hyphal length were observed in response to warming, even without changes in root mass or length (Rillig et al., 2002); the majority of studies investigating AMF response to increased temperature have found increased AMF colonization and hyphal length, although results vary considerably in different systems (Compant et al., 2010). We might expect to see positive effects in our soils, with more AMF biomarker in warmed soils. Furthermore, AM fungi have limited catabolic abilities and seem to contribute relatively little to the rhizosphere priming effect (Read & Perez-Moreno, 2003; Shahzad et al., 2015) Thus, increased C fixation and allocation to AM fungi could have offered a reasonable explanation for stable C stocks despite elevated respiration. However, we observed no significant relationship between temperature and relative or absolute abundance of NLFA 16:1ω5 (Figure 2.3d, Table 2.1). If primary production has increased in the low range of long-term warming, it does not seem to have benefitted the AM fungi. Increasing carbon input would not necessarily feed increasing AM fungal biomass – that carbon could also be allocated toward aboveground plant biomass and litter, root exudates, or bryophyte productivity – although some of these inputs might also stimulate priming effects and net C losses.

Higher relative abundance of AMF biomarker was in fact negatively correlated with soils' total carbon content (Supplementary Materials, Figure 2.19). This is seemingly at odds with the finding that AM fungi enhance carbon sequestration by increasing aggregation and translocating soil C away from hotspots of microbial activity (Wilson et al., 2009; Yang et al., 2011). But it is also reasonable that AM fungi could become more dominant when there is less carbon available in the soil. While free-living microbes, more reliant on their saprotrophic abilities, are likely to starve, the AMF receive as much as 20% of their symbiont's carbon directly from its roots (Willis et al., 2013). They could effectively bypass competition for a

carbon pool that is rapidly depleted under elevated temperatures and respiration. This might be true even in areas of the site where plant biomass has decreased, as AM fungi can form larger networks at some distance from their associated plants. Single genets of AM fungi have been found to cover areas up 10 m in diameter and facilitate P transport between plants at a distance of 0.5m (Chiariello et al., 1982; Lekberg et al., 2010; Rosendahl & Stukenbrock, 2004). An AM fungus could transport carbon across its hyphae to forage and compete successfully at some distance from its plant symbiont.

PLFAs showed a decrease in unsaturation under long-term warming (Figure 2.17), which is consistent with our expectation that microbes resynthesize membrane lipids as a response to temperature changes (Wixon & Balser, 2013). At least some taxa exhibit acclimation in membrane composition. In the absence of any acclimation mechanism, increasing temperatures would cause lipid membranes to become both more fluid and more permeable (Marr & Ingraham, 1962). This has implications for the basic integrity of the cell and the organism's ability to contain or exclude various chemicals. Stable membrane permeability is key for generation of an electrochemical gradient during respiration, where proton leakage would lead to a reduction in respiratory efficiency. However, the membranes of bacteria and archaea with a wide range of optimal growth temperatures exhibit similar proton permeability at their respective growth temperature (Van De Vossenberg et al., 1995). Furthermore, pure cultures of bacteria grown at different temperatures display structural changes which result in nearly constant proton permeability at the growth temperature (Van De Vossenberg et al., 1999). Despite the significance of these acclimation mechanisms, it is also possible for a change in saturation to occur coincidentally as a result of a change in community composition; for example, an increased abundance of fungi, whose marker PLFAs all contain one or more double bonds, would affect this metric. However, the trend is also consistent within taxa: newly-produced Gm- marker PLFAs had fewer double bonds at higher incubation temperatures (Table 2.2).

PLFAs can undergo other warming-related structural changes besides increasing saturation, such as increasing fatty acid chain length and increasing ratio of iso- to anteiso-branching (Sohlenkamp & Geiger, 2015; Van De Vossenberg et al., 1999). Mechanisms for regulating membrane fluidity are known to vary among taxa. The ratio of unsaturated fatty acids to saturated fatty acids is the most important modification in gram-negative bacteria, while gram-positive bacteria are more likely to alter fatty acid chain length (Yoon et al., 2015). This modification, too, is visible in our data: the average length of newly-produced Firmicute PLFAs

increases with incubation temperature in both the field- and short-term warmed samples (Table 2.2).

Some authors have calculated ratios comparing the amount of a particular fatty acid in the NLFA fraction to its counterpart in the PLFA fraction, using this as an indicator for environmental conditions and microbes' responses to them. For example, the fungal NLFA 18:1ω9 might increase after glucose addition without a corresponding increase in PLFA 18:1ω9, such that a high NLFA:PLFA ratio might indicate high availability of labile carbon (Bååth, 2003). However, we see no pattern of changes in NLFA:PLFA ratios either across all fatty acids, within fatty acids associated to a particular taxonomic group, or within individual fatty acids

2.4.5 Conclusion

Our soils show no evidence of compensatory adaptation in activity rates following long-term warming. Furthermore, we find enhancement of growth in long-term warmed soils, reflecting that some microbes can capitalize on the effects of soil warming, which accelerate biological reactions and disrupt soil structure. At lower warming intensities, we find no carbon losses despite elevated. This suggests changes in carbon input and vegetation, even in the absence of air warming. Vegetation changes might also relate to changes in microbial community composition, and could be explored further.

Deuterium labelling allowed us to measure not only biomass, but also growth of specific microbial taxa using PLFAs. We show that PLFAs respond rapidly to warming, reflecting changes in taxon-specific growth as well as acclimation to preserve constant membrane fluidity. Long-term warming biomass data reflects similar trends, such as reduced Gm-bacterial biomass. However, biomass changes also appear linked to the indirect effects of warming via substrate losses, with a notable increase in Actinobacteria. Further research might attempt to link the taxa we studied, and even the growth responses of individual species to warming, with traits implicated in thermal adaptation, such as regulation mechanisms for or inherent differences in protein synthesis machinery.

The patterns we observe in long-term responses to warming provide insights for conceptual and mathematical models of biogeochemical cycling in a warming world. The differences we observe among taxa, across time scales, and between respiration and growth also highlight the complexity of microbial feedbacks to warming and the necessity of studying microbial processes to make predictions about global change.

2.5 Supplementary Material

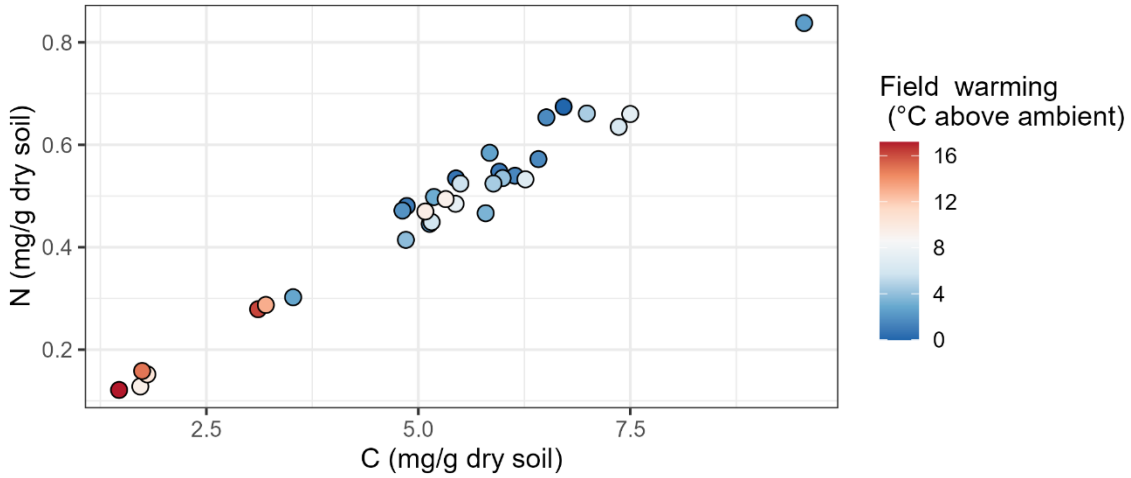

Figure 2.18 Total N against total C in field warming soils.

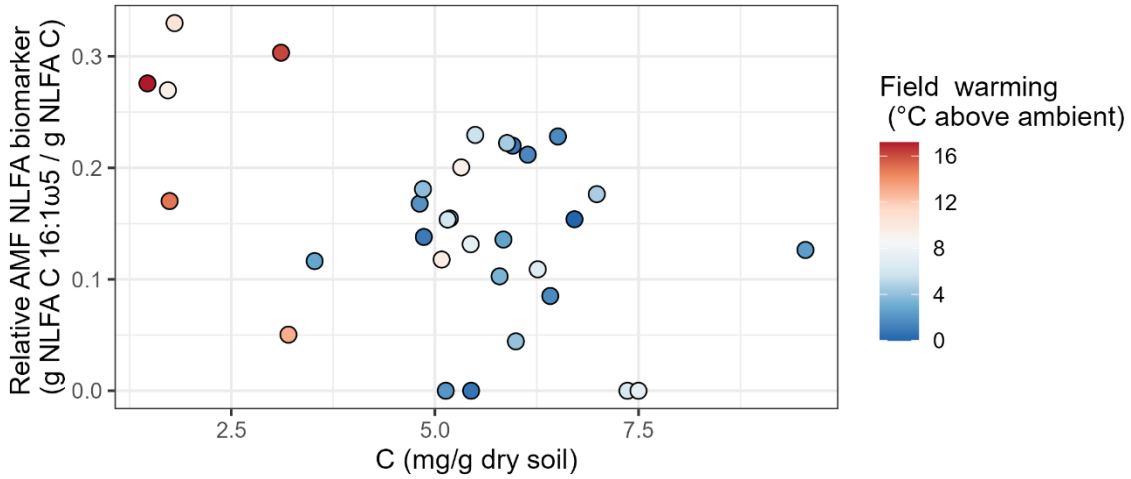

Figure 2.19 AMF NLFA biomarker in the field-warmed soils plotted against total carbon content.

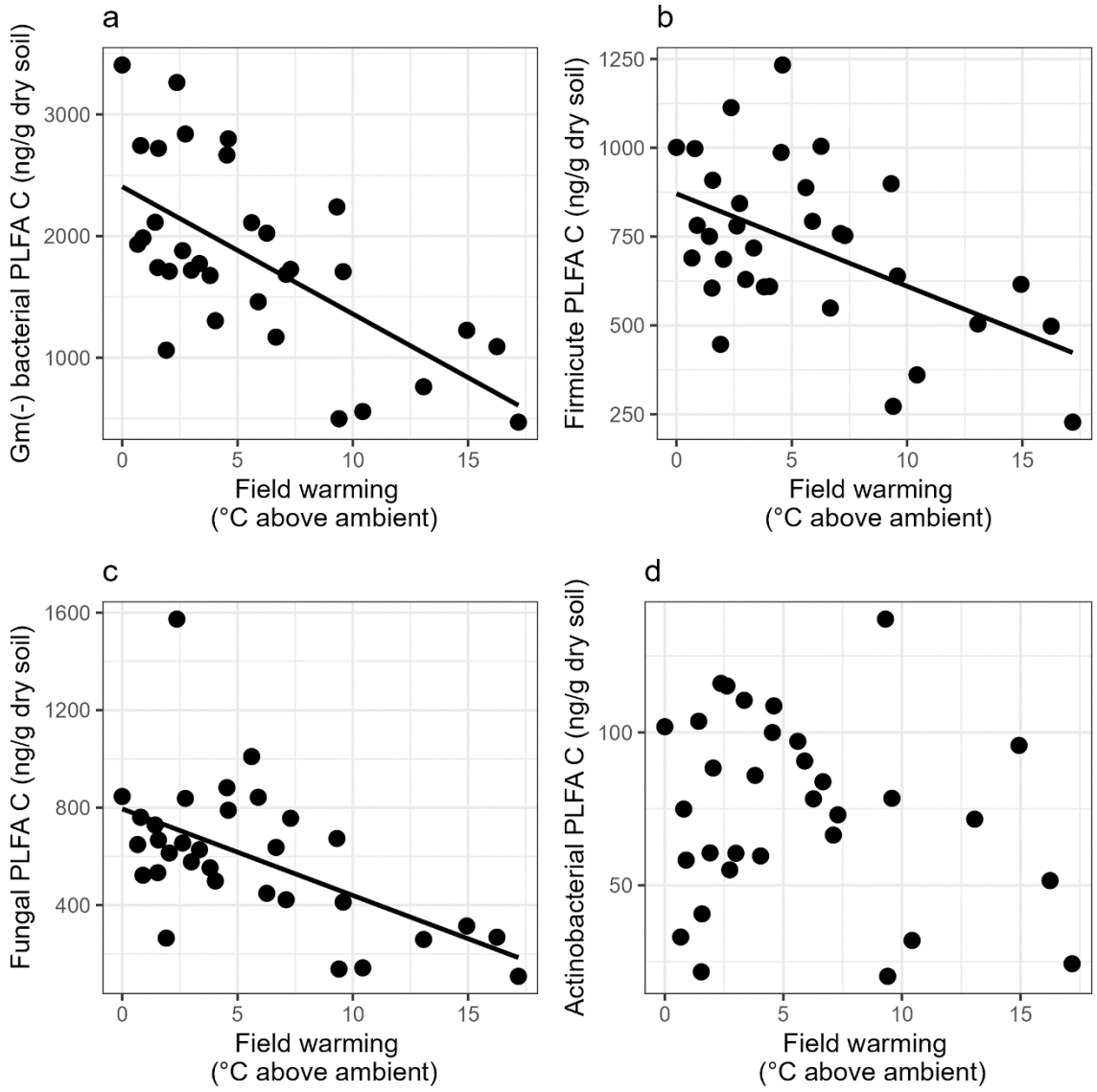

Figure 2.20 Total biomass of PLFA biomarkers for (a) gram-negative bacteria, (b) Firmicutes, (c) Fungi, and (d) Actinobacteria, plotted against field. Lines represent simple linear regressions for those taxa whose absolute abundance was significantly correlated with warming.

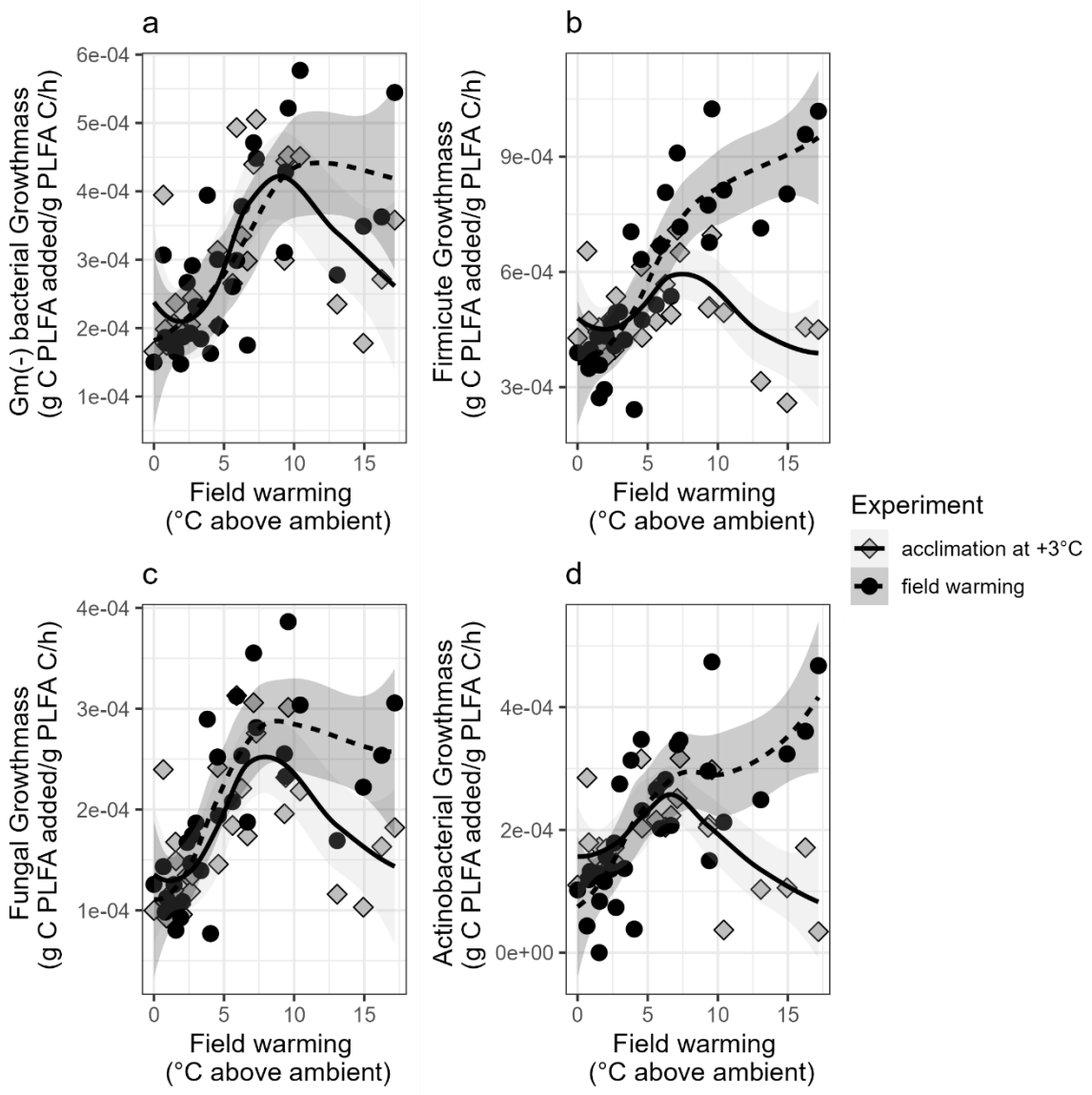

Figure 2.21 Mass-specific growth rates of PLFA biomarkers for (a) gram-negative bacteria, (b) Firmicutes, (c) Fungi, and (d) Actinobacteria, plotted against field warming, for long-term warmed samples incubated at either field-like temperatures or +3°C. Lines represent LOESS regressions, shading represents 95% confidence intervals.

SUMMARIES

2.6 Summary

Soil is the largest terrestrial carbon store, exceeding vegetation and atmospheric carbon combined. As such, the exchange of carbon between soil and atmosphere is a major control for the greenhouse gas effect. Warming is expected to cause large-scale soil carbon losses as soil respiration increases following warming. However, studies have found that this increase in respiration is typically only short-term, with respiration rate eventually returning to pre-warming levels. Two main hypotheses explain the short-term nature of thermal respiration increases: (i) The supply of labile carbon declines following increased microbial activity and becomes a limiting factor, or (ii) thermal adaptation, in which soil microbes exposed to long-term warming have lower mass-specific growth and respiration rates than microbes in soils at ambient temperatures. The current body of literature is inconclusive, with evidence in support of both hypotheses.

This study examined microbial respiration and growth rates at a long-term geothermal warming site in Iceland. Total respiration rate was higher in long-term warmed soils than in ambient soils incubated at the same temperature. However, mass-specific respiration rate was similar between ambient and warmed soils, suggesting that carbon losses, and not thermal adaptation, were responsible for long-term reduction in total respiration. Furthermore, mass-specific growth rates were higher in long-term warmed soils than ambient soils, suggesting adaptations which allowed for enhanced microbial activity. Warmed soils exhibited changes in microbial communities, including decreasing relative biomass of gram-negative bacteria. Differences between the growth responses of specific taxonomic groups, as measured by deuterium incorporation into phospholipid fatty acids, indicate that temperature has a direct and immediate effect on community composition and growth. These results contribute to our understanding of warming's impacts on soil microbial communities across timescales and highlight the complexity of this climate feedback.

2.7 Zusammenfassung

Böden sind der größte terrestrische Kohlenstoffspeicher und enthalten mehr Kohlenstoff als Vegetation und die Atmosphäre zusammen. Dementsprechend hat der Austausch von Kohlenstoff zwischen Boden und Atmosphäre einen wichtigen Einfluss auf den Treibhauseffekt. Es wird erwartet, dass die Klimaerwärmung zu massiven Verlusten von Kohlenstoff aus dem Boden führen wird, da die mikrobielle Respiration mit steigenden Temperaturen zunimmt. Studien zeigen jedoch, dass diese Steigerung typischerweise nur kurzfristig anhält, und die Respiration schließlich auf das vorherige Niveau zurückkehrt. Zwei führende Hypothesen erklären den kurzfristigen Charakter der durch Erwärmung gesteigerten Respiration: (i) Beschleunigte mikrobielle Aktivität führt zu einem schnelleren Verbrauch des verfügbaren Kohlenstoffes, was die mikrobielle Aktivität einschränkt, oder (ii) Anpassung an die Temperatur, wobei die biomassespezifische Respiration und das biomassespezifische Wachstum bei Mikroben in langfristig erwärmten Böden gesenkt sind. Die aktuelle Forschungslage ist uneindeutig, und verschiedene Studien liefern Nachweise für beide Hypothesen.

Diese Studie untersuchte Respiration und Wachstum bei einem langfristig geothermisch erwärmten Forschungsstandort in Island. Die Gesamtrespirationsrate war in langfristig erwärmten Böden höher als in nicht erwärmten Böden, die bei der gleichen Temperatur inkubiert wurden. Im Gegensatz dazu war die massespezifische Respiration ähnlich zwischen erwärmten und nicht erwärmten Böden. Dieses Ergebnis deutet darauf hin, dass Kohlenstoffverluste -- und nicht thermische Anpassung -- die verminderte Gesamtrespiration verursachen. Zudem war das massespezifische Wachstum höher in langfristig erwärmten Böden als in nicht erwärmten Böden, was auf Anpassungen hinweist, die die mikrobielle Aktivität steigern. Erwärmte Böden weisen auch veränderte mikrobielle Gemeinschaften auf, beispielsweise eine verringerte relative Biomasse gramnegativer Bakterien. Unterschiede zwischen den Wachstumsreaktionen verschiedener Taxa, gemessen durch den Deuterium-Einbau in Phospholipid-Fettsäuren, deuten darauf hin, dass die Temperatur eine direkte und unmittelbare Auswirkung auf die Zusammensetzung und das Wachstum der mikrobiellen Gemeinschaft hat. Diese Ergebnisse bieten Einblicke in die Auswirkung erhöhter Temperaturen auf Böden über verschieden Zeitskalen hinweg und verdeutlichen die Komplexität dieser Klimarückkopplung.

3 REFERENCES

Allison, S. D., & Treseder, K. K. (2008). Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology*, *14*(12), 2898–2909. https://doi.org/10.1111/J.1365-2486.2008.01716.X

Alteio, L. V., Séneca, J., Canarini, A., Angel, R., Jansa, J., Guseva, K., Kaiser, C., Richter, A., & Schmidt, H. (2021). A critical perspective on interpreting amplicon sequencing data in soil ecological research. *Soil Biology and Biochemistry*, *160*(July). https://doi.org/10.1016/j.soilbio.2021.108357

Anthony, M. A., Knorr, M., Moore, J. A. M., Simpson, M., & Frey, S. D. (2021). Fungal community and functional responses to soil warming are greater than for soil nitrogen enrichment. *Elementa: Science of the Anthropocene*, *9*(1). https://doi.org/10.1525/elementa.2021.000059

Arndt, K. A., Santos, M. J., Ustin, S., Davidson, S. J., Stow, D., Oechel, W. C., Tran, T. T. P., Graybill, B., & Zona, D. (2019). Arctic greening associated with lengthening growing seasons in Northern Alaska. *Environmental Research Letters*, *14*(12). https://doi.org/10.1088/1748-9326/ab5e26

Aronson, E. L., & McNulty, S. G. (2009). Appropriate experimental ecosystem warming methods by ecosystem, objective, and practicality. *Agricultural and Forest Meteorology*, *149*(11), 1791–1799. https://doi.org/10.1016/j.agrformet.2009.06.007

Bååth, E. (2003). The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. *Microbial Ecology*, *45*(4), 373–383. https://doi.org/10.1007/s00248-003-2002-y

Bailey, V. L., Pries, C. H., & Lajtha, K. (2019). What do we know about soil carbon destabilization? In *Environmental Research Letters* (Vol. 14, Issue 8). Institute of Physics Publishing. https://doi.org/10.1088/1748-9326/ab2c11

Baldrian, P., & López-Mondéjar, R. (2014). Microbial genomics, transcriptomics and proteomics: New discoveries in decomposition research using complementary methods. *Applied Microbiology and Biotechnology*, *98*(4), 1531–1537. https://doi.org/10.1007/s00253-013-5457-x

Bao, X., Zhu, X., Chang, X., Wang, S., Xu, B., Luo, C., Zhang, Z., Wang, Q., Rui, Y., & Cui, X. (2016). Effects of Soil Temperature and Moisture on Soil Respiration on the Tibetan Plateau. *PLOS ONE*, *11*(10), e0165212.

Barrett, R. D. H., & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in Ecology & Evolution*, *23*(1), 38–44. https://doi.org/10.1016/j.tree.2007.09.008

Beer, E., Eisenman, I., & Wagner, T. J. W. (2020). Polar Amplification Due to Enhanced Heat Flux Across the Halocline. *Geophysical Research Letters*, *47*(4). https://doi.org/10.1029/2019GL086706

Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P., & Kauserud, H. (2010). ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. In *BMC Microbiology* (Vol. 10). http://www.biomedcentral.com/1471-2180/10/189

Birch, H. F. (1958). The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil*, *10*(1), 9–31. https://doi.org/10.1007/BF01343734

Blagodatskaya, E., Yuyukina, T., Blagodatsky, S., & Kuzyakov, Y. (2011). Turnover of soil organic matter and of microbial biomass under C3-C4 vegetation change: Consideration of 13C fractionation and preferential substrate utilization. *Soil*

Biology and Biochemistry, *43*(1), 159–166. https://doi.org/10.1016/j.soilbio.2010.09.028

Bonfante, P., & Anca, I. A. (2009). Plants, mycorrhizal fungi, and bacteria: A network of interactions. *Annual Review of Microbiology*, *63*(August), 363–383. https://doi.org/10.1146/annurev.micro.091208.073504

Bouchez, T., Blieux, A. L., Dequiedt, S., Domaizon, I., Dufresne, A., Ferreira, S., Godon, J. J., Hellal, J., Joulian, C., Quaiser, A., Martin-Laurent, F., Mauffret, A., Monier, J. M., Peyret, P., Schmitt-Koplin, P., Sibourg, O., D'oiron, E., Bispo, A., Deportes, I., … Ranjard, L. (2016). Molecular microbiology methods for environmental diagnosis. In *Environmental Chemistry Letters* (Vol. 14, Issue 4, pp. 423–441). Springer Verlag. https://doi.org/10.1007/s10311-016-0581-3

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

Bradford, M. A., Davies, C. A., Frey, S. D., Maddox, T. R., Melillo, J. M., Mohan, J. E., Reynolds, J. F., Treseder, K. K., & Wallenstein, M. D. (2008). Thermal adaptation of soil microbial respiration to elevated temperature. *Ecology Letters*, *11*(12), 1316–1327. https://doi.org/10.1111/j.1461-0248.2008.01251.x

Bradford, M. A., McCulley, R. L., Crowther, Thomas. W., Oldfield, E. E., Wood, S. A., & Fierer, N. (2019). Cross-biome patterns in soil microbial respiration predictable from evolutionary theory on thermal adaptation. *Nature Ecology & Evolution*, *3*(2), 223–231. https://doi.org/10.1038/s41559-018-0771-4

Bradford, M. A., Watts, B. W., & Davies, C. A. (2010). Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. *Global Change Biology*, *16*(5), 1576–1588. https://doi.org/https://doi.org/10.1111/j.1365-2486.2009.02040.x

Bramley-Alves, J., Wanek, W., French, K., & Robinson, S. A. (2015). Moss δ13C: An accurate proxy for past water environments in polar regions. *Global Change Biology*, *21*(6), 2454–2464. https://doi.org/10.1111/gcb.12848

Brooks, J. R., Flanagan, L. B., Buchmann, N., & Ehleringer, J. R. (1997). Carbon isotope composition of boreal plants: Functional grouping of life forms. *Oecologia*, *110*(3), 301–311. https://doi.org/10.1007/s004420050163

Bruns, T. D., & Shefferson, R. P. (2004). Evolutionary studies of ectomycorrhizal fungi: Recent advances and future directions. *Canadian Journal of Botany*, *82*(8), 1122–1132. https://doi.org/10.1139/B04-021

Bundschuh, J., Maity, J. P., Nath, B., Baba, A., Gunduz, O., Kulp, T. R., Jean, J. S., Kar, S., Yang, H. J., Tseng, Y. J., Bhattacharya, P., & Chen, C. Y. (2013). Naturally occurring arsenic in terrestrial geothermal systems of western Anatolia, Turkey: Potential role in contamination of freshwater resources. *Journal of Hazardous Materials*, *262*, 951–959. https://doi.org/10.1016/j.jhazmat.2013.01.039

Callaghan, T. V., Johansson, M., Brown, R. D., Groisman, P. Y., Labba, N., Radionov, V., Bradley, R. S., Blangy, S., Bulygina, O. N., Christensen, T. R., Colman, J. E., Essery, R. L. H., Forbes, B. C., Forchhammer, M. C., Golubev, V. N., Honrath, R. E., Juday, G. P., Meshcherskaya, A. V., Phoenix, G. K., … Wood, E. F. (2011). Multiple effects of changes in arctic snow cover. *Ambio*, *40*(SUPPL. 1), 32–45. https://doi.org/10.1007/S13280-011-0213-X/FIGURES/4

Canarini, A., Fuchslueger, L., Schnecker, J., Metze, D., Nelson, D. B., Kahmen, A., Watzka, M., Pötsch, E. M., Schaumberger, A., Bahn, M., & Richter, A. (2024). Soil fungi

remain active and invest in storage compounds during drought independent of future climate conditions. *Nature Communications*. https://doi.org/10.1038/s41467-024-54537-y

Carini, P., Marsden, P. J., Leff, J. W., Morgan, E. E., Strickland, M. S., & Fierer, N. (2016). Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nature Microbiology*, *2*(December 2016). https://doi.org/10.1038/nmicrobiol.2016.242

Caro, T. A., McFarlin, J., Jech, S., Fierer, N., & Kopf, S. (2023). Hydrogen stable isotope probing of lipids demonstrates slow rates of microbial growth in soil. *Proceedings of the National Academy of Sciences*, *120*(16), e2211625120. https://doi.org/10.1073/pnas.2211625120

Chen, I.-C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid Range Shifts of Species Associated with High Levels of Climate Warming. *Science (American Association for the Advancement of Science)*, *333*, 1026. https://doi.org/10.1126/science.1206432

Chen, J., Luo, Y., García-Palacios, P., Cao, J., Dacal, M., Zhou, X., Li, J., Xia, J., Niu, S., Yang, H., Shelton, S., Guo, W., & van Groenigen, K. J. (2018). Differential responses of carbon-degrading enzyme activities to warming: Implications for soil respiration. *Global Change Biology*, *24*(10), 4816–4826. https://doi.org/10.1111/gcb.14394

Chen, S., Bai, Y., Lin, G., Huang, J., & Han, X. (2007). Variations in 13C values among major plant community types in the Xilin River Basin, Inner Mongolia, China. *Australian Journal of Botany*, *55*(1), 48–54.

Chiariello, N., Hickman, J. C., & Mooney, H. A. (1982). Endomycorrhizal Role for Interspecific Transfer of Phosphorus in a Community of Annual Plants *American Association for the Advancement of Science*.

Choudoir, M. J., Narayanan, A., Rodriguez-Ramos, D., Simoes, R., Efroni, A., Sondrini, A., & Deangelis, K. M. (2014a). *Pangenomes reveal genomic signatures of microbial adaptation to experimental soil warming*.

Choudoir, M. J., Narayanan, A., Rodriguez-Ramos, D., Simoes, R., Efroni, A., Sondrini, A., & Deangelis, K. M. (2014b). *Pangenomes reveal genomic signatures of microbial adaptation to experimental soil warming*.

Collins, M., Knutti, R., Arblaster, J., Dufresne, J., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W., Johns, T., Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A., Wehner, M., Qin, D., Plattner, G., Tignor, M., Allen, S., Boschung, J., … Allen, M. R. (2013). *Long-term Climate Change: Projections, Commitments and Irreversibility*. Cambridge University Press.

Compant, S., Van Der Heijden, M. G. A., & Sessitsch, A. (2010). Climate change effects on beneficial plant-microorganism interactions. *FEMS Microbiology Ecology*, *73*(2), 197–214. https://doi.org/10.1111/j.1574-6941.2010.00900.x

Cooper, C. G., Cooper, M. D., Richards, M. P., & Schmitt, J. (2023). Geographic and seasonal variation in δ13C values of C3 plant arabidopsis: Archaeological implications. *Journal of Archaeological Science*, *149*(May 2022), 105709. https://doi.org/10.1016/j.jas.2022.105709

Dacal, M., Bradford, M. A., Plaza, C., Maestre, F. T., & García-Palacios, P. (2019). Soil microbial respiration adapts to ambient temperature in global drylands. *Nature Ecology & Evolution*, *3*(2), 232–238. https://doi.org/10.1038/s41559-018-0770-5

Deane-Coe, K. K., Mauritz, M., Celis, G., Salmon, V., Crummer, K. G., Natali, S. M., & Schuur, E. A. G. (2015). Experimental Warming Alters Productivity and Isotopic Signatures

of Tundra Mosses. *Ecosystems*, *18*(6), 1070–1082. https://doi.org/10.1007/s10021-015-9884-7

DeAngelis, K. M., Pold, G., Topçuoglu, B. D., van Diepen, L. T. A., Varney, R. M., Blanchard, J. L., Melillo, J., & Frey, S. D. (2015). Long-term forest soil warming alters microbial communities in temperate forest soils. *Frontiers in Microbiology*, *6*(FEB), 1–13. https://doi.org/10.3389/fmicb.2015.00104

De Boeck, H. J., Vicca, S., Roy, J., Nijs, I., Milcu, A., Kreyling, J., Jentsch, A., Chabbi, A., Campioli, M., Callaghan, T., Beierkuhnlein, C., & Beier, C. (2015). Global Change Experiments: Challenges and Opportunities. In *BioScience* (Vol. 65, Issue 9, pp. 922–931). Oxford University Press. https://doi.org/10.1093/biosci/biv099

Deslippe, J. R., Hartmann, M., Simard, S. W., & Mohn, W. W. (2012). Long-term warming alters the composition of Arctic soil microbial communities. *FEMS Microbiology Ecology*, *82*(2), 303–315. https://doi.org/10.1111/j.1574-6941.2012.01350.x

Dijkstra, F. A., Carrillo, Y., Pendall, E., & Morgan, J. A. (2013). Rhizosphere priming: A nutrient perspective. *Frontiers in Microbiology*, *4*(JUL), 1–8. https://doi.org/10.3389/fmicb.2013.00216

Dijkstra, P., Ishizu, A., Doucett, R., Hart, S. C., Schwartz, E., Menyailo, O. V., & Hungate, B. A. (2006). 13C and 15N natural abundance of the soil microbial biomass. *Soil Biology and Biochemistry*, *38*(11), 3257–3266. https://doi.org/10.1016/j.soilbio.2006.04.005

Dong, Y. P., Huang, H., Song, W., Sun, X. C., Wang, M., Zhang, W., Wang, K. L., Liu, C. Q., & Liu, X. Y. (2019). Natural 13C and 15N abundance of moss-substrate systems on limestones and sandstones in a karst area of subtropical China. *Catena*, *180*(April), 8–15. https://doi.org/10.1016/j.catena.2019.04.015

Donhauser, J., Niklaus, P. A., Rousk, J., Larose, C., & Frey, B. (2020). Temperatures beyond the community optimum promote the dominance of heatadapted, fast growing and stress resistant bacteria in alpine soils. *Soil Biology and Biochemistry*, *148*, 107873. https://doi.org/https://doi.org/10.1016/j.soilbio.2020.107873

Dore, M. H. I. (2005). Climate change and changes in global precipitation patterns: What do we know? *Environment International*, *31*(8), 1167–1181. https://doi.org/10.1016/j.envint.2005.03.004

Eliasson, P. E., McMurtrie, R. E., Pepper, D. A., Strömgren, M., Linder, S., & Ågren, G. I. (2005). The response of heterotrophic CO2 flux to soil warming. *Global Change Biology*, *11*(1), 167–181. https://doi.org/https://doi.org/10.1111/j.1365-2486.2004.00878.x

Elmendorf, S. C., Henry, G. H. R., Hollister, R. D., Björk, R. G., Bjorkman, A. D., Callaghan, T. V., Collier, L. S., Cooper, E. J., Cornelissen, J. H. C., Day, T. A., Fosaa, A. M., Gould, W. A., Grétarsdóttir, J., Harte, J., Hermanutz, L., Hik, D. S., Hofgaard, A., Jarrad, F., Jónsdóttir, I. S., … Wookey, P. A. (2012). Global assessment of experimental climate warming on tundra vegetation: Heterogeneity over space and time. *Ecology Letters*, *15*(2), 164–175. https://doi.org/10.1111/j.1461-0248.2011.01716.x

Epstein, H. E., Walker, M. D., Chapin, F. S., & Starfield, A. M. (2000). A transient, nutrient-based model of arctic plant community response to climatic warming. *Ecological Applications*, *10*(3), 824–841. https://doi.org/10.1890/1051-0761(2000)010[0824:ATNBMO]2.0.CO;2

Evans, S., Dieckmann, U., Franklin, O., & Kaiser, C. (2016). Synergistic effects of diffusion and microbial physiology reproduce the Birch effect in a micro-scale model. *Soil*

Biology and Biochemistry, *93*, 28–37. https://doi.org/10.1016/J.SOILBIO.2015.10.020

Evans, S. E., & Wallenstein, M. D. (2014). Climate change alters ecological strategies of soil bacteria. *Ecology Letters*, *17*(2), 155–164. https://doi.org/10.1111/ELE.12206

Fang, C., Verbrigghe, N., Sigurdsson, B. D., Ostonen, I., Leblans, N. I. W., Marañón-Jiménez, S., Fuchslueger, L., Sigurðsson, P., Meeran, K., Portillo-Estrada, M., Verbruggen, E., Richter, A., Sardans, J., Peñuelas, J., Bahn, M., Vicca, S., & Janssens, I. A. (2023). Decadal soil warming decreased vascular plant above and belowground production in a subarctic grassland by inducing nitrogen limitation. *New Phytologist*, *240*(2), 565–576. https://doi.org/10.1111/nph.19177

Fanin, N., Mooshammer, M., Sauvadet, M., Meng, C., Alvarez, G., Bernard, L., Bertrand, I., Blagodatskaya, E., Bon, L., Fontaine, S., Niu, S., Lashermes, G., Maxwell, T. L., Weintraub, M. N., Wingate, L., Moorhead, D., & Nottingham, A. T. (2022). Soil enzymes in response to climate warming: Mechanisms and feedbacks. *Functional Ecology*, *36*(6), 1378–1395. https://doi.org/10.1111/1365-2435.14027

Fan, Z., Neff, J. C., & Hanan, N. P. (2015). Modeling pulsed soil respiration in an African savanna ecosystem. *Agricultural and Forest Meteorology*, *200*, 282–292. https://doi.org/10.1016/j.agrformet.2014.10.009

Fernández-Alvarez, J. C., Pérez-Alarcón, A., Eiras-Barca, J., Rahimi, S., Nieto, R., & Gimeno, L. (2023). Projected changes in atmospheric moisture transport contributions associated with climate warming in the North Atlantic. *Nature Communications*, *14*(1), 6476. https://doi.org/10.1038/s41467-023-41915-1

Frey, S. D., Lee, J., Melillo, J. M., & Six, J. (2013). The temperature response of soil microbial efficiency and its feedback to climate. *Nature Climate Change 2013 3:4*, *3*(4), 395–398. https://doi.org/10.1038/nclimate1796

Frostegård, Å., Tunlid, A., & Bååth, E. (2011). Use and misuse of PLFA measurements in soils. *Soil Biology and Biochemistry*, *43*(8), 1621–1625. https://doi.org/10.1016/J.SOILBIO.2010.11.021

Garcia, M. O., Templer, P. H., Sorensen, P. O., Sanders-DeMott, R., Groffman, P. M., & Bhatnagar, J. M. (2020). Soil Microbes Trade-Off Biogeochemical Cycling for Stress Tolerance Traits in Response to Year-Round Climate Change. *Frontiers in Microbiology*, *11*, 507526. https://doi.org/10.3389/FMICB.2020.00616/BIBTEX

Gargallo-Garriga, A., Ayala-Roque, M., Sardans, J., Bartrons, M., Granda, V., Sigurdsson, B. D., Leblans, N. I. W., Oravec, M., Urban, O., Janssens, I. A., & Peñuelas, J. (2017). Impact of soil warming on the plant metabolome of Icelandic grasslands. *Metabolites*, *7*(3). https://doi.org/10.3390/metabo7030044

Giesemann, P., Eichenberg, D., Stöckel, M., Seifert, L. F., Gomes, S. I. F., Merckx, V. S. F. T., & Gebauer, G. (2020). Dark septate endophytes and arbuscular mycorrhizal fungi (Paris-morphotype) affect the stable isotope composition of 'classically' non-mycorrhizal plants. *Functional Ecology*, *34*(12), 2453–2466. https://doi.org/10.1111/1365-2435.13673

Glaser, B., Turrión, M. B., & Alef, K. (2004). Amino sugars and muramic acid - Biomarkers for soil microbial community structure analysis. *Soil Biology and Biochemistry*, *36*(3), 399–407. https://doi.org/10.1016/j.soilbio.2003.10.013

Gorka, S., Darcy, S., Horak, J., Imai, B., Mohrlok, M., Salas, E., Richter, A., Schmidt, H., Wanek, W., Kaiser, C., & Canarini, A. (2023). Beyond PLFA: Concurrent extraction of neutral and glycolipid fatty acids provides new insights into soil microbial

communities. *Soil Biology and Biochemistry*, *187*. https://doi.org/10.1016/j.soilbio.2023.109205

Gunina, A., & Kuzyakov, Y. (2015). Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate. In *Soil Biology and Biochemistry* (Vol. 90, pp. 87–100). Elsevier Ltd. https://doi.org/10.1016/j.soilbio.2015.07.021

Guo, Q., Wang, Y., & Liu, W. (2008). B, As, and F contamination of river water due to wastewater discharge of the Yangbajing geothermal power plant, Tibet, China. *Environmental Geology*, *56*(1), 197–205. https://doi.org/10.1007/s00254-007-1155-2

Hartley, I., Heinemeyer, A., Evans, S., & Ineson, P. (2007). The effect of soil warming on bulk soil vs. rhizosphere respiration. *Global Change Biology*, *13*, 2654–2667. https://doi.org/10.1111/j.1365-2486.2007.01454.x

Hartley, I. P., Heinemeyer, A., & Ineson, P. (2007). Effects of three years of soil warming and shading on the rate of soil respiration: Substrate availability and not thermal acclimation mediates observed response. *Global Change Biology*, *13*(8), 1761–1770. https://doi.org/10.1111/j.1365-2486.2007.01373.x

He, Y., Zhou, X., Jia, Z., Zhou, L., Chen, H., Liu, R., Du, Z., Zhou, G., Shao, J., Ding, J., Chen, K., & Hartley, I. P. (2023). Apparent thermal acclimation of soil heterotrophic respiration mainly mediated by substrate availability. *Global Change Biology*, *29*(4), 1178–1187. https://doi.org/10.1111/gcb.16523

Hickling, R., Roy, D. B., Hill, J. K., Fox, R., & Thomas, C. D. (2006). The distributions of a wide range of taxonomic groups are expanding polewards. *Global Change Biology*, *12*(3), 450–455. https://doi.org/10.1111/j.1365-2486.2006.01116.x

Hodson, E., Shahid, F., Basinger, J., & Kaminskyj, S. (2009). Fungal endorhizal associates of Equisetum species from Western and Arctic Canada. *Mycological Progress*, *8*(1), 19–27. https://doi.org/10.1007/s11557-008-0574-0

Hopkins, F. M., Filley, T. R., Gleixner, G., Lange, M., Top, S. M., & Trumbore, S. E. (2014). Increased belowground carbon inputs and warming promote loss ofsoil organic carbon through complementary microbial responses. *Soil Biology and Biochemistry*, *76*, 57–69. https://doi.org/10.1016/j.soilbio.2014.04.028

Jansa, J., & Treseder, K. K. (2017). Introduction: Mycorrhizas and the Carbon Cycle. In *Mycorrhizal Mediation of Soil: Fertility, Structure, and Carbon Storage*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-804312-7.00019-X

Jenkinson, D. S., Brookes, P. C., & Powlson, D. S. (2004). Measuring soil microbial biomass. *Soil Biology and Biochemistry*, *36*(1), 5–7. https://doi.org/10.1016/j.soilbio.2003.10.002

Jun Liu, X. A., Han, S., Frey, S. D., Melillo, J. M., & Zhou, J. (2024). Microbial responses to long-term warming differ across soil microenvironments 1 2 Running title: Aggregates regulate microbial functional genes. *The ISME Journal*. https://doi.org/10.1093/ismeco/ycae051/7641696

Khorsand Rosa, R., Oberbauer, S. F., Starr, G., Parker La Puma, I., Pop, E., Ahlquist, L., & Baldwin, T. (2015). Plant phenological responses to a long-term experimental extension of growing season and soil warming in the tussock tundra of Alaska. *Global Change Biology*, *21*(12), 4520–4532. https://doi.org/10.1111/gcb.13040

Kirschbaum, M. U. F. (1995). The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. *Soil Biology and Biochemistry*, *27*(6), 753–760. https://doi.org/10.1016/0038-0717(94)00242-S

Kirschbaum, M. U. F. (2004). Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? *Global Change Biology*, *10*(11), 1870–1877. https://doi.org/https://doi.org/10.1111/j.1365-2486.2004.00852.x

Knorr, W., Prentice, I. C., House, J. I., & Holland, E. A. (2005). Long-term sensitivity of soil carbon turnover to warming. *Nature*, *433*(7023), 298–301. https://doi.org/10.1038/nature03226

Kramer, C., & Gleixner, G. (2008). Soil organic matter in soil depth profiles: Distinct carbon preferences of microbial groups during carbon transformation. *Soil Biology and Biochemistry*, *40*(2), 425–433. https://doi.org/10.1016/j.soilbio.2007.09.016

Kreyling, J., Grant, K., Hammerl, V., Arfin-Khan, M. A. S., Malyshev, A. V., Peñuelas, J., Pritsch, K., Sardans, J., Schloter, M., Schuerings, J., Jentsch, A., & Beierkuhnlein, C. (2019). Winter warming is ecologically more relevant than summer warming in a cool-temperate grassland. *Scientific Reports*, *9*(1), 1–9. https://doi.org/10.1038/s41598-019-51221-w

Kuzyakov, Y. (2010). Priming effects: Interactions between living and dead organic matter. *Soil Biology and Biochemistry*, *42*(9), 1363–1371. https://doi.org/10.1016/j.soilbio.2010.04.003

Lambers, H., Mougel, C., Jaillard, B., & Hinsinger, P. (2009). Plant-microbe-soil interactions in the rhizosphere: An evolutionary perspective. *Plant and Soil*, *321*(1–2), 83–115. https://doi.org/10.1007/s11104-009-0042-x

Lang, S. I., Cornelissen, J. H. C., Shaver, G. R., Ahrens, M., Callaghan, T. V., Molau, U., Ter Braak, C. J. F., Hölzer, A., & Aerts, R. (2012). Arctic warming on two continents has consistent negative effects on lichen diversity and mixed effects on bryophyte diversity. *Global Change Biology*, *18*(3), 1096–1107. https://doi.org/10.1111/j.1365-2486.2011.02570.x

Leblans, N. I. W., Sigurdsson, B. D., Vicca, S., Fu, Y., Penuelas, J., & Janssens, I. A. (2017). Phenological responses of Icelandic subarctic grasslands to short-term and long-term natural soil warming. *Global Change Biology*, *23*(11), 4932–4945. https://doi.org/10.1111/gcb.13749

Legay, N., Baxendale, C., Grigulis, K., Krainer, U., Kastl, E., Schloter, M., Bardgett, R. D., Arnoldi, C., Bahn, M., Dumont, M., Pommier, T., Clément, J. C., & Lavorel, S. (2014). Contribution of above- and below-ground plant traits to the structure and function of grassland soil microbial communities. *Annals of Botany*, *114*(5), 1011–1021. https://doi.org/10.1093/aob/mcu169

Lekberg, Y., Bååth, E., Frostegård, Å., Hammer, E., Hedlund, K., Jansa, J., Kaiser, C., Ramsey, P. W., Řezanka, T., Rousk, J., Wallander, H., Welc, M., & Olsson, P. A. (2022). Fatty acid 16:1ω5 as a proxy for arbuscular mycorrhizal fungal biomass: current challenges and ways forward. *Biology and Fertility of Soils*, *58*(8), 835–842. https://doi.org/10.1007/s00374-022-01670-9

Lekberg, Y., Hammer, E. C., & Olsson, P. A. (2010). Plants as resource islands and storage units - adopting the mycocentric viewofarbuscularmycorrhizal networks. *FEMS Microbiology Ecology*, *74*(2), 336–345. https://doi.org/10.1111/j.1574-6941.2010.00956.x

Lett, S., Christiansen, C. T., Dorrepaal, E., & Michelsen, A. (2024). Moss species and precipitation mediate experimental warming stimulation of growing season N2 fixation in subarctic tundra. *Global Change Biology*, *30*(7), 1–16. https://doi.org/10.1111/gcb.17401

Lewe, N., Hermans, S., Lear, G., Kelly, L. T., Thomson-Laing, G., Weisbrod, B., Wood, S. A., Keyzers, R. A., & Deslippe, J. R. (2021). Phospholipid fatty acid (PLFA) analysis as a tool to estimate absolute abundances from compositional 16S rRNA bacterial metabarcoding data. *Journal of Microbiological Methods*, *188*. https://doi.org/10.1016/j.mimet.2021.106271

Lindo, Z., Nilsson, M. C., & Gundale, M. J. (2013). Bryophyte-cyanobacteria associations as regulators of the northern latitude carbon balance in response to global change. *Global Change Biology*, *19*(7), 2022–2035. https://doi.org/10.1111/gcb.12175

Liu, C., Tian, H., Gu, X., Li, N., Zhao, X., Lei, M., Alharbi, H., Megharaj, M., He, W., & Kuzyakov, Y. (2022). Catalytic efficiency of soil enzymes explains temperature sensitivity: Insights from physiological theory. *Science of the Total Environment*, *822*. https://doi.org/10.1016/j.scitotenv.2022.153365

Liu, Y., He, N., Wen, X., Xu, L., Sun, X., Yu, G., Liang, L., & Schipper, L. A. (2018). The optimum temperature of soil microbial respiration: Patterns and controls. *Soil Biology and Biochemistry*, *121*(March), 35–42. https://doi.org/10.1016/j.soilbio.2018.02.019

Lundin, E. J., Klaminder, J., Giesler, R., Persson, A., Olefeldt, D., Heliasz, M., Christensen, T. R., & Karlsson, J. (2016). Is the subarctic landscape still a carbon sink? Evidence from a detailed catchment balance. *Geophysical Research Letters*, *43*(5), 1988–1995. https://doi.org/10.1002/2015GL066970

Luo, C., Rodriguez-R, L. M., Johnston, E. R., Wu, L., Cheng, L., Xue, K., Tu, Q., Deng, Y., He, Z., Shi, J. Z., Yuan, M. M., Sherry, R. A., Li, D., Luo, Y., Schuur, E. A. G., Chain, P., Tiedje, J. M., Zhou, J., & Konstantinidis, K. T. (2014). Soil microbial community responses to a decade of warming as revealed by comparative metagenomics. *Applied and Environmental Microbiology*, *80*(5), 1777–1786. https://doi.org/10.1128/AEM.03712-13

Luo, X., Zhou, H., Satriawan, T. W., Tian, J., Zhao, R., Keenan, T. F., Griffith, D. M., Sitch, S., Smith, N. G., & Still, C. J. (2024). Mapping the global distribution of C4 vegetation using observations and optimality theory. *Nature Communications*, *15*(1). https://doi.org/10.1038/s41467-024-45606-3

Luo, Y., Sherry, R., Zhou, X., & Wan, S. (2009). Terrestrial carbon-cycle feedback to climate warming: experimental evidence on plant regulation and impacts of biofuel feedstock harvest. *GCB Bioenergy*, *1*(1), 62–74. https://doi.org/10.1111/j.1757-1707.2008.01005.x

Luo, Y., Wan, S., Hui, D., & Wallace, L. L. (2001). Acclimatization of soil respiration to warming in a tall grass prairie. *Nature*, *413*(6856), 622–625. https://doi.org/10.1038/35098065

Marr, A. G., & Ingraham, J. L. (1962). Effect of temperature on the composition of fatty acids in Escherichia coli. *Journal of Bacteriology*, *84*(6), 1260–1267. https://doi.org/10.1128/jb.84.6.1260-1267.1962

McBean, G., Alekseev, G., Chen, D., Førland, E., Fyfe, J., Groisman, P. Y. P., King, R., Melling, H., Vose, R., & Whitfield, P. P. H. (2005). Arctic climate: past and present. In *Arctic Climate Impact Assessment.* (pp. 21–60).

McGuire, A. D., Anderson, L. G., Christensen, T. R., Scott, D., Laodong, G., Hayes, D. J., Martin, H., Lorenson, T. D., Macdonald, R. W., & Nigel, R. (2009). Sensitivity of the carbon cycle in the Arctic to climate change. In *Ecological Monographs* (Vol. 79, Issue 4, pp. 523–555). https://doi.org/10.1890/08-2025.1

Meeran, K., Verbrigghe, N., Ingrisch, J., Fuchslueger, L., Müller, L., Sigurðsson, P., Sigurdsson, B. D., Wachter, H., Watzka, M., Soong, J. L., Vicca, S., Janssens, I. A., & Bahn, M. (2023). Individual and interactive effects of warming and nitrogen supply on CO2 fluxes and carbon allocation in subarctic grassland. *Global Change Biology*, *29*(18), 5276–5291. https://doi.org/10.1111/gcb.16851

Melillo, J. M., Steudler, P. A., Aber, J. D., Newkirk, K., Lux, H., Bowles, F. P., Catricala, C., Magill, A., Ahrens, T., & Morrisseau, S. (2002). Soil Warming and Carbon-Cycle Feedbacks to the Climate System. *Science*, *298*(5601), 2173–2176. https://doi.org/10.1126/science.1074153

Metze, D., Schnecker, J., de Carlan, C. L. N., Bhattarai, B., Verbruggen, E., Ostonen, I., Janssens, I. A., Sigurdsson, B. D., Hausmann, B., Kaiser, C., & Richter, A. (2024). Soil warming increases the number of growing bacterial taxa but not their growth rates. *Science Advances*, *10*(8), 6295. https://doi.org/10.1126/SCIADV.ADK6295

Meynzer, W., Janssens, I., Leblans, N., & Dauwe, S. (2016). The effect of temperature and nitrogen on plant community structure in Icelandic subarctic grassland ecosystems.

Montgomery, H. J., Monreal, C. M., Young, J. C., & Seifert, K. A. (2000). Determinination of soil fungal biomass from soil ergosterol analyses. *Soil Biology and Biochemistry*, *32*(8–9), 1207–1217. https://doi.org/10.1016/S0038-0717(00)00037-7

Muhr, J., Franke, J., & Borken, W. (2010). Drying-rewetting events reduce C and N losses from a Norway spruce forest floor. *Soil Biology and Biochemistry*, *42*(8), 1303–1312. https://doi.org/10.1016/j.soilbio.2010.03.024

M., van A. I., & Axel, O. P. (2003). Fungal Lipid Accumulation and Development of Mycelial Structures by Two Arbuscular Mycorrhizal Fungi. *Applied and Environmental Microbiology*, *69*(11), 6762–6767. https://doi.org/10.1128/AEM.69.11.6762-6767.2003

Navarro, A., Font, X., & Viladevall, M. (2011). Geochemistry and groundwater contamination in the La Selva geothermal system (Girona, Northeast Spain). *Geothermics*, *40*(4), 275–285. https://doi.org/10.1016/j.geothermics.2011.07.005

Nehls, U., Göhringer, F., Wittulsky, S., & Dietz, S. (2010). Fungal carbohydrate support in the ectomycorrhizal symbiosis: A review. *Plant Biology*, *12*(2), 292–301. https://doi.org/10.1111/j.1438-8677.2009.00312.x

Norby, R. J., Childs, J., Hanson, P. J., & Warren, J. M. (2019). Rapid loss of an ecosystem engineer: Sphagnum decline in an experimentally warmed bog. *Ecology and Evolution*, *9*(22), 12571–12585. https://doi.org/10.1002/ece3.5722

Oechel, W. C., Vourlitis, G. L., Hastings, S. J., Zulueta, R. C., Hinzman, L., & Kane, D. (2000). Acclimation of ecosystem CO2 exchange in the Alaskan Arctic. *Nature*, *406*(6799), 978–981.

Oliverio, A. M., Bradford, M. A., & Fierer, N. (2017). Identifying the microbial taxa that consistently respond to soil warming across time and space. *Global Change Biology*, *23*(5), 2117–2129. https://doi.org/10.1111/gcb.13557

Olsson, P. A. (1999). Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology*, *29*(4), 303–310. https://doi.org/10.1111/j.1574-6941.1999.tb00621.x

Olsson, P. A., & Lekberg, Y. (2022). A critical review of the use of lipid signature molecules for the quantification of arbuscular mycorrhiza fungi. *Soil Biology and Biochemistry*, *166*, 108574. https://doi.org/https://doi.org/10.1016/j.soilbio.2022.108574

Osaki, S., & Nakatsubo, T. (2021). Effects of climate warming on the production of the pioneer moss Racomitrium japonicum: seasonal and year-to-year variations. *Journal of Plant Research*, *134*(1), 115–126. https://doi.org/10.1007/s10265-020-01240-w

Osborne, C. P., Salomaa, A., Kluyver, T. A., Visser, V., Kellogg, E. A., Morrone, O., Vorontsova, M. S., Clayton, W. D., & Simpson, D. A. (2014). A global database of C4 photosynthesis in grasses. *New Phytologist*, *204*(3), 441–446. https://doi.org/https://doi.org/10.1111/nph.12942

Paterson, E. (2003). Importance of rhizodeposition in the coupling of plant and microbial productivity. *European Journal of Soil Science*, *54*(4), 741–750. https://doi.org/10.1046/j.1351-0754.2003.0557.x

Permin, A., Michelsen, A., & Rousk, K. (2022). Direct and indirect effects of warming on moss abundance and associated nitrogen fixation in subarctic ecosystems. *Plant and Soil*, *471*(1–2), 343–358. https://doi.org/10.1007/s11104-021-05245-9

Perotto, S., Girlanda, M., & Martino, E. (2002). Ericoid mycorrhizal fungi: Some new perspectives on old acquaintances. *Plant and Soil*, *244*(1–2), 41–53. https://doi.org/10.1023/A:1020289401610

Pessenda, L. C. R., Aravena, R., Melfi, A. J., Telles, E. C. C., Boulet, R., Valencia, E. P. E., & Tomazello, M. (1996). The use of carbon isotopes (13C,14C) in soil to evaluate vegetation changes during the holocene in Central Brazil. *Radiocarbon*, *38*(2), 191–201. https://doi.org/10.1017/S0033822200017562

Petersen, S. O., & Klug, M. J. (1994). Effects of Sieving, Storage, and Incubation Temperature on the Phospholipid Fatty Acid Profile of a Soil Microbial Community. *Applied and Environmental Microbiology*, *60*(7), 2421–2430. https://doi.org/10.1128/AEM.60.7.2421-2430.1994

Poeplau, C., Sigurdsson, P., & Sigurdsson, B. D. (2020). Depletion of soil carbon and aggregation after strong warming of a subarctic Andosol under forest and grassland cover. *Soil*, *6*(1), 115–129. https://doi.org/10.5194/soil-6-115-2020

Polar Regions. (2023). In *Climate Change 2022 – Impacts, Adaptation and Vulnerability* (pp. 2319–2368). Cambridge University Press. https://doi.org/10.1017/9781009325844.023

Pold, G., Billings, A. F., Blanchard, J. L., Burkhardt, D. B., Frey, S. D., Melillo, J. M., Schnabel, J., van Diepen, L. T. A., & deAngelis, K. M. (2016). Long-term warming alters carbohydrate degradation potential in temperate forest soils. *Applied and Environmental Microbiology*, *82*(22), 6518–6530. https://doi.org/10.1128/AEM.02012-16

Poursalavati, A., Javaran, V. J., Laforest-Lapointe, I., & Fall, M. L. (2023). Soil Metatranscriptomics: An Improved RNA Extraction Method Toward Functional Analysis Using Nanopore Direct RNA Sequencing. *Phytobiomes Journal*, *7*(1), 42–54. https://doi.org/10.1094/PBIOMES-12-22-0108-TA

Quan, P. L., Sauzade, M., & Brouzes, E. (2018). DPCR: A technology review. In *Sensors (Switzerland)* (Vol. 18, Issue 4). MDPI AG. https://doi.org/10.3390/s18041271

Ramsey, P. W., Rillig, M. C., Feris, K. P., Holben, W. E., & Gannon, J. E. (2006). Choice of methods for soil microbial community analysis: PLFA maximizes power compared to CLPP and PCR-based approaches. *Pedobiologia*, *50*(3), 275–280. https://doi.org/https://doi.org/10.1016/j.pedobi.2006.03.003

Rantanen, M., Karpechko, A. Y., Lipponen, A., Nordling, K., Hyvärinen, O., Ruosteenoja, K., Vihma, T., & Laaksonen, A. (2022). The Arctic has warmed nearly four times

faster than the globe since 1979. *Communications Earth and Environment*, *3*(1). https://doi.org/10.1038/s43247-022-00498-3

Read, D. J., & Perez-Moreno, J. (2003). Mycorrhizas and nutrient cycling in ecosystems - A journey towards relevance? *New Phytologist*, *157*(3), 475–492. https://doi.org/10.1046/j.1469-8137.2003.00704.x

Richardson, A. D., Keenan, T. F., Migliavacca, M., Ryu, Y., Sonnentag, O., & Toomey, M. (2013). Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agricultural and Forest Meteorology*, *169*, 156–173. https://doi.org/10.1016/j.agrformet.2012.09.012

Rillig, M. C., Wright, S. F., Shaw, M. R., & Field, C. B. (2002). Artificial climate warming positively affects arbuscular mycorrhizae but decreases soil aggregate water stability in an annual grassland. *Oikos*, *97*(1), 52–58. https://doi.org/10.1034/j.1600-0706.2002.970105.x

Rinnan, R., Michelsen, An., Bââth, E., & Jonasson, S. (2007). Fifteen years of climate change manipulations alter soil microbial communities in a subarctic heath ecosystem. *Global Change Biology*, *13*(1), 28–39. https://doi.org/https://doi.org/10.1111/j.1365-2486.2006.01263.x

Rinnan, R., Stark, S., & Tolvanen, A. (2009). Responses of vegetation and soil microbial communities to warming and simulated herbivory in a subarctic heath. *Journal of Ecology*, *97*(4), 788–800. https://doi.org/https://doi.org/10.1111/j.1365-2745.2009.01506.x

Rogers, H. H., Runion, G. B., & Krupa, S. V. (1994). Plant responses to atmospheric CO2 enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution (Barking, Essex : 1987)*, *83*(1–2), 155–189. https://doi.org/10.1016/0269-7491(94)90034-5

Romero-Olivares, A. L., Allison, S. D., & Treseder, K. K. (2017). Soil microbes and their response to experimental warming over time: A meta-analysis of field studies. *Soil Biology and Biochemistry*, *107*, 32–40. https://doi.org/10.1016/J.SOILBIO.2016.12.026

Romero-Olivares, A. L., Meléndrez-Carballo, G., Lago-Lestón, A., & Treseder, K. K. (2019). Soil metatranscriptomes under long-term experimental warming and drying: Fungi allocate resources to cell metabolic maintenance rather than decay. *Frontiers in Microbiology*, *10*(AUG). https://doi.org/10.3389/fmicb.2019.01914

Rosendahl, S., & Stukenbrock, E. H. (2004). Community structure of arbuscular mycorrhizal fungi in undisturbed vegetation revealed by analyses of LSU rDNA sequences. *Molecular Ecology*, *13*(10), 3179–3186. https://doi.org/10.1111/j.1365-294X.2004.02295.x

Roy Chowdhury, P., Golas, S. M., Alteio, L. V., Stevens, J. T. E., Billings, A. F., Blanchard, J. L., Melillo, J. M., & DeAngelis, K. M. (2021). The Transcriptional Response of Soil Bacteria to Long-Term Warming and Short-Term Seasonal Fluctuations in a Terrestrial Forest. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.666558

Rzepczynska, A. M., Michelsen, A., Olsen, M. A. N., & Lett, S. (2022). Bryophyte species differ widely in their growth and N2-fixation responses to temperature. *Arctic Science*, *8*(4), 1236–1251. https://doi.org/10.1139/as-2021-0053

Salas, E., Gorfer, M., Bandian, D., Eichorst, S. A., Schmidt, H., Horak, J., Rittmann, S. K. M. R., Schleper, C., Reischl, B., Pribasnig, T., Jansa, J., Kaiser, C., & Wanek, W. (2024). Reevaluation and novel insights into amino sugar and neutral sugar necromass

biomarkers in archaea, bacteria, fungi, and plants. *Science of the Total Environment*, *906*. https://doi.org/10.1016/j.scitotenv.2023.167463
Schaeffer, S. M., Homyak, P. M., Boot, C. M., Roux-Michollet, D., & Schimel, J. P. (2017). Soil carbon and nitrogen dynamics throughout the summer drought in a California annual grassland. *Soil Biology and Biochemistry*, *115*, 54–62. https://doi.org/10.1016/j.soilbio.2017.08.009
Schimel, J., Balser, T. C., & Wallenstein, M. (2007). Microbial stress-response physiology and its implications for ecosystem function. *Ecology*, *88*(6), 1386–1394. https://doi.org/10.1890/06-0219
Schimel, J. P. (2018). Life in dry soils: Effects of drought on soil microbial communities and processes. *Annual Review of Ecology, Evolution, and Systematics*, *49*, 409–432. https://doi.org/10.1146/annurev-ecolsys-110617-062614
Schindlbacher, A., Rodler, A., Kuffner, M., Kitzler, B., Sessitsch, A., & ZechmeisterBoltenstern, S. (2011). Experimental warming effects on the microbial community of a temperate mountain forest soil. *Soil Biology and Biochemistry*, *43*(7), 1417–1425. https://doi.org/https://doi.org/10.1016/j.soilbio.2011.03.005
Schindlbacher, A., Wunderlich, S., Borken, W., Kitzler, B., Zechmeister-Boltenstern, S., & Jandl, R. (2012). Soil respiration under climate change: Prolonged summer drought offsets soil warming effects. *Global Change Biology*, *18*(7), 2270–2279. https://doi.org/10.1111/j.1365-2486.2012.02696.x
Schlesinger, W. H., & Andrews, J. A. (2000). Soil respiration and the global carbon cycle. *Biogeochemistry*, *48*(1), 7–20. https://doi.org/10.1023/A:1006247623877/METRICS
Schütz, K., Nagel, P., Vetter, W., Kandeler, E., & Ruess, L. (2009). Flooding forested groundwater recharge areas modifies microbial communities from top soil to groundwater table. *FEMS Microbiology Ecology*, *67*(1), 171–182. https://doi.org/10.1111/j.1574-6941.2008.00608.x
Shahzad, T., Chenu, C., Genet, P., Barot, S., Perveen, N., Mougin, C., & Fontaine, S. (2015). Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species. *Soil Biology and Biochemistry*, *80*, 146–155. https://doi.org/10.1016/j.soilbio.2014.09.023
Sharma, M. P., & Buyer, J. S. (2015). Comparison of biochemical and microscopic methods for quantification of arbuscular mycorrhizal fungi in soil and roots. *Applied Soil Ecology*, *95*, 86–89. https://doi.org/https://doi.org/10.1016/j.apsoil.2015.06.001
Sheik, C. S., Beasley, W. H., Elshahed, M. S., Zhou, X., Luo, Y., & Krumholz, L. R. (2011). Effect of warming and drought on grassland microbial communities. *ISME Journal*, *5*(10), 1692–1700. https://doi.org/10.1038/ismej.2011.32
Sigurdsson, B. D., Leblans, N. I. W., Dauwe, S., Gudmundsdóttir, E., Gundersen, P., Gunnarsdóttir, G. E., Holmstrup, M., Ilieva-Makulec, K., Kätterer, T., Marteinsdóttir, B., Maljanen, M., Oddsdóttir, E. S., Ostonen, I., Peñuelas, J., Poeplau, C., Richter, A., Sigurdsson, P., Van Bodegom, P., Wallander, H., … Janssens, I. (2016). Geothermal ecosystems as natural climate change experiments: The ForHot research site in Iceland as a case study. *Icelandic Agricultural Sciences*, *29*(1), 53–71. https://doi.org/10.16886/IAS.2016.05

Singh, S., Mayes, M. A., Kivlin, S. N., & Jagadamma, S. (2023). How the Birch effect differs in mechanisms and magnitudes due to soil texture. *Soil Biology and Biochemistry*, *179*, 108973. https://doi.org/10.1016/J.SOILBIO.2023.108973

Sinsabaugh, R. L., Moorhead, D. L., Xu, X., & Litvak, M. E. (2017). Plant, microbial and ecosystem carbon use efficiencies interact to stabilize microbial growth as a fraction of gross primary production. *New Phytologist*, *214*(4), 1518–1526. https://doi.org/10.1111/nph.14485

Skrzypek, G., Kałużny, A., & Jędrysek, M. O. (2007). Carbon Stable Isotope Analyses of Mosses-Comparisons of Bulk Organic Matter and Extracted Nitrocellulose. *Journal of the American Society for Mass Spectrometry*, *18*(8), 1453–1458. https://doi.org/10.1016/j.jasms.2007.04.020

Smith, N. G., & Dukes, J. S. (2013). Plant respiration and photosynthesis in global-scale models: Incorporating acclimation to temperature and CO2. *Global Change Biology*, *19*(1), 45–63. https://doi.org/10.1111/j.1365-2486.2012.02797.x

Sohlenkamp, C., & Geiger, O. (2015). Bacterial membrane lipids: Diversity in structures and pathways. *FEMS Microbiology Reviews*, *40*(1), 133–159. https://doi.org/10.1093/femsre/fuv008

Söllinger, A., Ahlers, L. S., Dahl, M. B., Sigurosson, P., Le Noir De Carlan, C., Bhattarai, B., Gall, C., Martin, V. S., Rottensteiner, C., Motleleng, L. L., Breines, E. M., Verbruggen, E., Ostonen, I., Sigurdsson, B. D., Richter, A., & Tveit, A. T. (2024). Microorganisms in subarctic soils are depleted of ribosomes under short-, medium-, and long-term warming. *ISME Journal*, *18*(1). https://doi.org/10.1093/ismejo/wrae081

Söllinger, A., Séneca, J., Dahl, M. B., Motleleng, L. L., Prommer, J., Verbruggen, E., Sigurdsson, B. D., Janssens, I., Peñuelas, J., Urich, T., Richter, A., & Tveit, A. T. (2022). Down-regulation of the bacterial protein biosynthesis machinery in response to weeks, years, and decades of soil warming. *Science Advances*, *8*(12). https://doi.org/10.1126/sciadv.abm3230

Soong, J. L., Phillips, C. L., Ledna, C., Koven, C. D., & Torn, M. S. (2020). CMIP5 Models Predict Rapid and Deep Soil Warming Over the 21st Century. *Journal of Geophysical Research: Biogeosciences*, *125*(2). https://doi.org/10.1029/2019JG005266

Soudzilovskaia, N. A., van Bodegom, P. M., Terrer, C., Zelfde, M. van't, McCallum, I., Luke McCormack, M., Fisher, J. B., Brundrett, M. C., de Sá, N. C., & Tedersoo, L. (2019). Global mycorrhizal plant distribution linked to terrestrial carbon stocks. *Nature Communications*, *10*(1), 1–10. https://doi.org/10.1038/s41467-019-13019-2

Stewart, G. R., Turnbull, M. H., Schmidt, S., & Erskine, P. D. (1995). 13C natural abundance in plant communities along a rainfall gradient: A biological integrator of water availability. *Australian Journal of Plant Physiology*, *22*(1), 51–55. https://doi.org/10.1071/PP9950051

Strömgren, M. (2001). Soil-surface CO2 flux and growth in a boreal Norway spruce stand.

Stuecker, M. F., Bitz, C. M., Armour, K. C., Proistosescu, C., Kang, S. M., Xie, S. P., Kim, D., McGregor, S., Zhang, W., Zhao, S., Cai, W., Dong, Y., & Jin, F. F. (2018). Polar amplification dominated by local forcing and feedbacks. *Nature Climate Change*, *8*(12), 1076–1081. https://doi.org/10.1038/s41558-018-0339-y

Swanson, D. K., Lacelle, B., & Tarnocai, C. (2000). Temperature and the boreal-subarctic maximum in soil organic carbon. *Geographie Physique et Quaternaire*, *54*(2), 157–167. https://doi.org/10.7202/004874ar

Swift, R. S. (2001). SEQUESTRATION OF CARBON BY SOIL. *Soil Science*, *166*(11).
Takriti, M., Wild, B., Schnecker, J., Mooshammer, M., Knoltsch, A., Lashchinskiy, N., Eloy Alves, R. J., Gentsch, N., Gittel, A., Mikutta, R., Wanek, W., & Richter, A. (2018). Soil organic matter quality exerts a stronger control than stoichiometry on microbial substrate use efficiency along a latitudinal transect. *Soil Biology and Biochemistry*, *121*, 212–220. https://doi.org/10.1016/j.soilbio.2018.02.022
Van De Vossenberg, J. L. C. M., Driessen, A. J. M., Da Costa, M. S., & Konings, W. N. (1999). Homeostasis of the membrane proton permeability in Bacillus subtilis grown at di¡erent temperatures. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1419*(1), 97–104. www.elsevier.com/locate/bba
Van De Vossenberg, J. L. C. M., Ubbink-Kok, T., Elferink, M. G. L., Driessen, A. J. M., & Konings, W. N. (1995). Ion permeability of the cytoplasmic membrane limits the maximum growth temperature of bacteria and archaea. *Molecular Microbiology*, *18*(5), 925–932. https://doi.org/10.1111/j.1365-2958.1995.18050925.x
Van Loock, S. (2017). Short-term and long-term changes of vegetation biomass in response to natural soil warming and nitrogen availability in a subarctic grassland. Universiteit Antwerpen.
Verbrigghe, N., Meeran, K., Bahn, M., Canarini, A., Fransen, E., Fuchslueger, L., Ingrisch, J., Janssens, I. A., Richter, A., Sigurdsson, B. D., Soong, J. L., & Vicca, S. (2022). Long-term warming reduced microbial biomass but increased recent plant-derived C in microbes of a subarctic grassland. *Soil Biology and Biochemistry*, *167*. https://doi.org/10.1016/j.soilbio.2022.108590
Waghmode, T. R., Chen, S., Li, J., Sun, R., Liu, B., & Hu, C. (2018). Response of nitrifier and denitrifier abundance and microbial community structure to experimental warming in an agricultural ecosystem. *Frontiers in Microbiology*, *9*(MAR), 3389. https://doi.org/10.3389/fmicb.2018.00474
Walker, T. N., Kaiser, C., Strasser, F., Herbold, C. W., Leblans, N., 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/s415580180259x
Wallenstein, M., Allison, S. D., Ernakovich, J., Steinweg, J. M., & Sinsabaugh, R. (2010). *Controls on the Temperature Sensitivity of Soil Enzymes: A Key Driver of In Situ Enzyme Activity Rates*. 245–258. https://doi.org/10.1007/978-3-642-14225-3_13
Wang, B., & Qiu, Y. L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*, *16*(5), 299–363. https://doi.org/10.1007/s00572-005-0033-6
Wang, C., & Kuzyakov, Y. (2023). Energy use efficiency of soil microorganisms: Driven by carbon recycling and reduction. *Global Change Biology*, *29*(22), 6170–6187. https://doi.org/10.1111/gcb.16925
Wang, D., Wang, S., Du, X., He, Q., Liu, Y., Wang, Z., Feng, K., Li, Y., & Deng, Y. (2022). ddPCR surpasses classical qPCR technology in quantitating bacteria and fungi in the environment. *Molecular Ecology Resources*, *22*(7), 2587–2598. https://doi.org/10.1111/1755-0998.13644
Wang, N., Quesada, B., Xia, L., Butterbach-Bahl, K., Goodale, C. L., & Kiese, R. (2019). Effects of climate warming on carbon fluxes in grasslands— A global meta-analysis. *Global Change Biology*, *25*(5), 1839–1851. https://doi.org/10.1111/gcb.14603

Wang, Y., & Wooller, M. J. (2006). The stable isotopic (C and N) composition of modern plants and lichens from northern Iceland: with ecological and paleoenvironmental implications. *Jökull*, *56*(1), 27–37. https://doi.org/10.33799/jokull2006.56.027

Warren, C. R. (2016). Do microbial osmolytes or extracellular depolymerisation products accumulate as soil dries? *Soil Biology and Biochemistry*, *98*, 54–63. https://doi.org/10.1016/j.soilbio.2016.03.021

Weedon, J. T., Bååth, E., Rijkers, R., Reischke, S., Sigurdsson, B. D., Oddsdottir, E., Hal, van, Aerts, R., Janssens, I. A., & Bodegom, van. (2023). Community adaptation to temperature explains abrupt soil bacterial community shift along a geothermal gradient on Iceland. *Soil Biology and Biochemistry*, *177*, 108914. https://doi.org/https://doi.org/10.1016/j.soilbio.2022.108914

Wegener, G., Kellermann, M. Y., & Elvert, M. (2016). Tracking activity and function of microorganisms by stable isotope probing of membrane lipids. *Current Opinion in Biotechnology*, *41*, 43–52. https://doi.org/10.1016/j.copbio.2016.04.022

Wellington, E. M. H., Berry, A., & Krsek, M. (2003). Resolving functional diversity in relation to microbial community structure in soil: Exploiting genomics and stable isotope probing. *Current Opinion in Microbiology*, *6*(3), 295–301. https://doi.org/10.1016/S1369-5274(03)00066-3

Werth, M., & Kuzyakov, Y. (2010). 13C fractionation at the root-microorganisms-soil interface: A review and outlook for partitioning studies. *Soil Biology and Biochemistry*, *42*(9), 1372–1384. https://doi.org/10.1016/j.soilbio.2010.04.009

Wetang'ula, G. N., & Snorrason, S. S. (2005). Evaluation of Trace Element Levels and their Ecotoxicological Relevance in Geothermal Wastewater of Olkaria East Field, Kenya. In *Proceedings World Geothermal Congress*.

Wieder, W. R., Bonan, G. B., & Allison, S. D. (2013). Global soil carbon projections are improved by modelling microbial processes. *Nature Climate Change 2013 3:10*, *3*(10), 909–912. https://doi.org/10.1038/nclimate1951

Willis, A., Rodrigues, B. F., & Harris, P. J. C. (2013). The Ecology of Arbuscular Mycorrhizal Fungi. *Critical Reviews in Plant Sciences*, *32*(1), 1–20. https://doi.org/10.1080/07352689.2012.683375

Wilson, G. W. T., Rice, C. W., Rillig, M. C., Springer, A., & Hartnett, D. C. (2009). Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: Results from long-term field experiments. *Ecology Letters*, *12*(5), 452–461. https://doi.org/10.1111/j.1461-0248.2009.01303.x

Wixon, D. L., & Balser, T. C. (2013). Toward conceptual clarity: PLFA in warmed soils. *Soil Biology and Biochemistry*, *57*, 769–774. https://doi.org/10.1016/J.SOILBIO.2012.08.016

Wu, G. L., Cheng, Z., Alatalo, J. M., Zhao, J., & Liu, Y. (2021). Climate Warming Consistently Reduces Grassland Ecosystem Productivity. *Earth's Future*, *9*(6), 1–14. https://doi.org/10.1029/2020EF001837

Yang, H., Yuan, Y., Zhang, Q., Tang, J., Liu, Y., & Chen, X. (2011). Changes in soil organic carbon, total nitrogen, and abundance of arbuscular mycorrhizal fungi along a large-scale aridity gradient. *Catena*, *87*(1), 70–77. https://doi.org/10.1016/j.catena.2011.05.009

Ye, J.-S., Bradford, M. A., Dacal, M., Maestre, F. T., & García-Palacios, P. (2019). Increasing microbial carbon use efficiency with warming predicts soil heterotrophic

respiration globally. *Global Change Biology*, *25*(10), 3354–3364. https://doi.org/https://doi.org/10.1111/gcb.14738

Yoon, Y., Lee, H., Lee, S., Kim, S., & Choi, K. H. (2015). Membrane fluidity-related adaptive response mechanisms of foodborne bacterial pathogens under environmental stresses. *Food Research International*, *72*, 25–36. https://doi.org/10.1016/j.foodres.2015.03.016

Yukawa, T., Ogura-Tsujita, Y., Shefferson, R. P., & Yokoyama, J. (2009). Mycorrhizal diversity in Apostasia (Orchidaceae)indicates the origin and evolution of orchid mycorrhiza. *American Journal of Botany*, *96*(11), 1997–2009. https://doi.org/10.3732/ajb.0900101

Zengpu, L., Junran, J., & Changwen, W. (1994). Antagonism Between Ectomycorrhizal Fungi and Plant Pathogens. *Mycorrhizas for Plantation Forestry in Asia*.

Zhang, Y., Zheng, N., Wang, J., Yao, H., Qiu, Q., & Chapman, S. J. (2019). High turnover rate of free phospholipids in soil confirms the classic hypothesis of PLFA methodology. *Soil Biology and Biochemistry*, *135*, 323–330. https://doi.org/https://doi.org/10.1016/j.soilbio.2019.05.023

Zhang, Y., Zou, J., Meng, D., Dang, S., Zhou, J., Osborne, B., Ren, Y., Liang, T., & Yu, K. (2020). Effect of soil microorganisms and labile C availability on soil respiration in response to litter inputs in forest ecosystems: A meta-analysis. *Ecology and Evolution*, *10*(24), 13602–13612. https://doi.org/10.1002/ece3.6965

Zhang, Z., Wang, D., & Li, M. (2022). Soil respiration, aggregate stability and nutrient availability affected by drying duration and drying-rewetting frequency. *Geoderma*, *413*(35), 115743. https://doi.org/10.1016/j.geoderma.2022.115743

Zhao, J., Xie, X., Jiang, Y., Li, J., Fu, Q., Qiu, Y., Fu, X., Yao, Z., Dai, Z., Qiu, Y., & Chen, H. (2024). Effects of simulated warming on soil microbial community diversity and composition across diverse ecosystems. *Science of the Total Environment*, *911*(September 2023). https://doi.org/10.1016/j.scitotenv.2023.168793

Zheng, T., Miltner, A., Liang, C., Nowak, K. M., & Kästner, M. (2021). Turnover of gram-negative bacterial biomass-derived carbon through the microbial food web of an agricultural soil. *Soil Biology and Biochemistry*, *152*(January 2020). https://doi.org/10.1016/j.soilbio.2020.108070

Zhou, J., Liu, C., Shi, L., & Zamanian, K. (2024). Rhizosphere influence on microbial functions: consequence for temperature sensitivity of soil organic matter decomposition at early stage of plant growth. *Plant and Soil*, *494*(1–2), 95–109. https://doi.org/10.1007/s11104-023-06258-2

Zhou, Y. C., Fan, J. W., Zhong, H. P., & Zhang, W. Y. (2013). Relationships between altitudinal gradient and plant carbon isotope composition of grassland communities on the Qinghai-Tibet Plateau, China. *Science China Earth Sciences*, *56*(2), 311–320. https://doi.org/10.1007/s11430-012-4498-9

Zhu, B., & Cheng, W. (2011). Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. *Global Change Biology*, *17*(6), 2172–2183. https://doi.org/10.1111/j.1365-2486.2010.02354.x

Zimov, S. A., Schuur, E. A. G., Iii, F. S. C., Zimov, S. A., Schuur, E. A. G., & Chapin, F. S. (2006). *Permafrost and the Global Carbon Budget Linked*. *312*(5780), 1612–1613.