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Decreased growth of wild soil microbes after 15 years of transplant-induced warming in a montane meadow

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Abstract

The carbon stored in soil exceeds that of plant biomass and atmospheric carbon and its stability can impact global climate. Growth of decomposer microorganisms mediates both the accrual and loss of soil carbon. Growth is sensitive to temperature and given the vast biological diversity of soil microorganisms, the response of decomposer growth rates to warming may be strongly idiosyncratic, varying among taxa, making ecosystem predictions difficult. Here, we show that 15 years of warming by transplanting plant–soil mesocosms down in elevation, strongly reduced the growth rates of soil microorganisms, measured in the field using undisturbed soil. The magnitude of the response to warming varied among microbial taxa. However, the direction of the response—reduced growth—was universal and warming explained twofold more variation than did the sum of taxonomic identity and its interaction with warming. For this ecosystem, most of the growth responses to warming could be explained without taxon-specific information, suggesting that in some cases microbial responses measured in aggregate may be adequate for climate modeling. Long-term experimental warming also reduced soil carbon content, likely a consequence of a warming-induced increase in decomposition, as warming-induced changes in plant productivity were negligible. The loss of soil carbon and decreased microbial biomass with warming may explain the reduced growth of the microbial community, more than the direct effects of temperature on growth. These findings show that direct and indirect effects of long-term warming can reduce growth rates of soil microbes, which may have important feedbacks to global warming.

KEYWORDS
field qSIP, soil microbe response to ecosystem warming

1 | INTRODUCTION

Microbes influence carbon storage in soil, simultaneously reducing carbon through respiration, while contributing to its accrual and stabilization by producing new biomass and extracellular products (Bardgett et al., 2008; Liang et al., 2017; Schimel & Schaeffer, 2012). Warming could affect the balance of these processes, causing soils to store or release sufficient carbon to influence the pace of climate change (Davidson & Janssens, 2006; Melillo et al., 2017), a feedback between climate and carbon that has considerable uncertainty in earth system models (Bradford et al., 2016; Luo et al., 2016; Wieder et al., 2013). Modeling how microorganisms react to changes in temperature currently rests on empirical observations of the aggregated microbial community (Davidson et al., 2006; Sinsabaugh et al., 2013), yet these responses can be inconsistent, contributing to the uncertainty around the climate carbon feedback. For example, experimental warming often increases soil respiration (Carey et al., 2016; Rustad et al., 2001; Schindlbacher et al., 2011), though this effect can attenuate over time (Bradford et al., 2008; Melillo et al., 2017; Romero-Olivares et al., 2017). Warming can reduce bacterial abundance (Allison et al., 2010; Bradford et al., 2008; Melillo et al., 2017; Vanhala et al., 2011), increase it (Bell et al., 2010; Zhou et al., 2012), or have no effect (DeAngelis et al., 2015; Schindlbacher et al., 2011). Averaged across 25 experiments, warming had no significant effect on bacterial biomass over time, though considerable variation was observed (Romero-Olivares et al., 2017). Similarly, nitrogen mineralization generally increases with warming, but these findings also vary greatly by study and ecosystem (Bai et al., 2013; Rustad et al., 2001). It is possible that microbial biodiversity contributes to variation in the warming effect. Testing this idea is challenging because measures of microbial processes aggregate the responses of thousands of individual microbial populations that grow, respire, and expire at different rates (Blazewicz et al., 2020; Koch et al., 2018; Morrissey et al., 2019; Papp et al., 2018). Knowing responses of individual populations to temperature could help reduce uncertainty in the climate-carbon feedback, an idea that has motivated several recent proposals to incorporate microbial physiology, activity, and diversity into models (Delgado-Baquerizo et al., 2016; Wang et al., 2017; Wieder et al., 2013).

While temperature can influence soil microbial community composition (Zhou et al., 2016), coupling warming effects on the microbial community to soil carbon balance remains challenging due to the complexity and diversity of the underlying community (Delgado-Baquerizo et al., 2018; Fierer & Jackson, 2006; Torsvik & Øvreås, 2002), lack of quantitative measures of individual populations, and dynamic effects of warming duration (Melillo et al., 2017). Soil warming experiments have detected changes in microbial populations via 16S rRNA gene relative abundance, phospholipid fatty acid (PLFA) profiles, and fatty acid methyl esters (FAME) analysis (DeAngelis et al., 2015; Frey et al., 2008; Melillo et al., 2017; Oliverio et al., 2017; Yu et al., 2018; Zhang et al., 2005). However, if the goal is to understand the process rates that impact carbon in soils, measures of microbial community composition alone may be insufficient (Schimel & Schaeffer, 2012). Furthermore, whereas most measurements of microbial activity occur in the laboratory under disturbed conditions (e.g., sieved, roots removed), our understanding of soil responses to warming will advance from measurements of microorganisms in the field, where they experience the environmental conditions and biological interactions characteristic of their habitats. Responses to experimental treatments, including warming, are more likely to be predictive of actual global change if microbial taxa are measured in intact assemblages, experiencing field conditions.

Here, we measured taxon-specific microbial growth in a subalpine meadow in northern Arizona (Figure S1) that had been subject to 15 years of experimental warming (Blankinship et al., 2010). The long-term warming experiment used intact plant–soil mesocosms and a transplant design, whereby a set of intact mesocosms were transplanted to a lower elevation (i.e., simulating warmer conditions), while the other set was transplanted at the same elevation to serve as controls, hereafter referred to "warmed" and "control," respectively. While transplanting often alters multiple environmental factors, not just temperature (Link et al., 2003; Waldrop & Firestone, 2006; Zumsteg et al., 2013), we manipulated precipitation to match control plots, allowing us to isolate the effect of climate warming and warming-induced changes to the soil environment. Mean annual air temperature was 3.4 ± 0.89°C warmer in transplants than controls from the onset of the transplant experiment in 2002 to this microbial growth study in 2017. This magnitude of warming corresponds to projected warming above pre-industrial levels for the middle of the 21st century (Masson-Delmotte et al., 2018). We used field quantitative stable isotope probing (qSIP) with ^{18}O-labeled water to quantify microbial growth. In this study, we refer to "microbes" as bacteria and archaea. This qSIP method measures the rate at which oxygen in water is assimilated into newly synthesized DNA (Hungate et al., 2015). ^{18}O enrichment of a microbial taxon's DNA is therefore a proxy for growth because oxygen from water is ultimately used for nucleic acid synthesis (Aanderud & Lennon, 2011; Schwartz, 2007). The ^{18}O-H_2O tracer was introduced to surface soil, simulating a modest rain event, and soils were collected 3 days later for DNA and isotopic analysis such that the period of growth measured occurred under field conditions, with minimal environmental soil disturbance (Figure S2). As far as we know, our study is the first to report field growth measurements of "wild" microbes.

2 | MATERIALS AND METHODS

2.1 | Site and transplant experiment description

This experiment took place within an existing plant–soil transplant experiment established in 2002 (Blankinship et al., 2010) along the C. Hart Merriam Elevation gradient located just north of Flagstaff, Arizona in only the highest elevation site (mixed conifer meadow) and its transplant to the warmer ponderosa pine meadow. Briefly, in 2002, 30 cm diameter and 30 cm long plant–soil mesocosms were retrieved via a hardened steel corer from the mixed conifer meadow

TABLE 1 Mean measurements during the 3-day field quantitative stable isotope probing (qSIP) warming experiment of an Arizona meadow

Site	Mean air temperature (°C)***	Mean soil temperature 5 cm (°C)**	Mean soil moisture (%) NS	Mean soil pH**	Mean bacterial 16S rRNA gene copies × 10^{11} g dry $soil^{-1}$*	Mean Archaeal 16S rRNA gene copies × 10^{9} g dry $soil^{-1}$**
Control	12.2 ± 0.5	14.1 ± 0.4	17.7 ± 1.9	6.34 ± 0.06	3.5 ± 0.2	4.3 ± 0.5
Warmed	15 ± 0.5	15.5 ± 0.3	18.6 ± 4	6.52 ± 0.03	1.4 ± 0.6	2.3 ± 0.4

Note: Mean air and soil temperature at 5 cm depth were obtained from weather stations of the Merriam Powell Center for Environmental Research at NAU that recorded temperature every hour. Soil moisture was measured from soil collected from the plant-soil transplant mesocosm, outside of each field qSIP microcosm core. Bacterial and archaeal 16S rRNA gene copies were measured using quantitative PCR. 16S rRNA gene abundances were measured in triplicate from 5 and 4 microcosm cores for the control and warmed microcosm cores, respectively. Asterisks represent significance from an unpaired *t* test: NS, not significant;
*$p < .05$; **$p < .01$; ***$p < .001$.

and transplanted nearby in the same site and at the next lowest elevation site. These plant–soil mesocosms from the 2002 experiment are hereafter referred to as "transplant mesocosms." The transplant mesocosms that remained in the highest ecosystem, the mixed conifer meadow are hereafter referred to as "control" and the mesocosms that were moved to the next lowest site, the ponderosa pine meadow are hereafter referred to as "warmed." The mixed conifer meadow (Figure S2) is at an elevation of 2620 m with a mean annual temperature of 6.7 ± 2.3°C (2002–2017) and a mean annual soil temperature at 5 cm of 7.6 ± 1.4°C (2009–2017; soil temperature data were not available for the period 2002–2008 because of sensor errors). Dominant herbaceous plants in the mixed conifer meadow include *Muhlenbergia montana*, *Festuca arizonica*, and *Blepharoneuron tricholepsis* (Blankinship et al., 2010). The ponderosa pine meadow is at an elevation of 2344 m with a mean annual temperature of 10.2 ± 2.6°C (2002–2017) and a mean annual soil temperature at 5 cm of 9.7 ± 1.9°C (2009–2017). The plant types at the ponderosa pine meadow include *Aristida purpurea*, *Bouteloua gracilis*, and *Festuca arizonica* (Blankinship et al., 2010). The soil type in this study was Pachic Udic Argiboroll with a loam texture (Blankinship et al., 2010). After 9 years of ecosystem warming, these soils had no significant differences in extractable carbon, but they did have a significant increase in extractable nitrogen (Mau et al., 2018).

To compensate for lower annual precipitation at the warmer site (Ponderosa), rain collector funnels provided 20% supplemental precipitation, an amount calibrated to simulate native ecosystem precipitation events (Blankinship et al., 2010). Precipitation events were concurrent between native and transplanted plots, with a 2.6% deviation in cumulative precipitation from 2011 to 2017 (Figure S3). Consistent with this, soil moisture content in the native and transplanted mesocosms did not differ when our qSIP measurements began (Table 1). In contrast, average monthly soil temperatures were higher at the transplant, warmed site (Ponderosa) compared with the native site (Figure S4) with an average soil temperature increase of 2.1 ± 0.78°C.

2.2 | Intact core field microcosm experiment description

This field qSIP microcosm experiment took place May 26–29, 2017. Aluminum sleeves (2.5 cm diameter by 6 cm), here referred to as "microcosm cores," were pushed into random locations (plant interspaces) within each transplant mesocosm (Figure S2). Per transplant mesocosm, one field qSIP microcosm core received 3 ml of natural abundance ^{18}O water and one received 3 ml of 97.4 at% enriched ^{18}O water to the top 3 cm with a double side-port needle for even distribution. This was performed in five and four replicate transplant mesocosms at the mixed conifer and ponderosa pine sites, respectively. The field qSIP microcosm cores were left in place for 3 days to incubate in situ. The microcosm cores were removed, placed in sterile Whirl-Pak sample bags, flash frozen in liquid nitrogen, and stored at −80°C.

2.3 | Mean annual air and soil temperature and precipitation data

Daily precipitation and air and soil temperature data at 5 cm depth were obtained from weather stations maintained by the Merriam Powell Center for Environmental Research at Northern Arizona University (https://knb.ecoinformatics.org/view/paullheinrich.26.2). The MAT for soil at 5 cm was obtained between 2011 and 2017 and the air MAT was obtained between 2002 and 2017. Daily precipitation was obtained from January 2011 to May 2017.

2.4 | DNA extraction

The soil cores were thawed, and the top 3 cm were removed and homogenized for DNA extraction. DNA from 3 to 6 g of soil was extracted using the DNeasy PowerMax Soil Kit (Qiagen) following the kit protocol with minor adjustments. The bead tube with soil and directed solutions were vortexed on max power for 30 min as opposed to the 10 min as written in the manufacturer's protocol. The entire volume of each supernatant was transferred to the following step and solution C6 was heated to 65°C before DNA elution. The C6 solution was added to the column 1 ml at a time for a total of 3 ml, with a 5 min wait time between elutions to concentrate the DNA into a smaller volume as opposed to the 5 ml direction of the kit protocol. DNA concentration was measured using a NanoDrop Fluorometer (Thermoscientific).

Ultracentrifugation and DNA density separation: Five μg of DNA from each sample, 600 μl gradient buffer (Hungate et al., 2015), and 2.5 ml of saturated CsCl (density 1.9 g/ml) were added to a 3.3 ml OptiSeal ultracentrifuge tube (Beckman Coulter, Inc). The tubes were capped and inverted 5× and centrifuged in an Optima Max benchtop ultracentrifuge, using a Beckman TLN-100 rotor (Beckman Coulter, Inc) at 127,000 *g* (60,000 rpm) at 18°C for 72 h. Per sample, 22 DNA density fractions of volume 150 μl were collected, purified, and quantified as previously described (Hungate et al., 2015; Purcell et al., 2020).

2.5 | Quantitative PCR

qPCR was performed on each fraction and whole DNA (non-fractionated) sample using BioRad CFX-384 thermal cycler. Standards were prepared by amplifying DNA extracted from soil in this study using bacterial (EUB338F/EUB518R; Fierer et al., 2005) and archaeal (ARC915F/ARC1059R; Takai & Horikoshi, 2000; Yu et al., 2005) 16S rRNA gene primer sets. Triplicate reactions of 10 μl were quantified using the following reaction mix: 1X Forget me Not (Biotium), 0.2 μM each primer, 1 ng template, and remaining volume of molecular grade water. The following thermal cycling protocol was used for quantifying total bacterial 16S rRNA gene copies: 95°C for 2 min and 40 cycles of 95°C for 5 s, 59°C for 10 s, 72°C for 10 s, then a melt curve of 0.5°C intervals, 30 s each, 55–95°C to determine product specificity (Purcell et al., 2020). The following thermal cycling protocol was used for quantifying total archaeal 16S rRNA gene copies: 95°C for 2 min and 40 cycles of 95°C for 5 s, 62°C for 10 s, 72°C for 10 s, then a melt curve of 0.5°C intervals of 30 s from 55 to 95°C to determine product specificity (Purcell et al., 2020). A standard curve of 10^1–10^9 gene copies was included along with each plate. Data were used for further analysis if the R^2 value of the standard curve was ≥.97, amplification efficiency was 90%–110%, and the melt curve analysis revealed zero nonspecific product amplifications.

2.6 | 16S rRNA gene amplicon sequencing

16S rRNA gene libraries were prepared using a two-step PCR amplification method as previously described on each fraction and whole DNA (unfractionated) sample (Purcell et al., 2020). Sequencing primers used were 515FB/806RB that amplify the V4-V5 region (Apprill et al., 2015; Caporaso et al., 2011; Parada et al., 2016). All qSIP DNA fractions within the density range 1.646–1.723 (g/ml) were sequenced, capturing the entire DNA density curve. The DNA in each fraction was cleaned using isopropanol precipitation with glycogen. After normalizing amplicon DNA concentration, they were pooled and sequenced (2 × 150 bp pair-ended chemistry). Sequencing took place on an Illumina MiSeq platform at the Environmental Genetics and Genomics (EnGGEN) laboratory at Northern Arizona University, Flagstaff, Arizona (nau.edu/enggen).

2.7 | Bioinformatics analysis of 16S rRNA gene amplicons

Sequences were imported into QIIME2 (Bolyen et al., 2019), demultiplexed, and denoised using DADA2 (Callahan et al., 2016). Taxonomy was assigned using the SILVA 132 database (Quast et al., 2012) and QIIME2 pretrained classifier (Bokulich et al., 2018). The QIIME2 core diversity metrics pipeline was used to explore and visualize beta diversity measures of the whole community sequenced experimental replicates (Bolyen et al., 2019; Vázquez-Baeza et al., 2013; Figure S5). A feature table containing sequence reads of all ASVs for each qSIP fraction was exported from QIIME2 and then imported into R for further analysis. Relative abundance of a taxon was calculated as the mean relative abundance across all replicates in a treatment using the whole DNA sample (non-fractionated) samples.

2.8 | Determination of taxon-specific ^{18}O enrichment and relative growth rate

Example calculations and qSIP code to calculate taxon isotopic enrichment have been previously published (Finley et al., 2019; Hungate et al., 2015; Koch et al., 2018; Purcell et al., 2020). Laboratory data (including density, DNA concentration, bacterial

16S rRNA gene copies, and archaeal 16S rRNA gene copies for each qSIP fraction), taxonomic metadata, and the QIIME2 exported feature table were all imported into R. Previously developed code was utilized to calculate taxon isotopic enrichment (https://bitbucket.org/qsip/). This calculates the change in weighted average density for each taxon between the two isotope treatments, then the molecular weight change, and ultimately excess atom fraction calculated using 1000 bootstrapped iterations (Hungate et al., 2015; Koch et al., 2018; Purcell et al., 2020). Criteria for including taxa in the analysis included the following: (1) a taxon had to be present in the non-fractionated qSIP whole DNA sequenced samples and (2) a taxon had to be present in at least three qSIP fractions of two replicates of both natural abundance and heavy ^{18}O enriched water treatments per control or warmed treatment. We estimated taxon relative growth rate (RGR) as a function of the rate of ^{18}O assimilation into DNA (measured by study end point taxon ^{18}O excess atom fraction), assuming 60% of oxygen atoms in DNA were derived from water (Koch et al., 2018), assuming populations are at a steady state, and using measured ^{18}O soil water enrichment (Table S3). Taxon relative growth rate (RGR) = $^{18}O\text{-}EAF_{taxon}$/[(average soil water ^{18}O enrichment during 3-day study)*(0.6)*3 days]. RGR captures variation among taxa in the proportion of DNA that was newly synthesized during the incubation, and thus is an estimate of relative growth rate for individual microbial taxa (Li et al., 2019). For family-level RGR differences, a family had to have at least three members and RGR was calculated for each taxon in the family using their 1000 iterations of bootstrapped excess atom fraction difference.

2.9 | Soil water ^{18}O enrichment

The soil water ^{18}O enrichment was determined on an isotope ratio mass spectrometer (IRMS) for each soil sample that received either natural abundance water or water enriched in the heavy isotope of oxygen ($H_2{}^{18}O$). One gram of wet soil from each sample was homogenized by vortexing with 5 ml of nanopure water and was shaken at room temperature for 7 days at 200 rpm to allow oxygen isotope exchange. The soil-water slurry was centrifuged at 11,000 *g* for 10 min and 2 ml of the water supernatant without visible soil particles was analyzed on the IRMS for ^{18}O content. A mixing model was used to determine the qSIP soil incubation water ^{18}O enrichment (Table S1).

2.10 | Soil property measurements

Total soil carbon and nitrogen, pH, and gravimetric moisture content were measured from the same soils thawed from −80°C storage for DNA extraction (homogenized soil from the top 3 cm of each field qSIP microcosm core). For C, N, and gravimetric moisture content, quadruplicate 1 g of soil per treatment was dried at 105°C for 24 h. The soils for measuring total carbon and nitrogen were weighed and analyzed in quadruplicate per treatment with standards on an isotope ratio mass spectrometer (IRMS) at the Colorado Plateau Stable Isotope Lab at Northern Arizona University. To measure soil pH, 1 g of wet weight soil from each plot (n = 5 and 4 for the control and warmed, respectively) was added to 3 ml deionized water, homogenized and left at room temperature for 30 min prior to measurement with an electrode.

2.11 | Statistical analyses

All statistical analyses were performed in R version 3.5.2 (Team, 2018) and R studio version 1.2.5019 (Team, 2019). To test for significant differences between the two treatments for total carbon, nitrogen, relative growth rate, and bacterial and archaeal 16S rRNA gene copies, unpaired, two-sided *t*-tests were performed. For the linear regression of 16S rRNA gene relative abundance and relative growth rate, the following model was used: model <- lm(log(RelativeAbundance) ~ RGR. For the linear mixed-effects model, the lme4 package (Bates et al., 2014) in R was used to estimate the variance associated with the warming treatment, taxon identity, and their interaction for measuring the relative growth rate response as follows: lme.model <- lmer(RGR ~ 1 + (1|treatment:taxonID) + (1|treatment) + (1|taxonID), data = data). The data used were taxon growth rates represented by relative growth rate (RGR) per replicate of all shared taxa between the warmed and control treatment.

3 | RESULTS

3.1 | Impact of ecosystem warming on soil properties and the microbial community

Warming reduced total soil carbon and nitrogen content (Figure 1) and decreased microbial growth and abundance (Figure 1; Table 1). There were no significant differences in alpha diversity (Table S2). However, beta diversity using the whole microbial community data indicated that community composition was altered with warming (Figure S5; Table S3). During the tracer study, the mean soil moisture prior to $H_2{}^{18}O$ water addition at both sites was not significantly different (Table 1). Transplanted soils were slightly higher in pH (Table 1).

3.2 | Impact of ecosystem warming on individual taxa

In addition to the observed decline in growth with warming of the aggregate microbial assemblage, all taxonomic families shared between the control and warmed plots responded to warming in a uniform direction with slower growth (Figure 2). The taxa shared between the treatments represented 74.9% of the control and 92.7% of the warmed community, indicating fewer unique taxa growing in the warmed treatment. The families with the largest reduction in growth

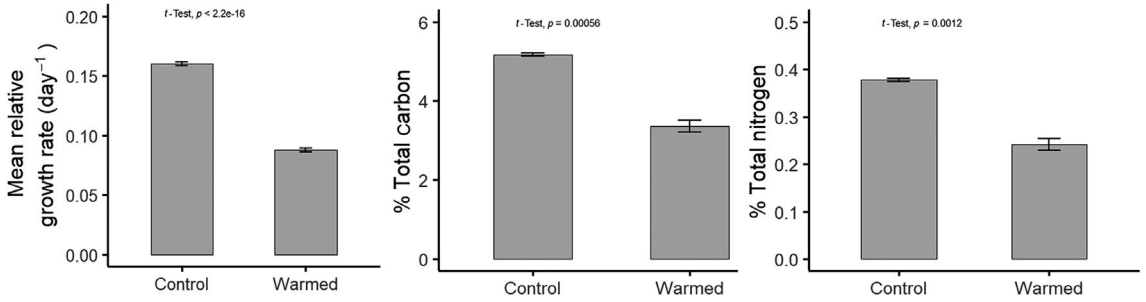
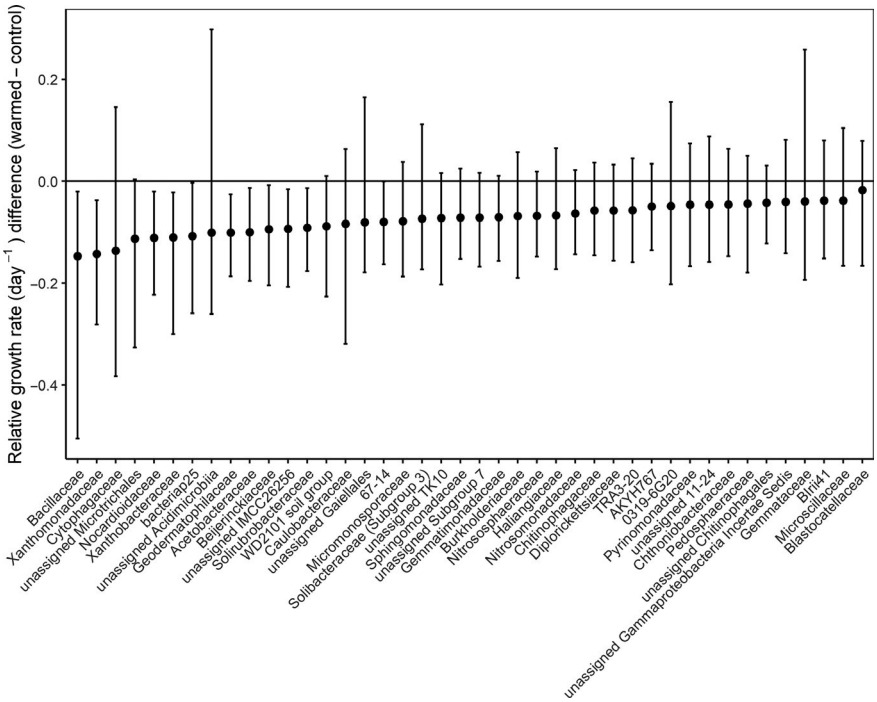


FIGURE 1 Measures of soil microbial relative growth rate (day^{-1}) and total carbon and nitrogen from the 3-day in situ field quantitative stable isotope probing (qSIP) warming experiment with ^{18}O enriched water in an Arizona meadow. The mean relative growth rate (RGR) of all taxa present in the control and warmed treatments (left panel). The mean percent of total carbon (middle panel) and nitrogen (right panel) present in the control and warmed soil qSIP microcosms. Error bars indicate standard errors and *p* values indicate *t* test results

FIGURE 2 The differences in relative growth rate (day^{-1}) of shared taxa by taxonomic family between the warmed and control soils of a 3-day in situ field quantitative stable isotope probing (qSIP) warming experiment with ^{18}O enriched water in an Arizona meadow. Each point represents the median RGR of a taxonomic family where a minimum of three taxa were represented, with 95% confidence intervals of the bootstrapped difference. Points below zero indicate slower growth rate of that family in the warmed treatment



rate with warming include Bacillaceae and Xanthomonadaceae (Figure 2). Using variance partitioning with a linear mixed-effects model, we found that the overall effect of warming explained 44.4% of the observed growth response, more than explained by taxonomic identity (4.8%) and the interaction between warming and taxonomic identity (11.7%). Thus, the reduction in growth rates of the whole microbial community in response to warming was more important than observed taxon-specific responses.

3.3 | Relationship between microbial growth and 16S rRNA gene relative abundance

The taxa that grew the fastest in both the warmed and control treatments were the rarer taxa exhibiting low 16S rRNA gene relative abundances (Figure 3). 16S rRNA gene relative abundance was negatively related to relative growth rate, where low abundance taxa tended to grow faster (Figure 3). Moreover, changes in taxonomic family 16S rRNA gene relative abundance with warming varied, where some families exhibited increases, decreases, or no change in relative abundance (Figure 4). In contrast, the warming response of family relative growth rate exhibited a uniform direction of decreased growth (Figure 4). For example, in response to warming, the *Burkholderiaceae* family had the largest increase in relative abundance with an observed decrease in relative growth rate (day^{-1}; Figure 4).

4 | DISCUSSION

The decline in microbial growth rate we observed occurred in response to 15 years of experimental treatment, which includes direct and indirect effects of warming. For example, warming altered the plant community (Wu et al., 2012), nutrient cycling (Mau et al., 2018), and pH (Table 1), all of which can influence the microbial community (Berg & Smalla, 2009; Carrillo et al., 2017; Shen et al., 2013). Effects

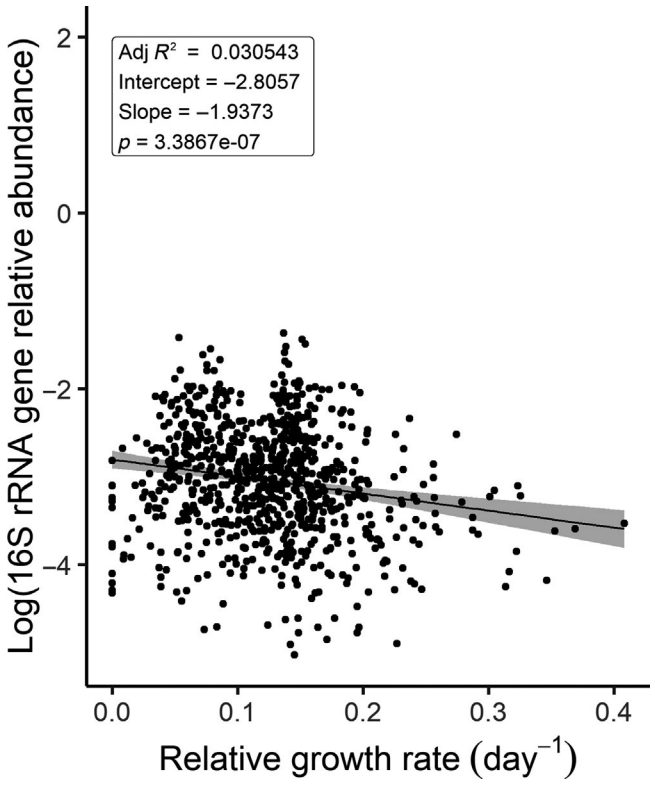


FIGURE 3 The relationship between taxon 16S rRNA gene relative abundance and relative growth rate. Points represent each taxon in either the control or warmed treatment of the 3-day in situ field quantitative stable isotope probing (qSIP) warming experiment with ^{18}O enriched water in an Arizona meadow

of warming on pH have been reported (Rousk et al., 2013; Yue et al., 2015), possibly associated with effects on decomposition and exchangeable cations (Mills et al., 2014), though the changes observed (~0.2) may be too small to have much effect on bacterial growth rates (Fernández-Calviño & Bååth, 2010). Warming frequently alters plant communities (Walther, 2010; Wu et al., 2011) and nutrient cycling (Butler et al., 2012; Melillo et al., 2011), and in this experiment, we previously showed how both direct and indirect effects of warming best explain plant growth responses over the first 10 years of the study (Wu et al., 2012). For these reasons, the reduction in microbial growth rates we observed cannot be attributed solely to the direct effects of temperature, but rather integrate both direct and indirect effects that occurred during the 15-year experiment.

A warming-induced decline in soil carbon has been suggested to explain the attenuated response of microbial respiration (Conant et al., 2011; Hartley et al., 2007; Kirschbaum, 2004; Romero-Olivares et al., 2017), decreased microbial abundance (Bradford et al., 2008; Che et al., 2018; Frey et al., 2008; Melillo et al., 2017), reduced biomass and growth (Walker et al., 2018), and restructured microbial communities (Yu et al., 2018). The uniform decrease in microbial growth rates that we measured contrasts with short-term warming responses that have been observed in laboratory settings, where whole community growth increased (as measured by the incorporation of thymidine or leucine) with temperature up to 25–30°C (Bárcenas-Moreno et al., 2009; Pietikäinen et al., 2005; Ranneklev & Bååth, 2001). We propose that warming-induced long-term shifts in community composition (DeAngelis et al., 2015; Pietikäinen et al., 2005; Vanhala et al., 2011), likely initial respiration increases (Romero-Olivares et al., 2017), declines of total soil carbon (Walker et al., 2018), led to the growth response we observed to deviate from the expectation predicted by short-term laboratory studies.

The growth rate variation associated with taxonomic identity and its interaction with warming could sort at different levels of phylogenetic rank, as seen in responses to rewetting (Schimel & Schaeffer, 2012) and along gradients of salinity (Martiny et al., 2015). In contrast, we found reduced growth in response to warming at the community level (Figure 1) and uniform reduced growth among families present in both the control and warmed treatments (Figure 2). Nevertheless, there was some variation in the magnitude of the growth response to warming among taxa. The taxa that responded most negatively to warming were common soil bacteria with cultured representatives, such as members of the families *Bacillaceae*, *Xanthobacteraceae*, and *Xanthomonadaceae* (Figure 2), with *Xanthomonadaceae* previously found to exhibit a negative response to short-term warming (Oliverio et al., 2017). A taxon classified within *Xanthomonadaceae* and the genus *Lysobacter* decreased its growth rate by 76.1%. This genus is ubiquitous in soil and includes described species that can produce antifungals against plant pathogens, degrade proteins, and produce enzymes to degrade complex carbon compounds (Kwak et al., 2015; Park et al., 2008; Qian et al., 2009). The family *Bacillaceae* slowed its growth by 87.3 (±16.7)% with warming, an ecologically widespread family notable for its endospore formation in response to environmental stress (Mandic-Mulec et al., 2015). Laboratory studies have also found taxonomic variation in the response of bacteria to warming, albeit with changes in 16S rRNA gene relative abundance, with some taxa increasing and others declining (Oliverio et al., 2017). Our relative abundance data also reveal variation in the direction of the response to warming, where some families had an increase, decrease, or no change in their relative abundance (Figure 4), contrasting with the direction of their growth rate (Figures 2 and 4). This indicates that changes in a taxon's 16S rRNA gene relative abundance do not reflect their warming-induced change in growth rate.

We also observed that rare taxa exhibited some of the fastest growth rates in both the control and warmed soils (Figure 3), indicating the importance of low abundance taxa. Low abundance taxa making up the "rare biosphere" can impact community structure and ecosystem function over space and time (Jousset et al., 2017; Shade et al., 2014), but it is still unknown whether rare taxa tend to be active or dormant. Our study gives direct evidence that rare taxa are not only active, but some of these rare taxa are the most active in the growing microbial community and rare taxa tended to grow faster (Figure 3).

Decreased total abundance (Table 1) and decreased growth (Figure 1) of the microbial community with warming likely slows the accumulation of soil carbon, as microbially derived carbon is a major source of soil organic matter (Kallenbach et al., 2016; Liang et al., 2017). The carbon loss observed may be compounded with slower carbon accumulation and further warming. Warming can reduce carbon

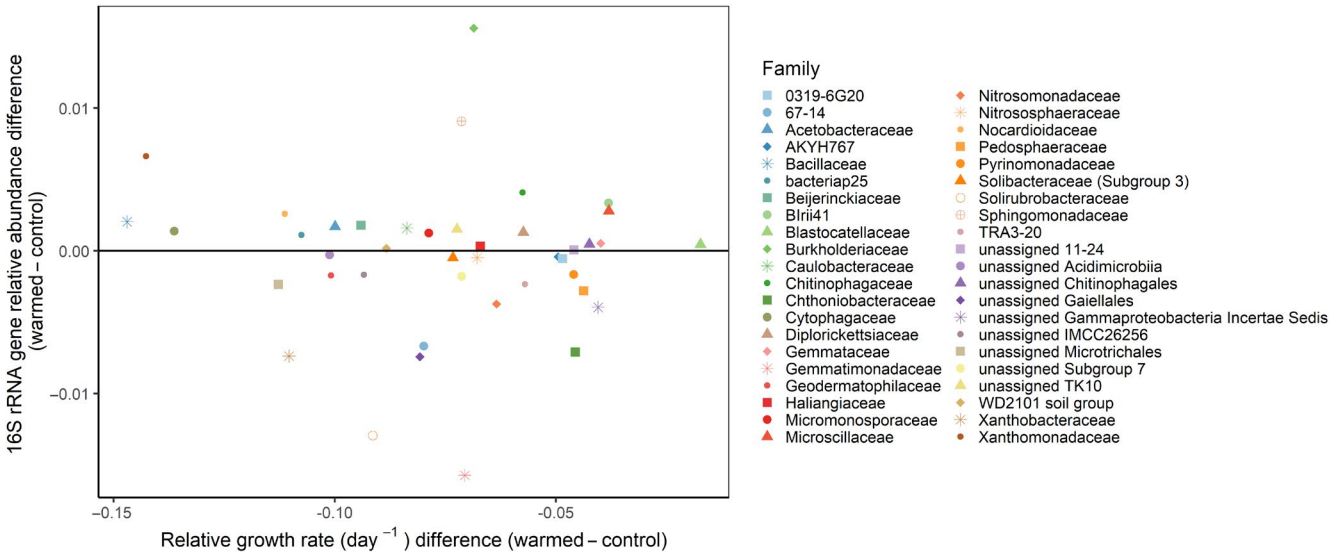


FIGURE 4 The differences in 16S rRNA gene relative abundances versus the difference in relative growth rate (day^{-1}) of shared taxonomic families between the warmed and control soils of a 3-day in situ field quantitative stable isotope probing (qSIP) warming experiment with ^{18}O enriched water in an Arizona meadow. Each point represents the sum of 16S rRNA gene relative abundance of a taxonomic family or median relative growth rate in the warmed minus the control treatment, where a minimum of three taxa were represented

use efficiency (Tucker et al., 2013), the proportion of assimilated carbon that is retained in biomass, and soil respiration has been found to have a higher temperature maximum than growth (Pietikäinen et al., 2005), thereby contributing to soil carbon loss. Growth is likely reduced in the warmed soils due to the decrease in available carbon (Demoling et al., 2007). The decline in growth observed here could coincide with a decrease in carbon use efficiency in conjunction with less carbon being available to assimilate which may explain the continued loss of soil carbon over time. Projected soil carbon loss with warming is highly sensitive to microbial growth, carbon use efficiency, and allocation to extracellular enzyme production (Allison et al., 2010). Increased allocation of carbon to extracellular products or maintenance instead of new biomass could contribute to reduced growth, and such shifts in allocation can occur because of stress and nutrient limitation (Lipson, 2015). Breaking down complex carbon compounds using extracellular enzymes may help to alleviate a decline in carbon substrates. Some of the active genera (e.g., *Lysobacter*, *Geobacillus*, and *Cytophaga*) in our experiment have representatives that are known to degrade complex substrates like xylan, lignin, and chitin (Brumm et al., 2015; López-Mondéjar et al., 2020; Park et al., 2008). Measurements that reveal how organisms allocate substrates to new cell growth, cell maintenance, protein synthesis, storage, and cell structures would help complete the picture of how warming alters microbial growth. Direct measures of growth of individual taxa, as shown here, provide new information to evaluate trait-based frameworks. However, the uniform direction response to warming across families challenges the idea of discrete life-history strategies and phylogeny for predicting microbial responses to a changing environment (Ho et al., 2017; Isobe et al., 2020; Malik et al., 2020).

Our work shows that it is possible to quantify how environmental change affects the growth rates of individual soil microbial taxa in the field without the artifacts introduced by laboratory-based assays. Since the majority of environmental microbes have not been cultured (Steen et al., 2019) and measuring taxon-specific activity remains challenging even in culture-independent techniques, field qSIP has broad potential for revealing taxonomic variation—or its absence—in response to environmental change, thereby exploring whether microbial biodiversity accounts for the variation in response observed in aggregate. Furthermore, qSIP has the power to compare population 16S rRNA gene relative abundance patterns with measures of their growth, which in our study indicated that the fastest growing taxa had some of the lowest 16S rRNA gene abundances (Figure 3) and changes in taxon relative abundance with warming are discordant with changes in growth rate (Figure 4). This provides evidence that estimating the ecological importance of taxa and their responses to environmental change based solely on 16S rRNA gene abundances may not be accurate. The findings presented here show that warming increased decomposition, which, over 15 years, caused a loss of soil carbon, suppressing microbial growth rates. Given the universal reduction in growth observed across taxa, our findings suggest that an aggregate measure of bacterial growth may suffice for modeling microbial contributions to carbon cycling responses of terrestrial ecosystems to warming.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Conceptualization: BAH, ES, MCM, NCG, JCM, PD, JPR, SJB, and EMM; Methodology: RLM, BAH, ES, BJK, NCG, JCM, and MCM; Investigation: AMP, MH, BJK, RLM, BAH, ES, and RLR; Visualization: AMP, BJK, and BAH; Funding acquisition: BAH, BJK, SJB, PD, MCM, JCM, JPR, ES, and EMM; Writing—original draft: AMP and BAH; Writing—review and editing: AMP, MH, BJK, RLM, SJB, PD, MCM, JCM, EMM, JPR, RLR, ES, NCG and BAH.

DATA AVAILABILITY STATEMENT

The dataset used to prepare Figure 1 (left panel) and Figure 2 are included as a supplemental file (Table S4). Other datasets generated and analyzed during the current study are available from the corresponding author (A. Purcell) on reasonable request. The 16S rRNA gene sequences are available in NCBI's SRA at BioProject accession PRJNA705223. The R code used to complete the analyses from this study is available from the corresponding author (A. Purcell) on reasonable request.

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REFERENCES

Aanderud, Z. T., & Lennon, J. T. (2011). Validation of heavy-water stable isotope probing for the characterization of rapidly responding soil bacteria. *Applied and Environmental Microbiology*, *77*(13), 4589–4596. https://doi.org/10.1128/AEM.02735-10

Allison, S. D., Wallenstein, M. D., & Bradford, M. A. (2010). Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience*, *3*(5), 336–340. https://doi.org/10.1038/ngeo846

Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology*, *75*(2), 129–137. https://doi.org/10.3354/ame01753

Bai, E., Li, S., Xu, W., Li, W., Dai, W., & Jiang, P. (2013). A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. *New Phytologist*, *199*(2), 441–451. https://doi.org/10.1111/nph.12252

Bárcenas-moreno, G., Gómez-brandón, M., Rousk, J., & Bååth, E. (2009). Adaptation of soil microbial communities to temperature: Comparison of fungi and bacteria in a laboratory experiment. *Global Change Biology*, *15*(12), 2950–2957. https://doi.org/10.1111/j.1365-2486.2009.01882.x

Bardgett, R. D., Freeman, C., & Ostle, N. J. (2008). Microbial contributions to climate change through carbon cycle feedbacks. *The ISME Journal*, *2*(8), 805–814. https://doi.org/10.1038/ismej.2008.58

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). Package lme4: Linear mixed-effects models using Eigen and S4. *Journal of Statistical Software*, *67*.

Bell, T. H., Klironomos, J. N., & Henry, H. A. L. (2010). Seasonal responses of extracellular enzyme activity and microbial biomass to warming and nitrogen addition. *Soil Science Society of America Journal*, *74*(3), 820–828. https://doi.org/10.2136/sssaj2009.0036

Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, *68*(1), 1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x

Blankinship, J. C., Brown, J. R., Dijkstra, P., Allwright, M. C., & Hungate, B. A. (2010). Response of terrestrial CH_4 uptake to interactive changes in precipitation and temperature along a climatic gradient. *Ecosystems*, *13*(8), 1157–1170. https://doi.org/10.1007/s10021-010-9391-9

Blazewicz, S. J., Hungate, B. A., Koch, B. J., Nuccio, E. E., Morrissey, E., Brodie, E. L., Schwartz, E., Pett-Ridge, J., & Firestone, M. K. (2020). Taxon-specific microbial growth and mortality patterns reveal distinct temporal population responses to rewetting in a California grassland soil. *The ISME Journal*, *14*(6), 1520–1532. https://doi.org/10.1038/s41396-020-0617-3

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

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

Bradford, M. A., 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., Wieder, W. R., Bonan, G. B., Fierer, N., Raymond, P. A., & Crowther, T. W. (2016). Managing uncertainty in soil carbon feedbacks to climate change. *Nature Climate Change*, *6*(8), 751–758. https://doi.org/10.1038/nclimate3071

Brumm, P., Land, M. L., Hauser, L. J., Jeffries, C. D., Chang, Y.-J., & Mead, D. A. (2015). Complete genome sequences of *Geobacillus* sp. Y412MC52, a xylan-degrading strain isolated from obsidian hot spring in Yellowstone National Park. *Standards in Genomic Sciences*, *10*(1), 1–9.

Butler, S. M., Melillo, J. M., Johnson, J. E., Mohan, J., Steudler, P. A., Lux, H., Burrows, E., Smith, R. M., Vario, C. L., Scott, L., Hill, T. D., Aponte, N., & Bowles, F. (2012). Soil warming alters nitrogen cycling in a New England forest: Implications for ecosystem function and structure. *Oecologia*, *168*(3), 819–828. https://doi.org/10.1007/s00442-011-2133-7

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, *108*(Suppl. 1), 4516–4522. https://doi.org/10.1073/pnas.1000080107

Carey, J. C., Tang, J., Templer, P. H., Kroeger, K. D., Crowther, T. W., Burton, A. J., Dukes, J. S., Emmett, B., Frey, S. D., Heskel, M. A., Jiang, L., Machmuller, M. B., Mohan, J., Panetta, A. M., Reich, P. B., Reinsch, S., Wang, X., Allison, S. D., Bamminger, C., … Tietema, A. (2016). Temperature response of soil respiration largely unaltered with experimental warming. *Proceedings of the National Academy*

of Sciences of the United States of America, *113*(48), 13797–13802. https://doi.org/10.1073/pnas.1605365113

Carrillo, Y., Bell, C., Koyama, A., Canarini, A., Boot, C. M., Wallenstein, M., & Pendall, E. (2017). Plant traits, stoichiometry and microbes as drivers of decomposition in the rhizosphere in a temperate grassland. *Journal of Ecology*, *105*(6), 1750–1765. https://doi.org/10.1111/1365-2745.12772

Che, R., Deng, Y., Wang, W., Rui, Y., Zhang, J., Tahmasbian, I., Tang, L. I., Wang, S., Wang, Y., Xu, Z., & Cui, X. (2018). Long-term warming rather than grazing significantly changed total and active soil procaryotic community structures. *Geoderma*, *316*, 1–10. https://doi.org/10.1016/j.geoderma.2017.12.005

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

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

Davidson, E. A., Janssens, I. A., & Luo, Y. (2006). On the variability of respiration in terrestrial ecosystems: Moving beyond Q10. *Global Change Biology*, *12*(2), 154–164.

DeAngelis, K. M., Pold, G., Topçuoğlu, 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*, 104. https://doi.org/10.3389/fmicb.2015.00104

Delgado-Baquerizo, M., Giaramida, L., Reich, P. B., Khachane, A. N., Hamonts, K., Edwards, C., Lawton, L. A., & Singh, B. K. (2016). Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. *Journal of Ecology*, *104*(4), 936–946. https://doi.org/10.1111/1365-2745.12585

Delgado-Baquerizo, M., Oliverio, A. M., Brewer, T. E., Benavent-González, A., Eldridge, D. J., Bardgett, R. D., Maestre, F. T., Singh, B. K., & Fierer, N. (2018). A global atlas of the dominant bacteria found in soil. *Science*, *359*(6373), 320–325.

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

Fernández-Calviño, D., & Bååth, E. (2010). Growth response of the bacterial community to pH in soils differing in pH. *FEMS Microbiology Ecology*, *73*(1), 149–156. https://doi.org/10.1111/j.1574-6941.2010.00873.x

Fierer, N., Jackson, J. A., Vilgalys, R., & Jackson, R. B. (2005). Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology*, *71*(7), 4117. https://doi.org/10.1128/AEM.71.7.4117-4120.2005

Fierer, N., & Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(3), 626–631. https://doi.org/10.1073/pnas.0507535103

Finley, B. K., Hayer, M., Mau, R. L., Purcell, A. M., Koch, B. J., van Gestel, N. C., Schwartz, E., & Hungate, B. A. (2019). Microbial taxon-specific isotope incorporation with DNA quantitative stable isotope probing. In M. G. Dumont & M. H. García (Eds.), *Stable isotope probing* (pp. 137–149). Springer.

Frey, S. D., Drijber, R., Smith, H., & Melillo, J. (2008). Microbial biomass, functional capacity, and community structure after 12 years of soil warming. *Soil Biology and Biochemistry*, *40*(11), 2904–2907. https://doi.org/10.1016/j.soilbio.2008.07.020

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

Ho, A., Di Lonardo, D. P., & Bodelier, P. L. E. (2017). Revisiting life strategy concepts in environmental microbial ecology. *FEMS Microbiology Ecology*, *93*(3), fix006. https://doi.org/10.1093/femsec/fix006

Hungate, B. A., Mau, R. L., Schwartz, E., Caporaso, J. G., Dijkstra, P., van Gestel, N., Koch, B. J., Liu, C. M., McHugh, T. A., Marks, J. C., Morrissey, E. M., & Price, L. B. (2015). Quantitative microbial ecology through stable isotope probing. *Applied and Environmental Microbiology*, *81*(21), 7570–7581. https://doi.org/10.1128/AEM.02280-15

Isobe, K., Bouskill, N. J., Brodie, E. L., Sudderth, E. A., & Martiny, J. B. H. (2020). Phylogenetic conservation of soil bacterial responses to simulated global changes. *Philosophical Transactions of the Royal Society B*, *375*(1798), 20190242. https://doi.org/10.1098/rstb.2019.0242

Jousset, A., Bienhold, C., Chatzinotas, A., Gallien, L., Gobet, A., Kurm, V., Küsel, K., Rillig, M. C., Rivett, D. W., Salles, J. F., van der Heijden, M. G. A., Youssef, N. H., Zhang, X., Wei, Z., & Hol, W. H. G. (2017). Where less may be more: How the rare biosphere pulls ecosystems strings. *The ISME Journal*, *11*(4), 853–862. https://doi.org/10.1038/ismej.2016.174

Kallenbach, C. M., Frey, S. D., & Grandy, A. S. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications*, *7*(1), 1–10. https://doi.org/10.1038/ncomms13630

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/10.1111/j.1365-2486.2004.00852.x

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

Kwak, M.-J., Kwon, S.-K., Yoon, J.-H., & Kim, J. F. (2015). Genome sequence of Lysobacter dokdonensis DS-58 T, a gliding bacterium isolated from soil in Dokdo, Korea. *Standards in Genomic Sciences*, *10*(1), 1–7. https://doi.org/10.1186/s40793-015-0116-8

Li, J., Mau, R. L., Dijkstra, P., Koch, B. J., Schwartz, E., Liu, X.-J., Morrissey, E. M., Blazewicz, S. J., Pett-Ridge, J., Stone, B. W., Hayer, M., & Hungate, B. A. (2019). Predictive genomic traits for bacterial growth in culture versus actual growth in soil. *The ISME Journal*, *13*(9), 2162–2172. https://doi.org/10.1038/s41396-019-0422-z

Liang, C., Schimel, J. P., & Jastrow, J. D. (2017). The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology*, *2*(8), 1–6. https://doi.org/10.1038/nmicrobiol.2017.105

Link, S. O., Smith, J. L., Halvorson, J. J., & Bolton, H. Jr (2003). A reciprocal transplant experiment within a climatic gradient in a semiarid shrub-steppe ecosystem: Effects on bunchgrass growth and reproduction, soil carbon, and soil nitrogen. *Global Change Biology*, *9*(7), 1097–1105. https://doi.org/10.1046/j.1365-2486.2003.00647.x

Lipson, D. A. (2015). The complex relationship between microbial growth rate and yield and its implications for ecosystem processes. *Frontiers in Microbiology*, *6*, 615. https://doi.org/10.3389/fmicb.2015.00615

López-Mondéjar, R., Tláskal, V., Větrovský, T., Štursová, M., Toscan, R., da Rocha, U. N., & Baldrian, P. (2020). Metagenomics and stable isotope probing reveal the complementary contribution of fungal and bacterial communities in the recycling of dead biomass in forest soil. *Soil Biology and Biochemistry*, *148*, 107875. https://doi.org/10.1016/j.soilbio.2020.107875

Luo, Y., Ahlström, A., Allison, S. D., Batjes, N. H., Brovkin, V., Carvalhais, N., Chappell, A., Ciais, P., Davidson, E. A., Finzi, A., Georgiou, K., Guenet, B., Hararuk, O., Harden, J. W., He, Y., Hopkins, F., Jiang, L., Koven, C., Jackson, R. B., … Zhou, T. (2016). Toward more realistic projections of soil carbon dynamics by Earth system

models. *Global Biogeochemical Cycles*, *30*(1), 40–56. https://doi.org/10.1002/2015GB005239

Malik, A. A., Martiny, J. B. H., Brodie, E. L., Martiny, A. C., Treseder, K. K., & Allison, S. D. (2020). Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. *The ISME Journal*, *14*(1), 1–9. https://doi.org/10.1038/s41396-019-0510-0

Mandic-Mulec, I., Stefanic, P., & Van Elsas, J. D. (2015). Ecology of bacillaceae. *Microbiology Spectrum*, *3*(2), TBS-0017-2013. https://doi.org/10.1128/microbiolspec.TBS-0017-2013

Martiny, J. B. H., Jones, S. E., Lennon, J. T., & Martiny, A. C. (2015). Microbiomes in light of traits: A phylogenetic perspective. *Science*, *350*(6261). https://doi.org/10.1126/science.aac9323

Masson-Delmotte, V., Zhai, P., Pörtner, H.-O., Roberts, D., Skea, J., Shukla, P. R., Pirani, A., Moufouma-Okia, W., Péan, C., Pidcock, R., Connors, S., Matthews, J. B. R., Chen, Y., Zhou, X., Gomis, M. I., Lonnoy, E., Maycock, T., Tignor, M., & Waterfield, T. (Eds.). (2018). IPCC, 2018: Summary for policymakers. *Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty*. World Meteorological Organization. 32 pp.

Mau, R. L., Dijkstra, P., Schwartz, E., Koch, B. J., & Hungate, B. A. (2018). Warming induced changes in soil carbon and nitrogen influence priming responses in four ecosystems. *Applied Soil Ecology*, *124*, 110–116. https://doi.org/10.1016/j.apsoil.2017.10.034

Melillo, J. M., Butler, S., Johnson, J., Mohan, J., Steudler, P., Lux, H., Burrows, E., Bowles, F., Smith, R., Scott, L., Vario, C., Hill, T., Burton, A., Zhou, Y.-M., & Tang, J. (2011). Soil warming, carbon–nitrogen interactions, and forest carbon budgets. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(23), 9508–9512. https://doi.org/10.1073/pnas.1018189108

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

Mills, R. T., Gavazov, K. S., Spiegelberger, T., Johnson, D., & Buttler, A. (2014). Diminished soil functions occur under simulated climate change in a sup-alpine pasture, but heterotrophic temperature sensitivity indicates microbial resilience. *Science of the Total Environment*, *473*, 465–472.

Morrissey, E. M., Mau, R. L., Hayer, M., Liu, X.-J., Schwartz, E., Dijkstra, P., Koch, B. J., Allen, K., Blazewicz, S. J., Hofmockel, K., Pett-Ridge, J., & Hungate, B. A. (2019). Evolutionary history constrains microbial traits across environmental variation. *Nature Ecology & Evolution*, *3*(7), 1064–1069. https://doi.org/10.1038/s41559-019-0918-y

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.

Papp, K., Hungate, B. A., & Schwartz, E. (2018). Microbial rRNA synthesis and growth compared through quantitative stable isotope probing with $H_2{}^{18}O$. *Applied and Environmental Microbiology*, *84*(8).

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*(5), 1403–1414. https://doi.org/10.1111/1462-2920.13023

Park, J. H., Kim, R., Aslam, Z., Jeon, C. O., & Chung, Y. R. (2008). Lysobacter capsici sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus Lysobacter. *International Journal of Systematic and Evolutionary Microbiology*, *58*(2), 387–392.

Pietikäinen, J., Pettersson, M., & Bååth, E. (2005). Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS Microbiology Ecology*, *52*(1), 49–58. https://doi.org/10.1016/j.femsec.2004.10.002

Purcell, A. M., Dijkstra, P., Finley, B., Hayer, M., Koch, B. J., Mau, R. L., Morrissey, E., Papp, K., Schwartz, E., & Stone, B. W. (2020). Quantitative stable isotope probing with $H_2{}^{18}O$ to measure taxon-specific microbial growth. *Soil Science Society of America Journal*, *84*(5), 1503–1518.

Qian, G.-L., Hu, B.-S., Jiang, Y.-H., & Liu, F.-Q. (2009). Identification and characterization of Lysobacter enzymogenes as a biological control agent against some fungal pathogens. *Agricultural Sciences in China*, *8*(1), 68–75. https://doi.org/10.1016/S1671-2927(09)60010-9

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

Ranneklev, S. B., & Bååth, E. (2001). Temperature-driven adaptation of the bacterial community in peat measured by using thymidine and leucine incorporation. *Applied and Environmental Microbiology*, *67*(3), 1116–1122. https://doi.org/10.1128/AEM.67.3.1116-1122.2001

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

Rousk, J., Smith, A. R., & Jones, D. L. (2013). Investigating the long-term legacy of drought and warming on the soil microbial community across five European shrubland ecosystems. *Global Change Biology*, *19*(12), 3872–3884. https://doi.org/10.1111/gcb.12338

Rustad, L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., Cornelissen, J., & Gurevitch, J. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, *126*(4), 543–562. https://doi.org/10.1007/s004420000544

Schimel, J., & Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Frontiers in Microbiology*, *3*, 348. https://doi.org/10.3389/fmicb.2012.00348

Schindlbacher, A., Rodler, A., Kuffner, M., Kitzler, B., Sessitsch, A., & Zechmeister-Boltenstern, 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/10.1016/j.soilbio.2011.03.005

Schwartz, E. (2007). Characterization of growing microorganisms in soil by stable isotope probing with $H_2{}^{18}O$. *Applied and Environmental Microbiology*, *73*(8), 2541.

Shade, A., Jones, S. E., Caporaso, J. G., Handelsman, J., Knight, R., Fierer, N., & Gilbert, J. A. (2014). Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. *mBio*, *5*(4). https://doi.org/10.1128/mBio.01371-14

Shen, C., Xiong, J., Zhang, H., Feng, Y., Lin, X., Li, X., Liang, W., & Chu, H. (2013). Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. *Soil Biology and Biochemistry*, *57*, 204–211. https://doi.org/10.1016/j.soilbio.2012.07.013

Sinsabaugh, R. L., Manzoni, S., Moorhead, D. L., & Richter, A. (2013). Carbon use efficiency of microbial communities: Stoichiometry, methodology and modelling. *Ecology Letters*, *16*(7), 930–939. https://doi.org/10.1111/ele.12113

Steen, A. D., Crits-Christoph, A., Carini, P., DeAngelis, K. M., Fierer, N., Lloyd, K. G., & Thrash, J. C. (2019). High proportions of bacteria and archaea across most biomes remain uncultured. *The ISME Journal*, *13*(12), 3126–3130. https://doi.org/10.1038/s41396-019-0484-y

Takai, K. E. N., & Horikoshi, K. (2000). Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. *Applied and Environmental Microbiology*, *66*(11), 5066–5072. https://doi.org/10.1128/AEM.66.11.5066-5072.2000

Team R. (2019). *RStudio: Integrated development for R*. RStudio, Inc., 2015. https://www.rstudio.com/products/rstudio

Team, R. C. (2018). *Core. R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Torsvik, V., & Øvreås, L. (2002). Microbial diversity and function in soil: From genes to ecosystems. *Current Opinion in Microbiology*, *5*(3), 240–245. https://doi.org/10.1016/S1369-5274(02)00324-7

Tucker, C. L., Bell, J., Pendall, E., & Ogle, K. (2013). Does declining carbon-use efficiency explain thermal acclimation of soil respiration with warming? *Global Change Biology*, *19*(1), 252–263. https://doi.org/10.1111/gcb.12036

Vanhala, P., Karhu, K., Tuomi, M., Björklöf, K., Fritze, H., Hyvärinen, H., & Liski, J. (2011). Transplantation of organic surface horizons of boreal soils into warmer regions alters microbiology but not the temperature sensitivity of decomposition. *Global Change Biology*, *17*(1), 538–550. https://doi.org/10.1111/j.1365-2486.2009.02154.x

Vázquez-Baeza, Y., Pirrung, M., Gonzalez, A., & Knight, R. (2013). EMPeror: A tool for visualizing high-throughput microbial community data. *GigaScience*, *2*(1), 2047–2217X. https://doi.org/10.1186/2047-217X-2-16

Waldrop, M. P., & Firestone, M. K. (2006). Response of microbial community composition and function to soil climate change. *Microbial Ecology*, *52*(4), 716–724. https://doi.org/10.1007/s00248-006-9103-3

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

Walther, G.-R. (2010). Community and ecosystem responses to recent climate change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1549), 2019–2024. https://doi.org/10.1098/rstb.2010.0021

Wang, K., Peng, C., Zhu, Q., Zhou, X., Wang, M., Zhang, K., & Wang, G. (2017). Modeling global soil carbon and soil microbial carbon by integrating microbial processes into the ecosystem process model TRIPLEX-GHG. *Journal of Advances in Modeling Earth Systems*, *9*(6), 2368–2384. https://doi.org/10.1002/2017MS000920

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

Wu, Z., Dijkstra, P., Koch, G. W., & Hungate, B. A. (2012). Biogeochemical and ecological feedbacks in grassland responses to warming. *Nature Climate Change*, *2*(6), 458–461. https://doi.org/10.1038/nclimate1486

Wu, Z., Dijkstra, P., Koch, G. W., Peñuelas, J., & Hungate, B. A. (2011). Responses of terrestrial ecosystems to temperature and precipitation change: A meta-analysis of experimental manipulation. *Global Change Biology*, *17*(2), 927–942. https://doi.org/10.1111/j.1365-2486.2010.02302.x

Yu, H., Ma, Q., Liu, X., Xu, Z., Zhou, G., & Shi, Y. (2018). Short- and long-term warming alters soil microbial community and relates to soil traits. *Applied Soil Ecology*, *131*, 22–28. https://doi.org/10.1016/j.apsoil.2018.07.006

Yu, Y., Lee, C., Kim, J., & Hwang, S. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnology and Bioengineering*, *89*(6), 670–679. https://doi.org/10.1002/bit.20347

Yue, H., Wang, M., Wang, S., Gilbert, J. A., Sun, X., Wu, L., Lin, Q., Hu, Y., Li, X., He, Z., Zhou, J., & Yang, Y. (2015). The microbe-mediated mechanisms affecting topsoil carbon stock in Tibetan grasslands. *The ISME Journal*, *9*(9), 2012–2020. https://doi.org/10.1038/ismej.2015.19

Zhang, W., Parker, K. M., Luo, Y. T., Wan, S., Wallace, L. L., & Hu, S. (2005). Soil microbial responses to experimental warming and clipping in a tallgrass prairie. *Global Change Biology*, *11*(2), 266–277. https://doi.org/10.1111/j.1365-2486.2005.00902.x

Zhou, J., Deng, Y. E., Shen, L., Wen, C., Yan, Q., Ning, D., Qin, Y., Xue, K., Wu, L., He, Z., Voordeckers, J. W., Nostrand, J. D. V., Buzzard, V., Michaletz, S. T., Enquist, B. J., Weiser, M. D., Kaspari, M., Waide, R., Yang, Y., & Brown, J. H. (2016). Temperature mediates continental-scale diversity of microbes in forest soils. *Nature Communications*, *7*(1), 1–10. https://doi.org/10.1038/ncomms12083

Zhou, J., Xue, K., Xie, J., Deng, Y. E., Wu, L., Cheng, X., Fei, S., Deng, S., He, Z., Van Nostrand, J. D., & Luo, Y. (2012). Microbial mediation of carbon-cycle feedbacks to climate warming. *Nature Climate Change*, *2*(2), 106–110. https://doi.org/10.1038/nclimate1331

Zumsteg, A., Bååth, E., Stierli, B., Zeyer, J., & Frey, B. (2013). Bacterial and fungal community responses to reciprocal soil transfer along a temperature and soil moisture gradient in a glacier forefield. *Soil Biology and Biochemistry*, *61*, 121–132. https://doi.org/10.1016/j.soilbio.2013.02.017

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