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

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Primary Research Article

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; Simsabaugh 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 13O-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). 18O 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 18O/H2O 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
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 $6.7 \pm 2.3^\circ C$ (2002–2017) and a mean annual soil temperature at 5 cm of $7.6 \pm 1.4^\circ C$ (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 $10.2 \pm 2.6^\circ C$ (2002–2017) and a mean annual soil temperature at 5 cm of $9.7 \pm 1.9^\circ C$ (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 $2.1 \pm 0.78^\circ C$.

### 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 inter-spaces) 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 $-80^\circ C$. 

### 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 × 10¹¹ g dry soil * | Mean Archaeal 16S rRNA gene copies × 10⁹ g dry soil ** |
|--------|-----------------------------|------------------------------------|---------------------------|----------------|------------------------------------------------------|------------------------------------------------------|
| Control| $12.2 \pm 0.5$              | $14.1 \pm 0.4$                     | $17.7 \pm 1.9$           | $18.6 \pm 4$  | $3.5 \pm 0.2$                                       | $1.4 \pm 0.6$                                       |
| Warmed | $15 \pm 0.5$                | $15.5 \pm 0.3$                     | $18.6 \pm 4$            | $18.6 \pm 4$  | $4.3 \pm 0.5$                                       | $2.3 \pm 0.4$                                       |

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 microcosms, respectively. Asterisks represent significance from an unpaired t test: NS, not significant; *p < .05; **p < .01; ***p < .001.
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/paulheinrich.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).

Ultranrentrifugation 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 5x and centrifuged in an Optima Max benchtop ultracentrifuge, using a Beckman TLN-100 rotor (Beckman Coulter, Inc). The tubes were capped and inverted 5x 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, 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 R² value of the standard curve was ≥ 0.97, 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 x 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 18O 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
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 soil weight 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 H2 18O 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...
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
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 (Bárdenas-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 microbiologically 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

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**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 18O enriched water in an Arizona meadow.
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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