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Microbial dormancy improves predictability of soil respiration at the seasonal time scale

Alejandro Salazar · J. T. Lennon · J. S. Dukes

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Abstract Global warming, in combination with altered precipitation patterns, is accelerating global soil respiration, which could in turn accelerate climate change. The biological mechanisms through which soil carbon (C) responds to climate are not well understood, limiting our ability to predict future global soil respiration rates. As part of a climate manipulation experiment, we tested whether differences in soil heterotrophic respiration (R_H) driven by season or climate treatment are linked to (1) relative abundances of microbes in active and dormant metabolic states, (2) net changes in microbial biomass and/or (3) changes in the relative abundances of microbial groups with different C-use strategies. We used a flow-cytometric single-cell metabolic assay to quantify the abundance of active and dormant microbes, and the phospholipid fatty acid method to determine microbial biomass and ratios of fungi:bacteria and Gram-positive:Gram-negative bacteria. R_H did not respond to climate treatments but was greater in the warm and dry summer than in the cool and less-dry fall. These dynamics were better explained when microbial data were taken into account compared to when only physical data (temperature and moisture) were used. Overall, our results suggest that R_H responses to temperature are stronger when soil contains more active microbes, and that seasonal patterns of R_H can be better explained by shifts in microbial activity than by shifts in the relative abundances of fungi and Gram-positive and Gram-negative bacteria. These findings contribute to our understanding of how and under which conditions microbes influence soil C responses to climate.

Keywords Boston-area climate experiment · Carbon-climate feedback · Microbial biomass · Precipitation change · Warming

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A. Salazar (✉) · J. S. Dukes
Department of Biological Sciences, Purdue University, 915 W. State Street, West Lafayette, IN, USA
e-mail: alejandro.salazar-villegas@fulbrightmail.org

A. Salazar · J. S. Dukes
Purdue Climate Change Research Center, Purdue University, West Lafayette, IN, USA

J. T. Lennon
Department of Biology, Indiana University, Jordan Hall 261B, Bloomington, IN, USA

J. S. Dukes
Department of Forestry and Natural Resources, Purdue University, 715 W. State Street, West Lafayette, IN, USA

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Introduction

Every year, microbes from terrestrial ecosystems emit approximately 54 Pg C into the atmosphere via soil heterotrophic respiration (Hashimoto et al. 2015). This is more than five times the amount of C released by fossil fuel emissions in 2016 (ca. 10 Pg C; Le Quéré et al. 2016). Even small increases in this soil C flux, if sustained, could contribute to accelerated global warming. Unless suppressed by dry conditions (Allison and Treseder 2008; Schindlbacher et al. 2012; Suseela et al. 2012), warming generally increases soil respiration (Carey et al. 2016). Evidence from the last 50 years (i.e., since the first soil respiration records) suggests that soil respiration, both total (i.e., plant roots and soil microbes, R_S; Bond-Lamberty and Thomson 2010; Zhao et al. 2017; Bond-Lamberty et al. 2018) and heterotrophic (i.e., microbes in root-free soil, R_H; Hashimoto et al. 2015; Bond-Lamberty et al. 2018) have been increasing with temperature at a rate of approximately 0.04 Pg C year^{-1} (Zhao et al. 2017); 0.1 Pg C year^{-1} in the last 3 decades (Bond-Lamberty and Thomson 2010). If changes in global temperature and precipitation regimes accelerate turnover of soil C without proportionally increasing C inputs from plant growth, this would generate a positive soil C-climate feedback.

The mechanisms through which global soil respiration is increasing are not clear, limiting our ability to predict whether this trend is going to accelerate, stabilize, or decrease. Current projections of feedbacks between terrestrial C and climate remain highly uncertain (Friedlingstein et al. 2014), which limits their usefulness to inform climate policy. Recent studies have raised the question of whether or not these uncertainties could be reduced by a better understanding of how microorganisms respond to climatic changes (Bardgett et al. 2008; Wieder et al. 2015).

Two of the most-studied microbial parameters that can be linked to R_H are microbial biomass (Illeris et al. 2003; Wang et al. 2003; Lee and Jose 2003; Liu et al. 2009; Zhou et al. 2011) and community composition (Zogg et al. 1997; Monson et al. 2006; Waldrop and Firestone 2006; Cleveland et al. 2007; Zhou et al. 2011; Don et al. 2017). Assuming that all microbes are active and respiring (e.g. under ideal environmental and nutritional conditions), one would expect soil R_H to increase with the abundance of soil microorganisms. This idea is supported by observations of simultaneous increases or decreases of microbial biomass and R_H in response to warming (Zhou et al. 2011), moisture (Illeris et al. 2003; Liu et al. 2009), substrate availability (Wang et al. 2003) and plant biomass removal (Zhang et al. 2005). However, other studies have failed to find a consistent relationship between microbial biomass and R_H (Waldrop and Firestone 2006; Waring and Hawkes 2015; Birge et al. 2015; Buchkowski et al. 2015), suggesting that microbial parameters besides biomass may contribute to changes in R_H. Hypotheses linking R_H and community composition postulate that a given set of external factors will distinctly favor the growth of particular microbial groups, based on their C needs and C use efficiencies. This idea is supported by observations of, for example, warming increasing the abundance of microbial functional populations specialized in the degradation of labile C, but not recalcitrant C (Zhou et al. 2011). The effects of warming on microbial groups with different C use strategies can change over time and can be modulated by factors such as the type and availability of soil nutrients (Frey et al. 2013; Treseder et al. 2016).

After microbial biomass and community composition, a third microbial parameter that has been increasingly proposed as an explanatory factor of R_H is microbial dormancy (Placella et al. 2012; Manzoni et al. 2014; Wang et al. 2014, 2015; Barnard et al. 2015; He et al. 2015; Salazar et al. 2018). In addition to growing, dying, and changing composition, microbial communities in soil can switch between active and dormant metabolic states (Stenström et al. 2001; Schimel et al. 2007; Lennon and Jones 2011). These metabolic switches can be triggered by environmental factors related to weather and climate, such as temperature and moisture (Barnard et al. 2015; Salazar et al. 2018), as well as by factors not addressed in this study, such as the concentration of nutrients (Blagodatskaya and Kuzyakov 2013) and the presence of pollutants (Bhupathiraju et al. 1999). Changes of metabolic state are generally faster than growth, death, and changes in composition (Blagodatskaya and Kuzyakov 2013). In part because of this, most experiments exploring the relationship between microbial dormancy and R_H have examined short temporal scales (Placella et al. 2012; Aanderud et al. 2015; Barnard et al. 2015; Salazar-Villegas et al. 2016). Much less is known about the importance of microbial dormancy for R_H at longer (e.g., seasonal)

temporal scales, which are more relevant for modeling purposes and society-level decision making.

In this study, we investigated whether seasonal R_H in a temperate old-field ecosystem is linked to changes in the metabolic state of microbial communities in soil; to net changes in microbial biomass; and/or to changes in the relative abundances of microbial groups that consume and emit soil C at different rates, namely, fungi and bacteria (Six et al. 2006; Sinsabaugh et al. 2016), and Gram-positive and Gram-negative bacteria (Lennon et al. 2012). Although there are other microbial processes that can affect R_H and that were not explicitly taken into account in our experimental design, such as the production and activity of extracellular enzymes (Steinweg et al. 2013), we expected that the parameters taken into account in this study would be important for explaining the variation in R_H across sampling times and treatments. In addition to the potential changes in total microbial biomass (Devi and Yadava 2006) and community composition (Waldrop and Firestone 2006) that can occur on a seasonal time scale, we expected seasonal changes in temperature and moisture to affect the metabolic state of microbial communities in soil. Specifically, we expected the abundance of active microbial biomass to be highest when environmental conditions were most optimal for microbial processes, and for this to help explain seasonal changes in soil respiration rates.

Methods

Study site

The study was conducted at the Boston-Area Climate Experiment (BACE), located at the University of Massachusetts' former Suburban Experiment Station in Waltham, Massachusetts (42°23.1′N, 71°12.9′W). The mean annual temperature and precipitation at the site are 10.3 °C and 1063 mm, respectively. The soil at BACE is classified as Mesic Typic Dystrudept (Haven series), with loamy topsoil (45% sand, 46% silt, 9% clay; gravel content: 7%) and a gravelly sandy loam subsoil. The plant community is dominated by non-native grasses and forbs (Hoeppner and Dukes 2012).

To guarantee that the measured R_H was caused by microbial activity and not by plant roots, we collected all of our samples from patches of soil that were isolated from roots and plant carbon inputs by "root-exclusion collars." These collars were made of 30-cm diameter plastic pipe that had been driven 30 cm into the soil in November 2007 (Suseela et al. 2012). The collars extended $\sim$ 4 cm above the soil surface. To prevent plant growth within these root-exclusion collars, we covered the soil surface within each collar with a circle of weed-blocking nylon mesh. This mesh was removed only during R_H measurements and soil sampling. Carbon inputs had been limited in this manner for the previous 9 years; by the fourth year of plant exclusion (2011), labile organic matter remaining in the soils was already substantially depleted in comparison to the surrounding soils in which plants grew, as shown by lower rates of substrate-induced respiration (Koyama et al. 2018). Thus, our use of these root-free soils with similar past C inputs enabled a controlled examination of microbial responses, but the sustained lack of plant inputs and the consequently depleted labile organic matter need to be kept in mind when interpreting our results.

Experimental design

The BACE manipulated climatic conditions in 36 square experimental plots, each 2 m $\times$ 2 m. A factorial combination of four levels of warming and three levels of precipitation created a total of 12 climate treatments. The experiment consisted of three replicate blocks. Within each block, plots were arranged linearly in three groups of four, with each group receiving one of the three precipitation treatments. The four plots within each group were spaced 1 m apart, with one plot receiving each of the four levels of warming. Each block was located under a single greenhouse frame that served as a mount for infrastructure related to the precipitation treatments.

Warming was applied with ceramic infrared heaters mounted 1 m above each corner of each plot, and facing towards the center of the plot and down at a 45° angle. The treatments corresponded to the wattage of the heaters surrounding each plot: unheated (0 W), low (200 W), medium (600 W), and high (1000 W) heat. The three heated plots within each group were wired to a single circuit, and the warming system was programmed to attempt to maintain a 4 °C difference between the canopy temperatures of the unheated and high heat plots within each group. The power supplied

to the heaters in each group was adjusted every 10 s based on the measured temperature difference between the unheated and high heat plots in that group. Canopy temperatures were measured with infrared radiometers (IRR-PN; Apogee Instruments, Logan, UT, USA) placed at a 45° downward angle, 1 m above the northern edges of the plots. The four warming levels approximately simulated the different warming scenarios projected by the Intergovernmental Panel on Climate Change (IPCC) for the end of this century (Stocker 2014).

The precipitation manipulation included ambient, dry (− 50% of all precipitation year-round), and wet (+ 50% growing season rainfall) treatments. Above the dry treatment, rainfall was captured by 15 cm-wide clear polycarbonate slats spaced 15 cm apart that were mounted on the greenhouse frames, > 2 m off the ground. From early May to mid-November, the removed rainfall was collected in tanks and immediately applied to the wet treatments with a sprinkler system. Further details of the experiment can be found in Hoeppner and Dukes (2012), Suseela et al. (2012), and Auyeung et al. (2013).

In situ soil measurements and sampling

We made in situ soil measurements (R_H, and microbial activity/dormancy) and collected samples for analysis in the laboratory in the summer (June) and fall (October) of 2016. For simplicity, we refer to these measurements by the season in which they were made, but it is important to recognize that they represent snapshots of the conditions at the moment of sampling and not an average of the respective months or seasons.

We measured R_H with a LICOR 6400 soil CO_2 flux chamber (LI-COR Biosciences, Inc. Lincoln, NE, USA), in small PVC collars (10 cm in diameter and 5 cm in height, 2 cm into the soil) that we had installed within the root-exclusion collars in April 2016. During the R_H measurements we also measured soil temperature (10 cm) using a thermocouple probe. We measured volumetric soil moisture on the same day using time-domain reflectometry waveguides installed vertically (0–10 cm depth) in the root-exclusion collars.

We used a soil sampler (2 cm diameter) to collect soil from the top 10 cm immediately after measuring R_H. We used 1 g of the soil for measuring in situ active and dormant microbial biomass (see below). We stored the rest of the soil in coolers with ice packs and transported them to Purdue University where we measured microbial biomass and fungi:bacteria and Gram-positive-Gram-negative ratios (see below). At Purdue, samples were stored at 4 °C for 4 to 6 weeks until all samples were processed.

Abundance of active and dormant microbes in soil

We used a flow-cytometric single-cell metabolic assay to quantify active and dormant microbial abundance (Del Giorgio and Gasol 2008). Immediately after measuring R_H and collecting soil samples in each plot, we mixed 1 g of soil with 9 mL of distilled, sterile water and vortexed this solution for 1 min. We filtered the solution (particle retention > 11 μm) to remove large debris. We sampled 0.8 mL of the filtered solution and added 0.1 mL of 4',6-diamidino-2-phenylindole (DAPI; 5 μg DAPI mL^{-1} final concentration) and 0.1 mL of 5-cyano-2,3-ditolyl tetrazolium chloride (CTC; 5 mM final concentration) to stain all and respiring-only cells, respectively. DAPI stains the DNA of all viable cells while only metabolically active cells can transform the electron acceptor CTC to the fluorophore CTC-formazan (Kaprelyants and Kell 1993). We mixed this solution in a shaker for 30 min and then stopped the reaction by storing the samples in coolers with ice packs. Immediately after processing all samples, we shipped them to the flow cytometry facility at Indiana University, USA. To check for potential auto-fluorescence in soil, we also analyzed negative-control unstained samples for each plot.

To estimate the abundance of active (i.e., DAPI and CTC co-labeled) and dormant (i.e., DAPI-only labeled) cells, we used an LSRII flow cytometer (Becton–Dickinson, San Jose, CA) equipped with Forward Scatter PMT (FSC-PMT) for improved resolution of small particles. We used the FACSDiva v.6.1.3 software for data analysis. DAPI was excited with a 20 mW 405 nm laser, and detected using a 450/50 filter, while CTC was excited with a 30 mW 488 nm laser, and detected using a 695/40 filter. To further resolve small particles, we set the LSRII window extension at 2.00 (rather than the default of 10.00). To minimize the amount of debris and background included in the sample analysis, we set thresholds at 1000 and 750 for FSC-PMT and SSC

parameters, respectively. We ran controls (unlabeled, DAPI, and CTC) for each sample, and saved 10,000 events per sample. The sample injection was rinsed after each sample in order to minimize any cross contamination between samples. An example of the analysis output (Fig. S1) is shown in the supplementary material.

We acknowledge three caveats with our approach for measuring abundance of active and dormant cells: (1) we did not measure dead cells, so our estimate of dormant cells could be overestimated by non-CTC stained cells that were not viable, (2) a fraction of the dormant cells in soil could have been activated when suspending samples in water for dye application, which would underestimate the proportion of inactive cells, and (3) Because CTC stains bacteria but not fungi and because we measured abundance of CTC/DAPI labeled cells based on light scattering characteristics of *Escherichia coli* (see Supplementary Material), our measurements of microbial activity reflect bacteria but not fungi. Based on these caveats, we made the following assumptions: We assumed that the cell structure of most dead cells was compromised, preventing the cells from retaining DAPI-labeled DNA and/or affecting its light scattering characteristics, and we therefore assumed that most dead cells were not counted as dormant. We also assumed that any activation of dormant cells during the exposure to the dyes was minimal (which seems reasonable considering the low fractions of active bacteria—see "Results" section) and similar for all samples (i.e., allowing comparisons among treatments). Because bacteria dominated the root-free soils considered in this study (see "Results" section), we suspect that measurements of bacterial activity are a reasonable indicator of overall soil microbial activity.

Microbial biomass and relative abundances of microbial groups

We measured microbial biomass, fungi:bacteria ratios, and Gram-positive:Gram-negative ratios with the phospholipid fatty acid (PLFA) method (Hurst et al. 1997). We estimated microbial biomass based on analysis of phospholipid phosphates ($PLPO_4$) and fungi:bacteria and Gram-positive:Gram-negative ratios based on analysis of phospholipids fatty acids (as in Acosta-Martinez et al. 1999). We extracted lipids from 5 g of soil (bags stored at 4 °C) using a chloroform/methanol/phosphate buffer and fractionated phospholipids using column chromatography. We measured $PLPO_4$ colorimetrically at 610 nm (DU®730 UV/VIS spectrophotometer, Beckman Coulter, Inc., Fullerton, CA) and fatty acids via gas chromatography–mass spectrometric detection (Agilent 7890, Agilent 5975 MSD, Agilent Technologies Inc., Santa Clara, CA).

We calculated fungi:bacteria ratio as the fungal PLFAs 18:2ω6, divided by the sum of the bacteria PLFAs 14:0, i15:0, a15:0, 15:0, 16:0, 10Me16:0, i17:0, a17:0, cy17:0, Me18:0, and cy19:0 (Bååth and Anderson 2003). Similarly, we calculated Gram-positive:Gram-negative ratios as the sum of Gram-positive PLFAs i15:0, a15:0, i17:0, and a17:0, divided by the sum of Gram-negative PLFAs cy19:0 and cy17:0 (Joynt et al. 2006).

The PLFA method does not allow a highly resolved analysis of the composition and structure of microbial communities in soil. However, previous studies have shown that respiration responses to the environments are conserved at fairly coarse phylogenetic scales (Lennon et al. 2012).

Statistical analysis

We first analyzed the effects of the warming and precipitation treatments (fixed effects) on R_H, using a mixed-effects model that included time as a fixed effect and block as a random effect. For this we used the *lmer* function from the *lme4* package (Bates et al. 2014) in R, version 3.3.1. We then used a multiple correlation analysis (*lm* function) to estimate how much of the seasonal (fixed effect) differences in R_H were explained by soil temperature, moisture, Total Microbial Biomass (TMB), Active Microbial Biomass (AMB), and the relative abundance of microbial groups (i.e., fungi:bacteria and Gram-positive:Gram-negative ratios). We used the *glmulti* function, from the *glmulti* package (Calcagno and de Mazancourt 2010), to select the best statistical model. We compared models based on the Bayesian Information Criterion (BIC), which accounts for differences in the number of explanatory variables among models.

Results

The warming and precipitation treatments affected environmental conditions, but had little effect on R_H in these relatively dry soils. Instead, R_H differed across seasons. These seasonal differences in R_H were explained better by temperature and the abundance of actively respiring cells than by environmental or microbial variables alone.

Effect of experimental warming on soil temperature

The warming treatments increased ($P < 0.05$; Table S1) soil temperature in both seasons (Fig. 1a, b). Soil temperature was affected by the precipitation treatments as well. Soil temperature was higher ($P < 0.05$) in the dry (and less plant-shaded) plots than in the ambient and wet plots, especially in the Fall ($P = 0.06$). In the summer, soil temperature ranged from 21.5 ± 1.2 °C in the unheated plots to 23.9 ± 2.0 °C in the high heated plots. In the fall, soil temperature ranged from 15.4 ± 1.5 °C in the unheated plots to 17.9 ± 1.7 °C in the high heated plots. Differences in soil temperature between unheated and high heated plots were ca. 2.5 °C in both seasons, while differences between seasons averaged ca. 6.0 °C. Overall, differences in soil temperature were larger between seasons than across warming treatments.

Effect of precipitation manipulation on soil moisture

Although there were differences ($P < 0.05$) in soil moisture across seasons and precipitation treatments (Fig. 1c, d; Table S2), soils were fairly dry (< 20% v/v) in all cases. Differences in soil moisture across treatments were larger ($P = 0.02$) in the fall than in the summer. In the fall, soils from the dry treatment (ca. 5% v/v) were drier than those from the ambient and wet plots (ca. 12% v/v in both precipitation treatments across all warming treatments). Averaged across treatments, soils were drier in the summer (6% v/v) than the fall (9% v/v; $P < 0.01$) (Fig. 1c, d).

Effects of warming and precipitation treatments on R_H

R_H did not differ ($P > 0.05$) across the warming and precipitation treatments but it differed ($P < 0.05$) between seasons (Fig. 1e, f; Table S3). In the summer, R_H averaged 2.88 ± 0.30 μmol m^{-2} s^{-1} across all the warming and precipitation treatments. By the fall, average R_H (1.16 ± 0.05 μmol m^{-2} s^{-1}) had decreased ($P < 0.05$) by more than 50%. Although in the fall R_H tended to increase from dry to wet plots (Fig. 1f), this trend (like all other differences across treatments) was not statistically significant (Table S3).

Effects of warming and precipitation treatments on microbial parameters

Microbial parameters differed between seasons but were unaffected or weakly affected by treatments. Although microbial biomass, active microbial biomass and fungi:bacteria ratios were marginally affected by experimental warming (Tables S4-6), differences in microbial parameters across treatments were small in comparison with the marked differences between seasons. From summer to fall, microbial biomass and Gram positive:Gram negative ratios increased by 60% (Fig. 2a, b; Table S4) and 16% (Fig. 2g, h; Table S7), respectively; and active microbial biomass and fungi:bacteria ratios decreased by 40% (Fig. 2c, d; Table S5) and 24% (Fig. 2e, f; Table S6), respectively.

Predictors of seasonal R_H

Seasonal differences in R_H were primarily explained by temperature and the abundance of metabolically active microbes in soil (Table 1). The statistical model that best fitted our data ($P < 0.05$, BIC = 69.7; Table S8) surprisingly suggests that decreases in R_H between the summer and fall were associated with increases ($P < 0.05$) in TMB, TMB having a different influence on R_H in each season ($P < 0.05$). For reasons that we discuss below, we also analyzed the second-best statistical model ($P < 0.05$, BIC = 81.5), which suggests that seasonal decreases in R_H from summer to fall were associated with decreases in temperature and in the abundance of actively respiring cells in soil (Table 1).

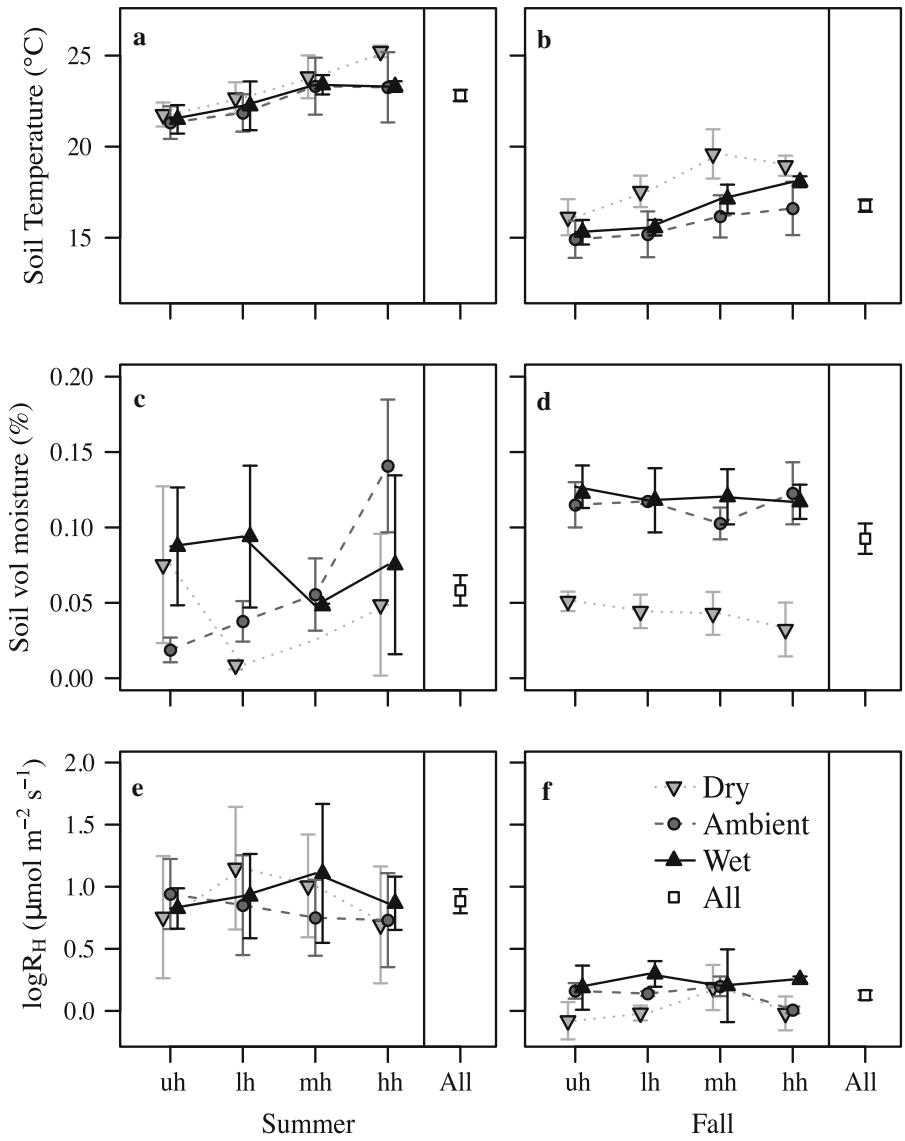

Fig. 1 Soil temperature (**a**, **b**), moisture (**c**, **d**), and R_H (**e**, **f**) in summer (**a**, **c**, **e**) and fall (**b**, **d**, **f**) across warming and precipitation treatments. uh: unheated, lh: low heat, mh: medium heat, and hh: high heat. Statistics in Tables S1, S2, and S3. Values are mean ± SE. No error bar is shown in the mh-dry treatment in panel *c* because of missing data

Overall, temperature and moisture explained seasonal soil respiration better than microbial processes alone, but incorporation of microbial data increased explanatory power (Fig. 3). Our results suggest that, on average, log(R_H) increased by 0.11 μmol m^{-2} s^{-1} per 1 °C increase in soil temperature (Table 1) and that the magnitude of this response increased with the abundance of active microbes in soil (Fig. 4). This model explained 35% (adjusted R^2) of the variation in R_H.

Discussion

Recently, the policy relevance of soil C-climate feedbacks has motivated a wide range of research on mechanisms that regulate soil C cycling, and their

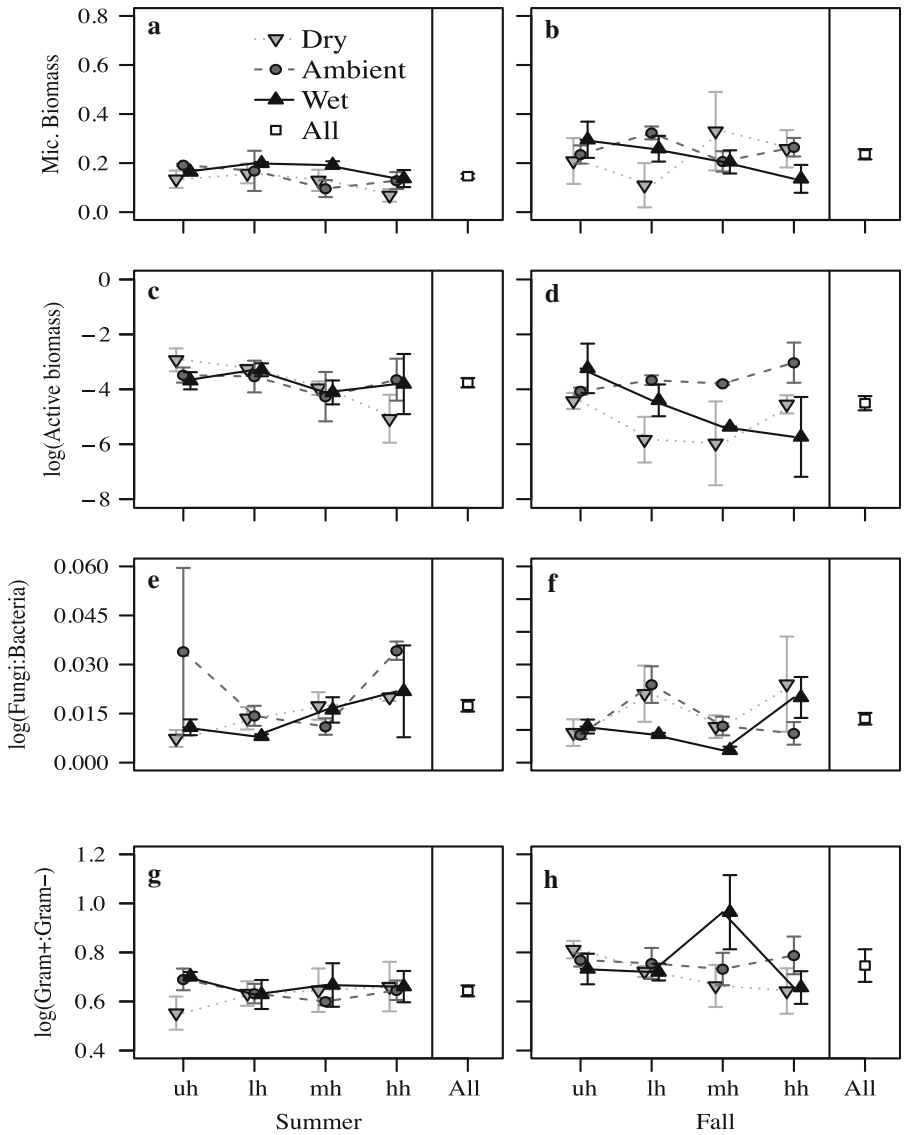

Fig. 2 Microbial biomass (**a**, **b**; in phospholipid phosphate g^{-1} soil), Active biomass (**c**, **d**; in phospholipid phosphate g^{-1} soil), Fungi:Bacteria ratios (**e**, **f**), and Gram positive:Gram negative ratios (**g**, **h**), in summer (**a**, **c**, **e**, **g**) and fall (**b**, **d**, **f**, **h**) across warming and precipitation treatments. uh: unheated, lh: low heat, mh: medium heat, and hh: high heat. Statistics in Tables S4, S5, S6, and S7. AMB, and Fungi:Bacteria and Gram positive:Gram negative ratios in statistical models were log transformed to meet assumptions. Values are mean ± SE. No error bars in lh-ambient treatment in panels *a* and *c* because of missing data

associated temporal scales. One of those mechanisms is the metabolic activation and deactivation of soil microbes in response to favorable and stressful environmental conditions. Previous studies have demonstrated that soil respiratory responses to temperature and moisture at a temporal scale of hours to days are associated with microbes switching between active and dormant metabolic states (Placella et al. 2012; Barnard et al. 2015; Salazar-Villegas et al. 2016). However, less is known about the importance of these mechanisms over longer timescales. The results of this study suggest that the abundance of active microbes in soil changes at the seasonal scale too, and that these shifts affect soil respiration rates.

The abundances of total and active microbes in soil can change across seasons. In our study, AMB (and

Table 1 Statistics of the best explanatory model for seasonal R_H

	Estimate	SE	t-value	P
Intercept	−0.894	0.614	−1.455	0.152
Temp	0.107	0.025	4.254	< 0.001***
Temp:moisture	−7.429	6.058	−1.226	0.226
Temp:log(AMB)	0.008	0.003	2.730	0.009**

Significance codes: P < 0.001'***', 0.001 < P < 0.01'**'

R_H) was greater in the summer than in the fall. However, TMB was greater in the fall than in the summer. Increases in TMB between June and October could have been partially caused by increases in soil moisture, which likely facilitated access of microbes to nutrients. Increases in soil moisture during the still warm end of the summer could have stimulated microbial growth, but decreases in temperature in the fall likely induced a large proportion of microbes in the soil to enter dormancy. This could explain why AMB was higher in the summer than in the fall, even though TMB was lower. We know of only one other study that simultaneously monitored TMB and AMB at the seasonal scale. In it, Van de Werf and Verstraete (1987) found TMB to increase by 29% from June to August in a fallow topsoil, while AMB remained practically unchanged (Van de Werf and Verstraete 1987). However, in winter-wheat soil both TMB and AMB increased from June to August (Van de Werf and Verstraete 1987). This suggests that seasonal changes in TMB and AMB can also be affected by soil type and/or agricultural practices (see also Girvan et al. 2003). Similarly, in the first year of a 2-year

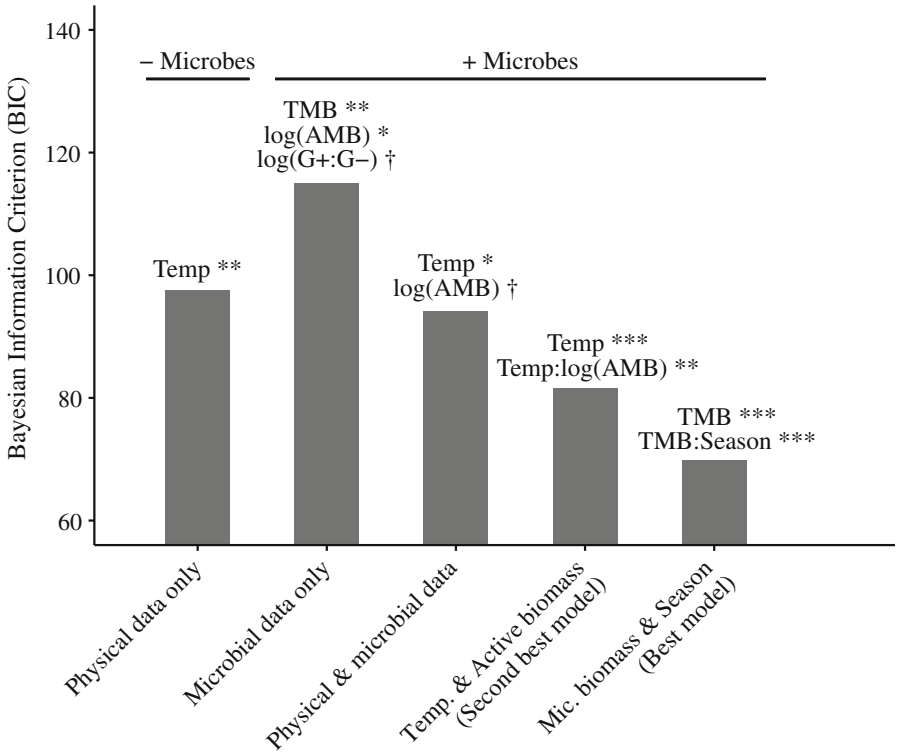

Fig. 3 Goodness of fit, as measured by the Bayesian Information Criterion (BIC), of models of log(R_H) that include different categories of explanatory variables. Lower scores indicate better model fits. Categories include models fitting log(R_H) as a function of only physical conditions (as a function of temperature and moisture; statistics in Table S10); only values related to microbes: TMB, log(AMB), log(Fungi:Bacteria), and log(Gram-positive:Gram-negative); statistics in Table S9); with microbes and physical conditions (as a function of temperature, moisture, TMB, log(AMB), log(Fungi:Bacteria), and log(Gram-positive:Gram-negative); statistics in Table S11); the second best model (as a function of temperature and the interaction between temperature and log(AMB); statistics in Table 1); and the best (but see discussion) model (as a function of TMB, and the interactions between log(AMB) and moisture, and TMB and season; statistics in Table S8). Significance codes: $P < 0.001$'***', $0.001 < P < 0.01$'**', $0.01 < P < 0.05$'*', $0.5 < P < < 0.1$'†'

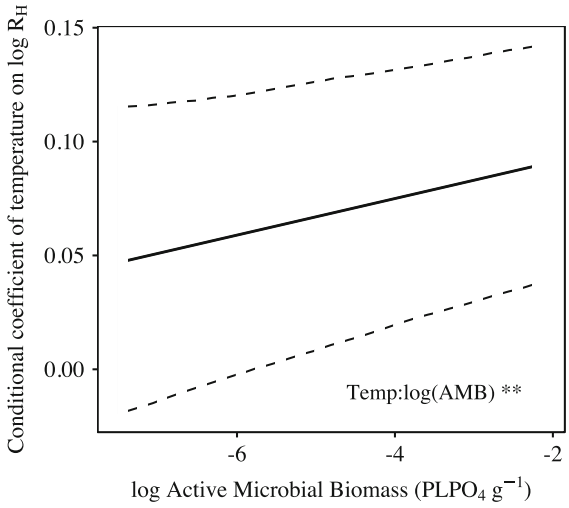

Fig. 4 Changes in the coefficient of soil temperature, in the two-way interaction term with AMB (Table 1), conditional on AMB (*interplot* function in R). AMB in statistical model was log transformed to meet assumptions. Dashed lines indicate 95% confidence intervals

warming study in a temperate forest (Schindlbacher et al. 2011), microbial biomass increased (both in heated and unheated treatments) by 30% from July to September, while microbial metabolic activity (measured as soil respiration rates per concentration of microbial biomass) decreased by 50%. Interestingly, in the second year, these trends reversed (Schindlbacher et al. 2011). Together, our results and these observations show that the amount of total and active microbial biomass in soil do not necessarily change in the same direction and at the same time across seasons.

Seasonal changes in microbial biomass can happen in parallel with changes in community composition. In our study, increases in TMB (and decreases in AMB) between summer and fall were accompanied, on average, by a decrease in fungi:bacteria ratios (Fig. 2e, f) and an increase in Gram positive:Gram negative ratios (Fig. 2g, h). The change in fungi:bacteria ratio could have been caused by warmer summer temperatures favoring fungi over bacteria (Zhang et al. 2005; Castro et al. 2010) and/or by faster bacterial growth between the summer and the fall as soil moisture increased (Fig. 1c, d). Although fungi usually play a key role in soil C cycling and can dominate the microbial community (Baldrian et al. 2012), the root-free soil from this experiment was dominated by bacteria (fungi:bacteria ratios were always < 0.1). Overall, our results suggest that changes within the bacterial community may have been more important for soil C cycling than relative changes in the abundance of fungi and bacteria.

Although in our study R_H did not respond to climate treatments, it differed between seasons, possibly due to the larger variation in temperature and moisture. For example, while differences in soil temperature between unheated and high heated plots were ca. 2.5 °C, between seasons they averaged 6.0 °C. Our multiple regression analysis suggests a few alternative, and possibly complementary, explanations for how the microbial parameters discussed above contribute to seasonal R_H. The statistical model that best fitted our data suggests a relationship between seasonal decreases in R_H with increases in TMB. This inverse relationship between TMB and R_H could result from seasonal differences in microbial carbon use efficiency (Tucker et al. 2013)—e.g. less C being respired and more being incorporated as microbial biomass in the cooler and wetter fall than in the drier and warmer summer. Alternatively, an inverse relationship between TMB and R_H could reflect pulses of R_H caused by active microbes recycling necromass C (Geyer et al. 2016). It is plausible that the more severe dryness in the summer than in the fall in our study, led to elevated microbial mortality and therefore to a larger abundance of necromass C accessible to active microbes. However, given the capacity of microbes to adjust their metabolism and remain viable under stressful conditions, the contributions of cell lysis to soil C fluxes is probably insignificant (Halverson et al. 2000). We do not know of any other biological process that could explain this result and therefore recommend caution when interpreting its causality. On the other hand, the statistical model that provided the second-best fit to our data suggests that seasonal decreases in R_H from summer to fall were driven by decreases in soil temperature and in the abundance of metabolically active microbes in soil. This is consistent with theory of microbial physiology (Stenström et al. 2001; Schimel et al. 2007; Lennon and Jones 2011) and with experiments conducted at short temporal scales (Placella et al. 2012; Aanderud et al. 2015; Barnard et al. 2015; Salazar-Villegas et al. 2016). If our one-time measurements of active/dormant biomass from summer and fall are representative of the respective seasonal averages, our results would indicate that temperature and microbial metabolism data alone are powerful in predicting seasonal R_H.

Although Gram-positive:Gram-negative ratios did not contribute to the best-fitting models of R_H (Tables 1, S8), changes within the bacterial community could help to explain the relationship between AMB and R_H. Increases in Gram-positive:Gram-negative ratio from summer to fall could have been associated with different capabilities of the bacterial groups to cope with moisture stress. Gram-positive bacteria have a peptidoglycan-rich cell wall that makes them more resistant to dry conditions than Gram-negative bacteria (Halverson et al. 2000; Fuchslueger et al. 2014). Considering that soils in our experiment were relatively dry (< 20% v/v) in both seasons, it is likely that Gram-negative bacteria were more severely affected by moisture stress than their thick-cell-wall counterparts. Some Gram-negative bacteria have higher maximum respiration rates than Gram-positive bacteria (e.g. Acidobacteria vs. Actinobacteria, respectively; Lennon et al. 2012). It is possible that from summer to fall there were larger decreases in the abundance of metabolically active microbes with high maximum respiration rates but low resistance to dryness (e.g. Acidobacteria), relative to bacterial groups with low maximum respiration rates but high resistance to dryness (e.g. Actinobacteria). However, not all Gram-negative bacteria have higher maximum respiration rates than Gram-positive bacteria. Gram-positive Firmicutes have higher maximum respiration rates than Gram-negative Acidobacteria, Bacteroidetes and Proteobacteria (Lennon et al. 2012). Therefore, decreases in R_H between summer and fall could also have been associated with metabolic deactivation of microbes with high maximum respiration rates and high resistance to dryness. We would need a more resolved composition analysis to know which (if any) of these alternative explanations was the case in our study. Nonetheless, our results suggest that, as soil moisture levels change, the abundance of microbial groups with different levels of resistance to dryness could influence the size of the microbial pool that remains metabolically active.

Finally, our findings suggest that the effect of temperature on R_H gets stronger with the abundance of metabolically active microbes in soil. Microbes that are pushed to enter dormancy by moisture and/or nutrient limitations are practically unaffected by changes in temperature. However, the metabolic rates (e.g. respiration) of active microbes are sensitive to temperature (Anderson and Domsch 1985), and therefore it is reasonable to expect that a greater abundance of active microbes in soil makes R_H more sensitive to temperature. Given that the soils used in this study were substantially depleted of labile organic matter, a constant input of labile carbon (e.g. via plant roots) would have probably ameliorated nutrient limitations and increased the proportion of active microbes across all climate treatments. Also, depending on input concentrations, it could have stimulated fungal and bacterial growth and/or metabolic rates (De Graaff et al. 2010). Considering this and the increased effect of temperature on R_H with active microbial biomass, we speculate that a constant input of labile C in C-depleted soils such as the ones used in this study would make R_H even more responsive to temperature. This builds on previous observations of R_H being less sensitive to warming (and precipitation) under dry, presumably water- and nutrient-limited conditions (Schindlbacher et al. 2012; Suseela et al. 2012; Koyama et al. 2018).

In summary, we found that (1) seasonal changes in total microbial biomass in soil do not necessarily reflect changes in the amount of microbial biomass that is metabolically active and capable of driving soil C processes, (2) the metabolic state of soil microbial communities can be more important for seasonal R_H than the relative abundances of microbial groups such as fungi and bacteria (Gram-positive and Gram-negative), and (3) the magnitude of the temperature effect on R_H increases with the abundance of metabolically active microbes in soil. This work builds on recent research distinguishing active from dormant microbes and highlighting the importance of the metabolically active community for microbe-driven processes. Although few studies to date have linked microbial metabolic state patterns with rates of soil CO_2 efflux, our findings suggest the possibility that recent increases in global soil respiration rates could be linked to climate-driven increases in the abundance of metabolically active microbes in soil.

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