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Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter?

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Abstract Climate models project that precipitation patterns will likely intensify in the future, resulting in increased duration of droughts and increased frequency of large soil rewetting events, which are stressful to the microorganisms that drive soil biogeochemical cycling. Historical conditions can affect contemporary microbial responses to environmental factors through the persistence of abiotic changes or through the selection of a more tolerant microbial community. We examined how a history of intensified rainfall would alter microbial functional response to drying and rewetting events, whether this historical legacy was mediated through altered microbial community composition, and how long community and functional legacies persisted under similar conditions. We collected soils from a long-term field manipulation (Rainfall Manipulation Plot Study) in Kansas, USA, where rainfall variability was experimentally amplified. We measured respiration, microbial biomass, fungal:bacterial ratios and bacterial community composition after collecting soils from the field experiment, and after subjecting them to a series of drying–rewetting pulses in the lab. Although rainfall history affected respiration and microbial biomass, the differences between field treatments did not persist throughout our 115-day drying–rewetting incubation. However, soils accustomed to more extreme rainfall did change less in response to lab moisture pulses. In contrast, bacterial community composition did not differ between rainfall manipulation treatments, but became more dissimilar in response to drying–rewetting pulses depending on their previous field conditions. Our results suggest that environmental history can affect contemporary rates of biogeochemical processes both through changes in abiotic drivers and through changes in microbial community structure. However, the extremity of the disturbance and the mechanism through which historical legacies occur may influence how long they persist, which determines the importance of these effects for biogeochemical cycling.

Keywords Drying–rewetting · Historical legacies · Lags · Precipitation variability · Stress · Resistance · Microbial community composition · RaMPS experiment

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Introduction

While soil moisture is an eminent control on the rates of biogeochemical processes in all terrestrial

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ecosystems, responses to moisture pulses driven by dynamic precipitation patterns are especially complex and difficult to predict (Collins et al. 2008). These drying–rewetting events can result in large pulses of soil CO_2 efflux that can strongly impact net ecosystem carbon (C) balance (Austin et al. 2004; Birch 1958) and in increased nitrogen (N) leaching (Gordon et al. 2008; Miller et al. 2005). Earth system climate models predict an impending intensification of the hydrologic cycle which will result in longer dry periods and more intense rainfall events (Huntington 2006). Under these conditions, the role of moisture pulses in regulating ecosystem function may become increasingly important, and changes in rainfall timing may alter the relationships between mean annual precipitation and rates of ecosystem processes (Knapp et al. 2002).

Since soil microorganisms are key drivers of biogeochemical cycling, the way they respond to changes in rainfall timing could be an important factor for predicting changes in ecosystem processes. Sudden changes in moisture are stressful to microbes, as they must expend energy to regulate osmotic pressure to their microenvironment. To achieve osmotic regulation as soils dry, many microbes synthesize solutes such as polyols and amino acids (Csonka 1989). As soil water potential increases rapidly after precipitation events, microbes must release solutes before osmotic pressure bursts cells (Wood et al. 2001). Fungi and bacteria have a wide range of tolerances to moisture stress, and have adopted many different strategies to cope with this stress (Van Gestel et al. 1993; Schimel et al. 1999). For example, fungi may be more drought tolerant than bacteria (with the exception of actinomycetes) because their hyphae can transfer moisture from water-filled micropores (de Boer et al. 2005; Harris 1981) whereas bacteria require water films for motility and substrate diffusion. These constitutive physiological adaptations to moisture pulses require a large investment of resources, and are likely to reduce population fitness in environments where they are less important to survival (Schimel et al. 2007). Therefore, as precipitation regimes intensify, frequent and extreme drying–rewetting events may select for microbial taxa that are more tolerant to desiccation stress, and these changes may result in a community that responds differently to moisture stress. On the other hand, the frequency of large magnitude drying–rewetting events may not drive changes in community composition or function: selection for stress tolerant taxa may occur with even a single drying–rewetting event and may persist over a period of years.

A ubiquitous underlying assumption for microbial communities is that fast turnover and widespread dispersion precludes any influence of antecedent conditions on contemporary structure and function (Allison and Martiny 2008). However, there is a growing body of evidence suggesting that, like plant communities, historical conditions influence responses of microbial communities to their environment (Waldrop and Firestone 2006; Fierer et al. 2003; Stres et al. 2010; Van Gestel et al. 1993). Although temporal lags in process rates could simply be mediated by the persistent changes of the drivers of microbial function, such as substrate quality or quantity, soil texture, or even moisture (through ecosystem water storage), these findings suggest that altered biotic potential through persistent changes in microbial community composition could be an additional mechanism fostering historical legacies. Indeed, microbial communities previously exposed to disturbances such as precipitation stress (Fierer et al. 2003), freeze–thaw cycles (Schimel et al. 2007; Stres et al. 2010), or redox fluctuations (De Angelis et al. 2010) have proven more resistant to these stresses than those that have not. In this way, whole microbial communities may "adapt" to a particular environment, and resultant shifts in community-level traits may alter relationships between environmental factors and function. Further, the timescale on which these legacies persist could determine their contribution to biogeochemical feedbacks and will influence our ability to predict ecosystem responses to novel climate regimes (Allison and Martiny 2008).

We were interested in isolating the effects of a single environmental change—intensified rainfall patterns—and testing whether historical exposure to these conditions altered microbial responses to drying–rewetting events that are more commonly experienced under the intensified precipitation regime. For this study, we collected soils from the Rainfall Manipulation Plot Study (RaMPS) in the U.S. tallgrass prairie, where the timing and quantity of precipitation events had been experimentally altered to simulate a more extreme rainfall regime (fewer, larger rainfall events separated by longer dry periods) for the previous 10 years. Harper et al. (2005) reported that the

experimentally increased duration of drought and intensity of rainfall events at this site led to a reduction in mean annual soil respiration. Soil moisture explained less than half of the variation in respiration rates, and although decreased plant C inputs was hypothesized to influence reduced respiration (Fay et al. 2002), the authors suggest that changes in whole-community microbial responses, brought on by the stress of the precipitation manipulation, may also be affecting respiration rates. It is unknown how the long-term modifications to the timing and magnitude of discrete rainfall events have altered microbial community composition and function in this experiment, whether community-level adaptations to climate persist in microbial communities, and whether microbial adaptation to precipitation regimes can affect soil respiration. With a coupled field-lab experiment, we were able to examine whether precipitation history altered functional response to drying–rewetting through persistent changes in environmental drivers or through community-level microbial adaptation either to precipitation changes or other environmental variables altered by precipitation.

We hypothesized that a history of rainfall intensification would cause changes in microbial respiration in response to drying–rewetting due to persistent changes in microbial community composition. As species sensitive to drying–rewetting would have already died or decreased in abundance, and tolerant species would remain, we predicted that soils that experienced altered rainfall timing would change less in response to drying–rewetting pulses in the lab, but that functional and compositional differences among field treatments would subside after soils are subjected to the same conditions for the duration of the 4-pulse incubation (115 days).

Methods

In order to test how different precipitation histories affect the response of soil microbial communities to drying–rewetting pulses, we subjected soils from an existing long-term rainfall manipulation in the tallgrass prairie to controlled drying–rewetting pulses in the lab, monitoring the function and composition of the community throughout the lab incubation (Fig. 1).

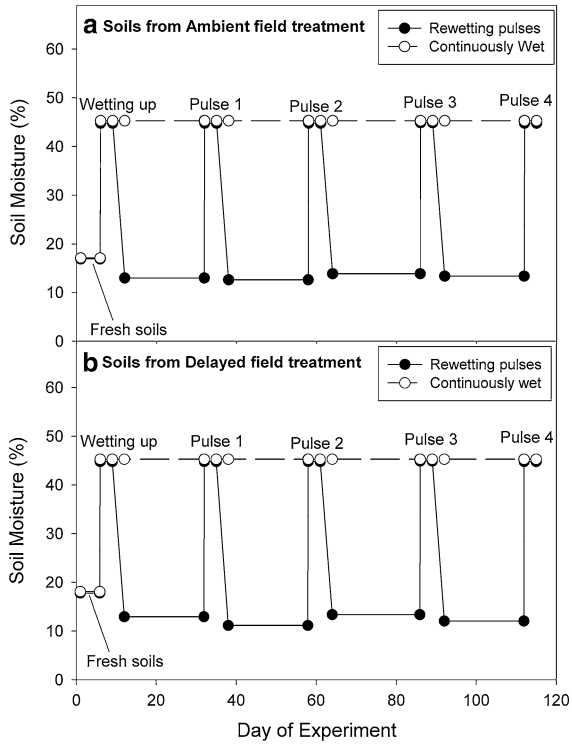

Fig. 1 Average soil moisture in lab incubation treatments throughout the experiment and time points of sample. Soils from Ambient (**a**) and Delayed (**b**) field manipulations were equivalently subject to either drying–rewetting pulses (*filled circles*, *solid line*) or kept continuously wet (*open circles*, *dashed line*). *Error bars* represent standard error of mean soils moisture at that time point, but often smaller than symbol

Field site and sampling

We sampled soils from the RaMPS at Konza Prairie Biological station in northeast Kansas (Fay et al. 2000). Twelve 7.6 × 7.6 m^2 plots were established in 1997 on annually burned native tallgrass prairie. In six "Delayed" rainfall treatment plots, rainfall timing was altered such that the dry periods were 50% longer than ambient conditions. Irrigation systems then re-applied all ambient rainfall that occurred in that period, creating larger, but less frequent, rainfall events in Delayed plots (Fay et al. 2000). Two cores were taken from each RaMPS plot in late December 2007, and homogenized to pass a 2 mm sieve. Soils from 0 to 10 cm depths were sent to Colorado State University and stored at −10°C until lab analysis.

Lab incubation

In early 2009, we set up a lab incubation that exposed soils from both field treatments to four drying and rewetting events that mimicked the conditions experienced for 10 years under the Delayed treatment in the field (Fig. 1). Pseudoreplicate cores from each plot were combined, and soils were thawed and allowed to thermally equilibrate over 5 days at 25°C. Initial soil moisture and water holding capacity (WHC) were determined on a small subsample of soil from each field plot. Incubations were run in duplicate; approximately 5 g soil was placed in sterile 50 ml tubes with septa in the lids to facilitate gas measurements. After temperature equilibration, we brought all soils to 45% gravimetric soil moisture using sterile distilled H_2O, and allowed them to incubate at this moisture with the caps on for 3 days. We then placed all tubes subject to drying–rewetting pulses in a fume hood with their lids off to air-dry for 3 days. We chose to wet up soils to 45% soil moisture (by weight) and allow 20 days between moisture pulses because these were average values obtained from 1998 to 2002 field data under the Delayed rainfall treatments at Konza Biological Station. Control ("continuously wet") treatments were not dried out and kept at this soil moisture for the duration of the experiment, and served as a comparison to dried and rewet samples to account for successional changes in microbial and soil properties over the course of the experiment. We subject dried–rewet soils to a total of four drying–rewetting periods, destructively harvesting samples from initial soils (Fresh, field-moist), after the initial wetting up period (field-moist soils brought to 45% soil moisture), after the first rewetting pulse (Pulse 1), and after the last rewetting pulse (Pulse 4) (Fig. 1). With the exception of the Fresh soils, all samples were harvested on the third day of incubation after the 45% soil moisture pulse, in order to facilitate comparisons among each time point and to the continuously wet control.

Respiration readings

We measured soil respiration rates by analyzing the accumulation of CO_2 in the headspace of the 50 ml tubes with a LiCor Infrared Gas Analyzer (IRGA). Readings were taken during the 3 days after a moisture pulse, and approximately weekly throughout the experiment on the continuously wet control.

Microbial biomass

Microbial biomass was determined by chloroform fumigation extractions (Vance et al. 1987). We placed a 4 g soil subsample into an acid-washed 50 ml tube and fumigated with chloroform for 5 days, while another 4 g subsample that was not fumigated acted as a control. Dissolved C and N were extracted from both subsamples by shaking 4 g soil subsamples in 10 ml of 0.5 M K_2SO_4 for 2 h then filtering through #40 Whatman filter papers. Extractions were analyzed on a Shimadzu TOC analyzer. Microbial biomass was determined by subtracting C and N in fumigated samples from non-fumigated control, and no correction factors were applied. Extractable C and N values were obtained from the non-fumigated control samples.

Quantitative PCR

We extracted soil DNA from each sample using the Power Soil DNA Isolation Kit (MoBio, Carlsbad, CA) according to the instructions of the manufacturer. We performed quantitative PCR reactions in triplicate using 96-well plates on an iCycler iQ thermal cycler (BioRad). Reactions consisted of 12.5 µl of Absolute QPCR SYBR Green mix (ABgene), 2.5 µl of 5 ng/µl bovine serum albumin (BSA), 0.25 µl of a 10 µM mixture of each primer (final volume 0.1 µM), 5 µl of template DNA, and PCR-grade H_2O to a final volume of 25 µl. For 16S rRNA bacterial genes, we used EUB338 (Lane 1991) and Eub518 (Muyzer et al. 1993) at an annealing temperature of 55°C; for fungal rRNA genes we used ITS1f (Gardes and Bruns 1993) and 5.8 s (Vilgalys and Hester 1990) at an annealing temperature of 53°C. Other conditions included: 95°C for 2 min, followed by 40 cycles of 95°C for 15 s, annealing temperature for 30 s, and 72°C for 30 s. We diluted DNA to 1 ng/µl for bacterial assays and 5 ng/µl for fungal assays, and adjusted to report copies per ng DNA.

We generated melting curves for each run to verify product specificity by increasing the temperature from 55 to 95°C. Standards were run in triplicate in each assay, and standard curves were developed using a serial dilution of genomic DNA extracted from pure

cultures. For all quantitative PCR assays there was a linear relationship between the log of the standard copy number and the calculated threshold cycle across the standard concentration range ($R^2 > 0.95$ in all cases).

Pyrosequencing of bacterial communities

We analyzed the bacterial community structure of Fresh, Pulse 1, and Pulse 4 soils (see Fig. 1) using a pyrosequencing-based analysis of the 16S rRNA gene in total soil DNA as described in Fierer et al. (2008). We amplified the 27–338 portion of the 16S rRNA gene using error-correcting bar-coded primers (Hamady et al. 2008). The forward primer contained a Roche 454 'A' pyrosequencing adapter, connected with a TC linker, and each reverse primer contained a unique 12-bp bar-coded sequence, Roche 454 'B' sequencing adapter, and a TC linker. PCR reactions were conducted with 0.5 μl (10 μM) of each forward and reverse primer, 3 μl template DNA, and 22.5 μl Platinum PCR SuperMix (Invitrogen, Carlsbad, CA), similar to Fierer et al. (2008). We amplified samples in triplicate, and pooled and cleaned them using a PCR Cleanup Kit (MoBio Laboratories, Carlsbad, CA), then sequenced them on a Roche FLX 454 pyrosequencing machine at the Environmental Genomics Core Facility at the University of South Carolina. Of 36 samples intended for pyrosequencing, 5 samples did not successfully amplify and therefore were not included in the 31 pooled barcoded samples submitted for sequencing.

We followed previously-described protocols to analyze pyrosequencing data (Fierer et al. 2008; Hamady et al. 2008; Lauber et al. 2009) using QIIME (Caporaso et al. 2010b). We first removed sequences <200 bp and with a quality score <25. We identified OTU's as 97% similarity and used the most abundant sequence per OTU as representative of that OTU. We aligned sequences using PyNAST (Caporaso et al. 2010a) and assigned taxonomies to sequences representative of each phylotype using the RDP Classifier (Wang et al. 2007).

Data analysis

We aimed to test how microbial communities from two different rainfall manipulations responded to a series of moisture pulses in the laboratory. Our experimental design consisted of 3 factors: field treatment (2 levels, Delayed and Ambient, fixed), time point in the lab incubation (4 levels, fixed), and a treatment by time point interaction, with 6 field replicates. To analyze univariate data, we first log-transformed data for certain variables (Microbial biomass C and N, Extractable C and N, fungal:bacterial ratio) to adjust for unequal variances. We then used a repeated measures model (SAS, proc mixed) to account for the correlation among plots over time in our lab incubation, with plots nested within treatment. When significant differences occurred in an ANOVA, we compared treatments separately within a time point and compared time points within treatments. We also used this model to compare changes in individual taxonomic groups in our community analysis.

To quantify how field treatments differed in variability in response to moisture pulses in the lab, we calculated the proportional change in response variable (Y) from one moisture pulse to the next $([Y_{t+1} - Y_t]/Y_t)$ for each sample. We also calculated the proportional change between Pulse 4 and the continuously wet control $([Y_{Pulse4} - Y_{Wet}]/Y_{Wet})$, which were measured at the same time point (the conclusion of the experiment), to describe the integrated effect of drying–rewetting compared to a continuously wet incubation, and the coefficient of variation (standard deviation divided by absolute value of the mean) to describe the samples' total variability throughout the lab incubation. We then compared Ambient and Delayed groups within the same univariate model as above.

To describe beta diversity and still account for differences in the number of sequences per sample, we constructed rarefaction curves that describe how the number of unique phylotypes (<97% sequence similarity) increased as sequences in a sample increased. We determined similarity of overall community composition among samples using Unifrac (Lozupone and Knight 2005). Unifrac calculates the fraction of branch length unique to a sample or environment compared to overall branch length, computing similarity distances using only presence or absence of a phylotype (unweighted), and including abundance of phylotype (weighted). The use of this distance metric allowed us to consider the phylogenetic relationship of groups when determining the similarity of one community to another.

After removing outliers, we created ordinations with Unifrac distances using Non-metric multidimensional scaling (NMDS) with the remaining 27 samples ($N = 3$–6 in each group), and tested for significance of differences between communities in different treatments and across time points using PerMANOVA (Anderson 2001) in Primer v6. PerMANOVA is a permutation-based multivariate analysis that can accommodate more complex and unbalanced sampling designs. This test calculates a pseudo F-statistic by comparing the total variance explained by sample identities (i.e. Time, Treatment) to that explained by random permutations of sample identities. As with univariate data from the same design, we tested the effect of Time (fixed), Treatment (fixed), Time × Treatment, and nested plots within treatments (random) on community similarity, and examined significance of pairwise comparisons within both Time and Treatment compared to 9,999 permutations. To examine more specific species responses, we also performed a Similarity Percentage (SIMPER) analysis (Clarke and Gorley 2006) to identify the relative contribution of each species to the differences in groups we observed using PerMANOVA.

Results

To determine whether a history of altered rainfall timing affected microbial community response to drying–rewetting, we measured variables that describe both the functional response and changes in the composition of microbial communities. We were interested in whether differences caused by rainfall manipulation persisted in the lab, whether this persistence could be explained by environmental variables or microbial community composition, and if a history of this stress caused variables to fluctuate less in response to moisture pulses.

Respiration

Respiration rates were highest in both Ambient and Delayed soils at the beginning of the lab incubation, and respiration pulses were smaller with each subsequent moisture pulse (Fig. 2). Soils from Ambient field treatments showed significantly higher respiration rates at the initial Wetting up period and after the second drying–rewetting pulse. Dry–rewet soils showed higher respiration pulses than continuously wet soil at the beginning of the experiment, but both the difference between field treatments (Ambient and Delayed) and the difference between pulsed and continuously wet soils was small at the end of the 115-day incubation (Pulse 4).

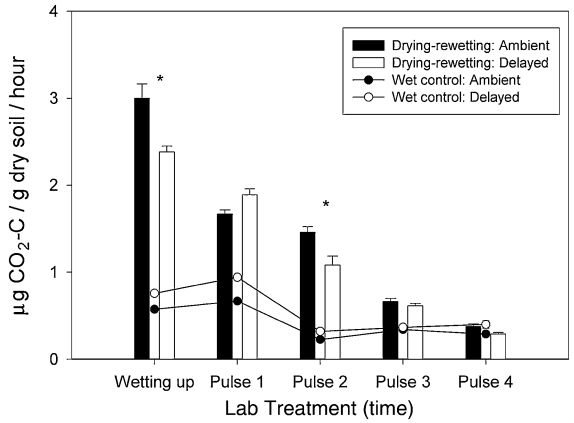

Fig. 2 Average respiration rate for soils from Ambient (*filled*) and Delayed (*open*) field plots when subject to drying rewetting pulses (*bars*) and continuously wet incubation (*symbols*). Rates for drying–rewetting incubations were calculated for the first 48 h after receiving each moisture pulse. *A significant difference ($P < 0.05$) between Ambient and Delayed treatments within that time point. *Error bars* are standard error for means ($N = 6$)

Microbial biomass

Historical treatment (Ambient or Delayed) also affected microbial biomass, but there were no significant differences by the end of the incubation. Microbial biomass C and N were significantly higher under Delayed rainfall timing manipulations at the time of sampling compared to soils from Ambient plots (Fig. 3), and responded differently to drying and rewetting in the lab. Microbial C increased after the first pulse in Ambient soils but was reduced by Pulse 4. Microbial N increased in Delayed soils after the first pulse but decreased in Ambient soils and in subsequent moisture pulses. Microbial C in Delayed soils was relatively unchanged by moisture pulses, but Microbial N in Delayed was more variable than Ambient across time points (Fig. 3; Table 1).

Extractable organic carbon (EOC) and nitrogen (EN)

There was a large increase of EOC (but not EN) during the first moisture pulse, especially in Ambient soils,

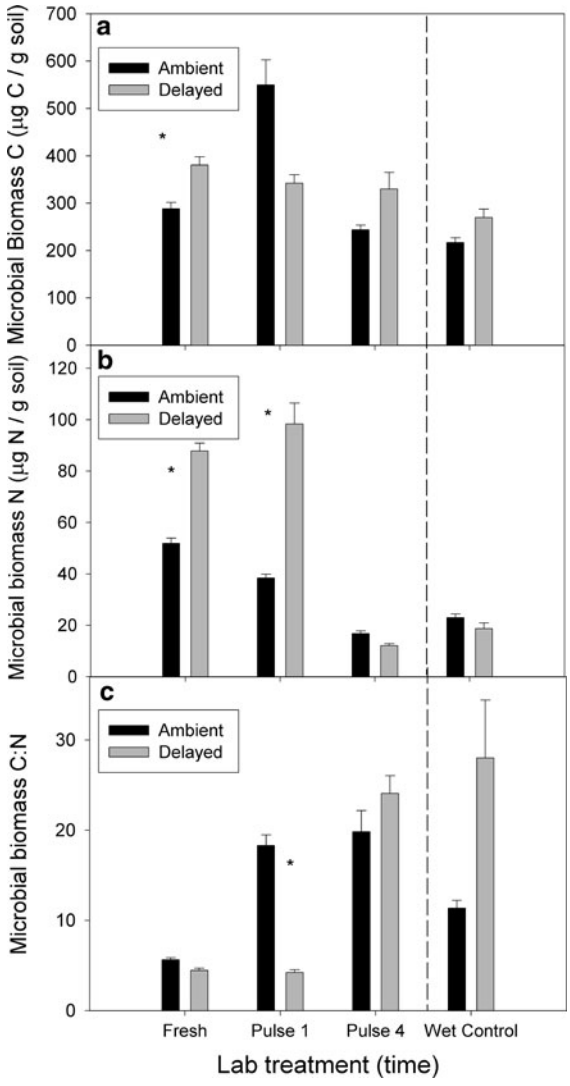

Fig. 3 Microbial biomass carbon (**a**), nitrogen (**b**) and carbon:nitrogen (**c**) throughout lab treatment as determined by chloroform-fumigation extractions of wet soil 3 days after soils from two field treatments received a moisture pulse. *A significant difference ($P < 0.05$) between Ambient and Delayed treatments within that time point. *Error bars* are standard error for means ($N = 6$)

and a later (Pulse 4) increase of N in soils from both field manipulations (Fig. 4). There was not a significant difference between EOC or EN in soils that had undergone drying rewetting pulses and those that were continuously wet, or significant differences between field treatments within any one time point. However, soils that experienced drying–rewetting in the field did have less variation in EOC in response to lab pulses (Fig. 4; Table 1).

Fungal:bacterial ratio

Soils that experienced Delayed rainfall timing had a higher fungal:bacterial ratios in Fresh soils and after Pulse 1 (Fig. 5). Fungal:bacterial ratio increased in both field treatments as pulses progressed in the lab, and pulsed soils had a higher ratio than soils kept in continuously wet conditions. Ambient soils changed more over the course of the lab incubation (Fig. 5; Table 1) and there were no significant differences in field treatments at the end of the incubation in soils that experienced drying–rewetting or between field treatments in the continuously wet incubation.

Bacterial community

Pyrosequencing resulted in 99,048 sequences and 14,207 unique phylotypes (1 phylotype = 97% similarity). Sequences per sample ranged from 41 to 7,485 with an average of 3,302. Four samples were removed from the community similarity and diversity analysis because they were outliers in our NMDS analysis, and also had less than 250 sequences per sample. Rarefaction curves continued to increase with additional sequences even up to 7,000 sequences (Online Resource 1), and diversity did not significantly differ between Ambient and Delayed soils in Fresh soils or after Pulse 1, but was higher in Ambient after Pulse 4 (Online Resource 2).

Bacteria dominated soil communities compared to Archaea, but this proportion was not affected by field or lab manipulations. The most abundant Phyla in all groups were Actinobacteria (23%), Proteobacteria (23%), Verrucomicrobia (14%), and Acidobacteria (11%) (Fig. 6), and there were trends of higher variability across time points in Delayed soils compared to Ambient (Table 1). According to our SIMPER analysis, a species from Verrucomicrobia (in the Xiphinematobacteriaceae family) most strongly contributed to differences among groups, which was more abundant in both Delayed soils compared to Ambient and in soils at Pulse 4 compared to Fresh (Online Resource 3). Other notable groups that contributed to differences among treatments were Acidobacteriaceae (increased by Pulse 4), and

Table 1 Summary of resistance of microbial communities from different long-term rainfall manipulation (Ambient and Delayed rainfall timing) subject to multiple drying–rewetting pulses in the lab

Parameter	Field rainfall manipulation	Proportional change between two time points in lab manipulation					CV[b]	Pulse 4–wet control[c]
		Fresh–Pulse 1	Pulse 1–2	Pulse 2–3	Pulse 3–4	Pulse 1–4[a]		
Respiration	Ambient	**−0.439**[a]	**−0.106**	−0.516	1.019	6.31	0.702	−0.003
	Delayed	**−0.209**	**−0.405**	−0.404	1.254	4.08	0.710	−0.100
Microbial biomass C	Ambient	**1.071**				−0.363	**0.410**	0.164
	Delayed	**0.147**				−0.222	**0.268**	0.410
Microbial biomass N	Ambient	−0.223				−0.561	0.416	0.037
	Delayed	0.182				−0.650	0.668	−0.332
Extractable C	Ambient	**8.975**				−0.564	**0.769**	0.098
	Delayed	**4.014**				−0.663	**0.619**	−0.071
Extractable N	Ambient	−0.029				1.479	0.553	0.148
	Delayed	0.185				1.358	0.562	0.217
Fungal:bacterial	Ambient	**2.068**				**1.101**	**0.784**	−0.342
	Delayed	**0.991**				**0.290**	**0.428**	−0.351
Community Dissimilarity[d]	Ambient	0.2098				0.2333		
	Delayed	0.2181				0.2990		

Bold indicates a significant difference ($P < 0.1$) between the proportional change (or CV) in Ambient and that in Delayed

[a] All variables other than respiration were measured only at Fresh, Pulse 1 and Pulse 4 time points, so proportional change could not be calculated among each time point

[b] Coefficient of Variation of all time points measured (excluding wet control)

[c] $Y_{Pulse\ 4} - Y_{Wet\ Control}/Y_{Wet\ Control}$

[d] Average Weighted Unifrac distance in ordination space between two communities of two groups. We could not test for significance of degree of change

Alphaproteobacteria (Rhizobales more abundant in Delayed but decreased in response to lab drying–rewetting).

When communities were analyzed for similarity based on Unifrac distances, there was significant variation within groups (Fig. 7), but lab treatment explained more similarity among samples than field treatment (Ambient or Delayed) (Table 2). PerMANOVA pairwise comparisons (among time points within treatments and between treatments within time points) revealed that no communities were significantly different using Unweighted Unifrac differences. When taking relative abundance into account (Weighted Unifrac), treatments were significantly different at Pulse 4 ($P < 0.05$) and in Fresh soils ($P < 0.1$). Soils from Delayed treatments changed from Pulse 1 to Pulse 4, showing greater differences as the lab incubation progressed, but Ambient soils did not significantly change over time (Tables 1, 2).

Discussion

Did historical conditions influence microbial response to drying rewetting?

While there is little doubt that soil microbial activity responds quickly to changes in environmental conditions, the role of environmental history in driving contemporary rates of microbially-mediated processes is largely unknown. Previous studies have documented differences in microbial function induced by historical legacies in climate (Fierer and Schimel 2002; Fierer et al. 2003), litter quality (Ayres et al. 2009; Keiser et al. 2010), or disturbance regime (Tobor-Kaplon et al. 2006), but the mechanisms driving these legacies is often unclear.

Our study provides evidence that, while soil moisture at any instant is the dominant driver of microbial function, the historical soil moisture

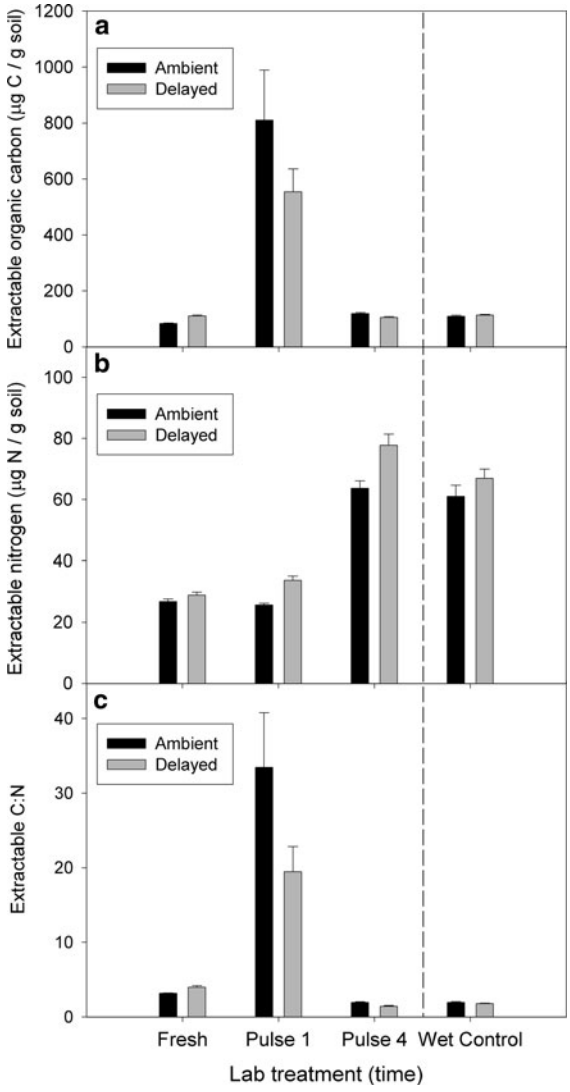

Fig. 4 Mean extractable organic carbon (**a**) and nitrogen (**b**) throughout lab treatment. *Error bars* are standard error for means ($N = 6$)

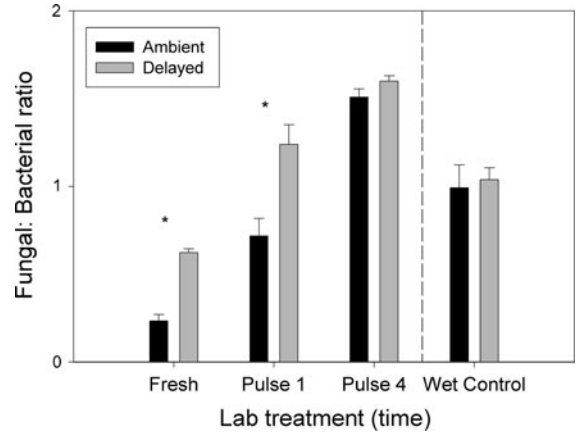

Fig. 5 Fungal to bacterial ratio as determine by quantitative PCR. *A significant difference ($P < 0.05$) between Ambient and Delayed treatments within that time point. *Error bars* are standard error for means ($N = 6$)

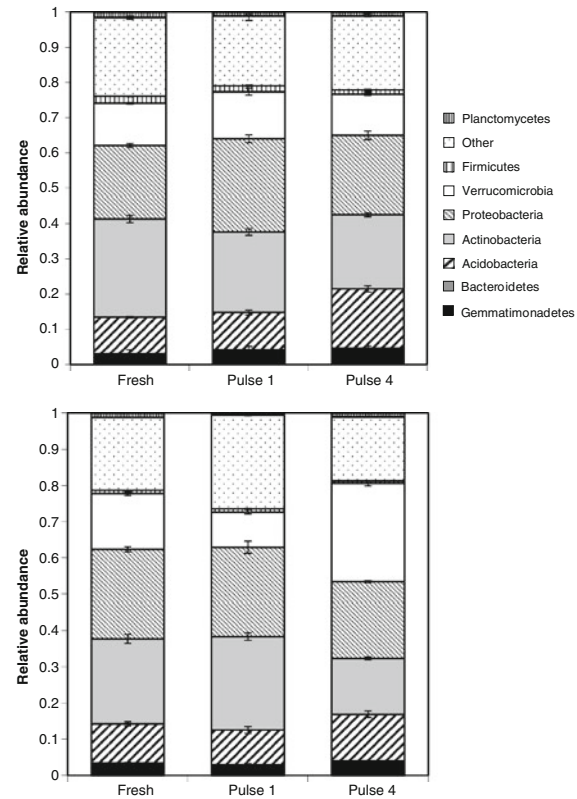

Fig. 6 Relative abundance of the dominant Phyla in soils from Ambient (**a**) and Delayed (**b**) rainfall timing manipulations at different time points in a drying–rewetting lab incubation. Relative abundance is the abundance of a particular sequence relative to the total number of sequences in that sample. *Error bars* are standard error for means ($N = 6$)

regime also affects the response of soil microbes to drying and rewetting events. For example, we observed a lower respiration rate following initial soil rewetting in Delayed soils compared to Ambient soils (Fig. 2). This could be explained by persistent changes in other drivers like microbial biomass or substrate availability, but these pools did not explain a reduction in respiration in Delayed soils at the beginning of the experiment (Figs. 3, 4). The different historical precipitation regime induced by these rainfall timing manipulations may have altered the aggregate community-level traits (sensu

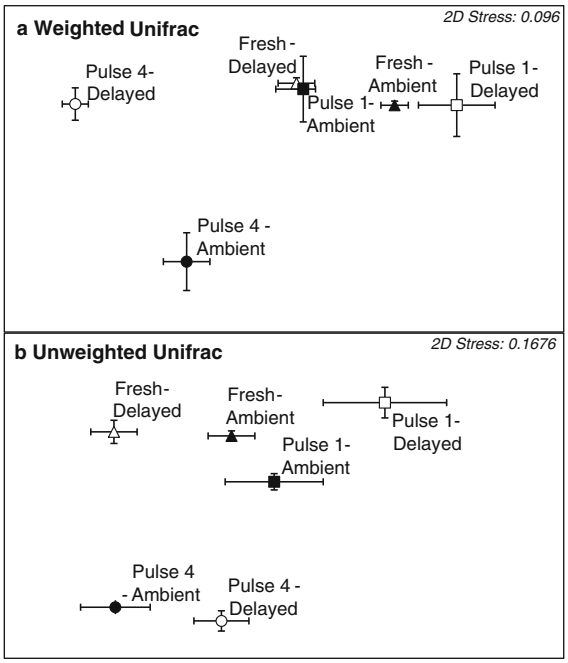

Fig. 7 Bacterial community composition similarity among groups calculated from Weighted (**a**) and Unweighted (**b**) Unifrac distances by non-metric multidimensional scaling. Symbol fill indicates field treatment (Ambient, *filled* and Delayed, *open*) and shapes indicate lab time point (Fresh, Pulse 1, and Pulse 4 as *triangles*, *squares*, and *circles*)

Wallenstein and Hall 2011) that control soil respiration including C use efficiency, soil moisture sensitivity and stress tolerance. These changes are most likely driven by changes in the relative abundance and activity of taxa that differ in physiology (Wallenstein and Hall 2011), which could occur at any phylogenetic level, depending on the degree to which these traits are conserved across evolutionary history. In our study, differences in community-level responses to the initial rewetting in our experiment could be attributed to the higher fungal:bacterial ratio in soils from the Delayed treatment (Fig. 5). In this manner, historical precipitation regimes can act as a distal control on contemporary rates of microbial processes (e.g. respiration) by modifying the traits of microbial communities that act as transducers between contemporary abiotic drivers (e.g. soil moisture, substrate availability) and microbial function (as proposed for denitrification by (Wallenstein et al. 2006).

Do historical effects persist under the same conditions?

The relative importance of environmental history on contemporary process rates depends, in part, on the degree to which historical effects persist following environmental change. In this study, the ecological importance of historical precipitation regime depends on whether the differences in moisture pulse response between Delayed and Ambient soils that we observed during the initial pulse persisted when the soils were subjected to the same moisture pulse regime. We predicted that soil microbial communities adapted to extreme rainfall patterns (i.e. drying–rewetting events of greater magnitude) would change less in response to drying–rewetting pulses than those that experienced ambient rainfall, and that Ambient soils would become more similar to Delayed through time as they adapted to moisture pulses. Consistent with this hypothesis, we found that respiration, biomass-C and extractable-C changed less in Delayed soils than Ambient soils throughout the 115-day laboratory experiment (Table 1), and that the effect of precipitation history declined throughout the experiment such that initial differences among soils from different field treatments were negligible by the end of the lab experiment.

Other studies suggest that the effects of drying rewetting events may cause changes in C-mineralization long after the moisture pulse (Fierer and Schimel 2002; Schimel et al. 1999). Fierer and Schimel (2002) showed that differences in function persisted 6 weeks after drying–rewetting, with little convergence once subjected to the same conditions. Our incubation extended longer than this, and although we examined how control (Ambient) and stressed (Delayed) soils responded to a stress (instead of how they recover), we did observe similar respiration rates, suggesting that the effects of a decade of an altered precipitation regime on respiration may not persist beyond a single growing season in this particular prairie ecosystem. The persistence of historical legacies observed by Fierer and Schimel (2002) was at least partially explained by differences in substrate availability, which did not differ at the end of our experiment. Thus, the persistence of historical effects may depend on the mechanism through which historical legacies are generated.

Table 2 PerMANOVA results for Main effects and Pairwise comparisons within the Trt × Time interaction (field treatments within each time point and time points within each field treatment)

Distance metric	Test	Factor	Pairwise comparison	Mean distance[a]	*P*-value[b]
Weighted Unifrac	Main effects	Trt			0.278
		Time			**0.001**
		Trt × Time			**0.085**
	Pairwise within time	Fresh	Ambient–Delayed	0.1766	**0.0771**
		Pulse 1	Ambient–Delayed	0.2294	0.2109
		Pulse 4	Ambient–Delayed	0.2244	**0.0486**
	Pairwise within Trt	Ambient	Fresh–Pulse 1	0.2098	0.2486
		Ambient	Pulse 1–Pulse 4	0.2333	0.2121
		Ambient	Fresh–Pulse 4	0.2412	0.1200
		Delayed	Fresh–Pulse 1	0.2181	0.2043
		Delayed	Pulse 1–Pulse 4	0.2990	**0.0464**
		Delayed	Fresh–Pulse 4	0.2338	**0.0383**
Unweighted Unifrac	Main effects	Trt			0.6606
		Time			**0.0440**
		Trt × Time			**0.0665**
	Pairwise within time	Fresh	Ambient–Delayed	0.6239	0.1639
		Pulse 1	Ambient–Delayed	0.6565	0.4874
		Pulse 4	Ambient–Delayed	0.6505	0.4428
	Pairwise within Trt	Ambient	Fresh–Pulse 1	0.6397	0.3869
		Ambient	Pulse 1–Pulse 4	0.6510	0.3502
		Ambient	Fresh–Pulse 4	0.6485	0.2068
		Delayed	Fresh–Pulse 1	0.6654	0.2094
		Delayed	Pulse 1–Pulse 4	0.6720	0.3635
		Delayed	Fresh–Pulse 4	0.6592	0.2491

[a] Pairwise mean distances were derived from different distance metrics (Weighted and Unweighted Unifrac) and therefore are only comparable within that distance matrix

[b] Bold indicates $P < 0.1$

Mechanisms of historical legacies

There are two mechanisms by which historical conditions may have affected contemporary microbial function in this experiment. First, the experimental intensification of precipitation regime induced by RaMPS could have caused changes in plant and soil properties that persisted after soils were removed from the field and placed under identical conditions in the laboratory. Our laboratory experiment isolated the effects of drying–rewetting by subjecting two soils that differed only in historical moisture regime (i.e. no other previous ecosystem differences that would result in soil texture or chemical differences) to drying–rewetting in a controlled lab environment in the absence of plants and other environmental drivers. Therefore, any changes that occurred reflect direct responses to shifts in precipitation, or indirect responses such as shifts in plant growth or chemistry affecting the quantity and quality of C inputs to soils. Although Fay et al. (2002) found decreased aboveground net primary production under Delayed rainfall in the field, we did not observe a difference in soluble (labile) C or N in initial soil measurements from each treatment. Therefore, we do not believe the persistence of differences in respiration between soils with different histories were primarily due to differences in substrate. Increased drying–rewetting can alter other abiotic factors such as soil physical structure (Adu and Oades 1978) that may also persist, although it is unlikely these changes significantly affected respiration rates because soils were initially identical

and many of these variables change on much longer timescales (Jenny 1941).

The second mechanism by which environmental history can affect contemporary microbial function is through changes in the composition and aggregate physiology of microbial communities. Altered precipitation patterns could induce community-level adaptation to the stress associated with drought and intensified rain events. This biotic selection could be driven directly by osmotic stress, or indirectly through abiotic factors that shifted under altered precipitation timing. Changes in community structure, such as the differences in fungal:bacterial ratio that we observed in this study, are likely to alter the aggregate function of microbial communities (Wallenstein and Hall 2011). Although we did not explicitly test fungal versus bacterial tolerance to drying or rewetting, increases in fungal:bacterial ratios do suggest that fungi and bacteria have differing sensitivities to drying–rewetting, as other studies have also suggested (Bapiri et al. 2010; Hawkes et al. 2010; Yuste et al. 2010). Ratios converged by Pulse 4, and Delayed soils changed less in response to drying–rewetting (Fig. 5; Table 1), suggesting biotic community adaptation to drying–rewetting stress could be captured at this broad level, and possibly explaining the persistence of observed differences in respiration rate.

Historical legacies in bacterial community composition

While our data shows that the fungal:bacterial ratio increased as a direct result of increased drying–rewetting, a more detailed investigation of bacterial community composition revealed only subtle differences in community structure between field treatments, but increasingly dissimilar communities when subjected to identical conditions in the lab (Fig. 7a, b). Bacterial community data from pyrosequencing do not support a biotic mechanism for the historical legacies we observed in function, although precipitation history clearly influenced bacterial community composition throughout the timescale of the incubation, and this lack of initial dissimilarity does not preclude this mechanism's expression on different timescales and through other microbially-mediated functions.

We suggest two reasons why a 10-year rainfall timing manipulation may not have resulted in more distinct bacterial communities. First, it is possible that most taxa in the tallgrass prairie soils are pre-adapted to some degree of moisture fluctuation, and the increased magnitude induced by these manipulations did not induce further selection. Other studies have observed no significant change in bacterial community composition under rainfall manipulations (Cruz-Martinez et al. 2009; Landesman and Dighton 2010). The differences we observed, either from field or lab treatments, emerged due to changes in relative abundance of particular taxa, rather than the presence or absence of certain taxa (as quantified by Unweighted Unifrac distances, Fig. 7b; Table 2). Delayed soils might have been better adapted to drying–rewetting. However, since the magnitude of the pulses that occurred in this precipitation regime also occurred in the natural historical climate, although less frequently, Ambient soils may have also contained the microbial taxa that allowed the extant community to adapt to laboratory moisture pulses quickly.

Second, the lack of detectable effects of the RaMPS experiment on plant community structure and function may have buffered soil microbial communities from direct drying–rewetting selection pressures. Plant community properties, which remained relatively unchanged under this rainfall manipulation, have been shown to stabilize microbial dynamics; for example, plant diversity has been shown to diminish changes in microbial biomass and denitrification rates across seasons (McGill et al. 2010). In the absence of plant-mediated environmental buffering, exposure to direct drying–rewetting in the lab may have may have induced stronger selection on community composition. Consistent with this hypothesis, subtle differences observed in field soil communities under the RaMPS appeared to drive divergent trajectories for community composition in the lab. For example, a greater abundance of a Verrucomicrobia species in Delayed soils most strongly contributed to whole-community dissimilarity of Delayed and Ambient soils at Pulse 4 (Online Resource 3). This increase in abundance with each subsequent lab pulse could have emerged from this species' slightly greater abundance in Delayed plots in the field which enabled them to capitalize on preferred conditions once plant-mediated buffers were removed.

Individual responses of certain species to drying–rewetting pulses, when examined across time, varied

significantly (see Sparklines in Online Resource 3). It is possible that the divergence in community composition we observed, and general variability within samples, may relate to the nature of drying–rewetting as a disturbance. Unlike the Verrocomicrobia example discussed above, an Acidobacteria species that was more abundant under altered rainfall timing in the field changed very little in the lab, perhaps reflecting an alternative strategy of shifting resource allocation from growth to structural stability, instead of capitalizing on short-lived optimal conditions. A climate shift toward more extreme conditions (intensified rainfall) may more strongly induce diverse life strategies compared to a unidirectional shift (drought), which may result in more specialization (see Wallenstein and Hall 2011). Other studies have suggested similar delineation of life strategies as a framework for predicting responses of microbial communities to disturbance (Fierer et al. 2007; Van Gestel et al. 1993).

Methodological idiosyncrasies could also have influenced measured trends in community composition and the absence of a link between community composition and function. First, tolerance to drying and rewetting may not have been expressed on the phylogenetic level we chose (97% similarity for OTU's) because it requires complex mechanisms involving multiple genes. Keiser et al. (2010) examined the effect of historical substrate exposure on function and community composition on this phylogenetic level and also found community composition, which converged under similar conditions, did not follow a similar trajectory as function, as historical legacies in litter type continued to affect decomposition rate after 100 days. Second, we only sequenced bacterial communities, and fungi could display unique and strong responses to moisture stress. Efforts to determine the phylogenetic level at which microbial stress tolerance is expressed will be important for the development of predictive frameworks. In addition, assessing overall microbial community composition (as opposed to only the active members) may mask discreet changes in species assemblage that are better linked to function (or stress tolerance) (McMahon et al. 2011). A final methodological concern is whether communities were affected by long-term storage at −10°C. Although physical effects on soils from the same site were likely similar, certain microbial communities could be more sensitive to cold-stress than others, and this could alter microbial community composition and responses to moisture upon rewetting (Gonzalez-Quinones et al. 2009; Lee et al. 2007). However, as we found no significant difference (yet communities were also not statistically the same), it is unlikely cold storage either affected soils differently or selected for species in a systematic way.

Implications of historical legacies for predicting ecosystem responses to novel climates

A current challenge for ecologists is to establish whether existing relationships between abiotic factors and community and ecosystem properties can be extrapolated over time to predict ecosystem-atmosphere feedbacks and the direction and rate of global change. Our results suggest, as other studies have, that historical conditions do play a role in determining the functional and composition response of microbial communities to environmental factors (Fierer and Schimel 2002; Fierer et al. 2003; Gulledge and Schimel 1998; Lundquist et al. 1999). In our study, differences in respiration rates—that could not be explained by substrate availability or microbial biomass—persisted when soils were incubated under the same conditions, but for less than 115 days. The increase in frequency of stressful conditions that already occur within an ecosystem's historical range of variability might cause lags in function, perhaps mediated by changes in community composition (in this case fungal:bacterial ratio), but these lags will be short. Historical conditions may more strongly influence contemporary functional response when disturbances are further outside an environment's historical range of variability, crossing potential thresholds, or when acting through indirect drivers like changes in plant properties. In contrast to short functional lags, effects of historical precipitation continued to cause differences in bacterial community composition through the end of our experiment. This suggests that biologically-mediated legacies at least have the potential to cause longer functional lags, perhaps in functions controlled by narrow phylogenetic groups (Schimel 1995; McGuire et al. 2010). Thus, legacies of environmental conditions may affect microbially-mediated processes on different timescales, and vary in magnitude for different functions. More detailed descriptions of the temporal dynamics of microbial responses could improve predictions for how

microbially-mediated processes will respond to global changes (see Treseder et al. 2011).

These results call for further work to 1. isolate direct and indirect mechanisms of historical conditions on responses of microbial communities through coupled field-lab studies (see Brown et al. 2011) 2. determine the phylogenetic level at which adaptations to stress, and functional linkages, are expressed, and 3. identify factors controlling the timescale on which historical legacies affect contemporary microbial responses. Under novel climate regimes, historical legacies may impair our ability to predict ecosystem responses with current predictive relationships. Some studies have begun to investigate whether C dynamics under moisture pulses can be better predicted using explicit microbial mechanisms (Lawrence et al. 2009; Li et al. 2010). Our results suggest that microbial adaptation to climate conditions may influence this response as well, and further research is needed to quantify how microbial legacies to climate could affect predicted changes in C flux at the ecosystem scale (Todd-Brown and Allison 2011).

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