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Is bacterial moisture niche a good predictor of shifts in community composition under long-term drought?

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Abstract. Both biogeographical and rainfall manipulation studies show that soil water content can be a strong driver of microbial community composition. However, we do not yet know if these patterns emerge because certain bacterial taxa are better able to survive at dry soil moisture regimes or if they are due to other drought-sensitive ecosystem properties indirectly affecting microbial community composition. In this study, we evaluated (1) whether bacterial community composition changed under an 11-year drought manipulation and (2) whether shifts under drought could be explained by variation in the moisture sensitivity of growth among bacterial taxa (moisture niche partitioning). Using 454 pyrosequencing of 16S rRNA, we observed shifts in bacterial community composition under drought, coincident with changes in other soil properties. We wet-up dry soils from drought plots to five moisture levels, and measured respiration and the composition of actively growing communities using bromodeoxyuridine (BrdU) labeling of DNA. The field drought experiment affected the composition of the active community when incubated at different moisture levels in the laboratory, as well as short-term (36-hour) respiration rates. Independent of history, bacterial communities also displayed strong niche partitioning across the wet-up moisture gradient. Although this indicates that moisture has the potential to drive bacterial community composition under long-term drought, species distributions predicted by response to moisture did not reflect the community composition of plots that were subjected to long-term drought. Bacterial community structure was likely more strongly driven by other environmental factors that changed under long-term drought, or not shaped by response to water level upon wet-up. The approach that we present here for linking niches to community composition could be adapted for other environmental variables to aid in predicting microbial species distributions and community responses to environmental change.

Key words: bacterial community composition; bromodeoxyuridine (BrdU); drought; grassland; microbial communities; niche partitioning; precipitation manipulation; pyrosequencing; rainfall; shortgrass steppe; soil.

Introduction

Several recent studies have shown that changes in precipitation patterns can alter the composition of soil microbial communities (Williams and Rice 2007, Clark et al. 2009, Hawkes et al. 2011, Hueso et al. 2012), suggesting that soil functioning may also be responsive to climate change. Biogeographical patterns of some microbial taxa are also highly correlated with soil water content (Frey et al. 1999, Lauber et al. 2009), which is not surprising given the importance of water for microbial functioning. However, other studies report that microbial communities are highly resistant to changes in rainfall (Griffiths et al. 2003, Landesman and Dighton 2010), even when subject to changes over many years (Cruz-Martinez et al. 2009). These contradictory observations suggest that we need to examine the ecological processes that lead to changes in microbial community composition in some systems to better understand how they will respond to altered precipitation patterns, which in many parts of the world is likely to be characterized by an increased frequency and duration of drought.

The most parsimonious explanation for changes in microbial composition in response to increased drought is niche selection, whereby taxa that are drought tolerant increase in relative abundance compared to drought-sensitive taxa. As a result, entire soil microbial communities could develop drought tolerance through time (de Vries et al. 2012, Goransson et al. 2013), which could lead to increased rates of microbial activity at low soil moistures. The occurrence of moisture-niche selection under drought has been difficult to test, given the high diversity of microbial communities, and the limited

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PLATE 1. The shortgrass steppe ecosystem in northeastern Colorado, USA, is characterized by short-stature grasses, including the dominant grass *Bouteloua gracilis*, *Opuntia polyacantha* cactus, and dwarf-shrub *Artemisia frigida*. Mean annual precipitation is 341 mm. Precipitation events are highly variable and predominantly occur in the summer months. Photo credit: Sallie G. Sprague.

a priori information on the drought tolerance of specific taxa. To date, our best evidence that microbial taxa differ in moisture-related niches comes from culture-based studies. For example, taxa differ in their ability to enter into dormant states, or their ability to quickly respond to optimal conditions, which are likely to affect survival under rapid changes in soil water potential (Lennon 2012). It is unclear whether these observations from microbial cultures affect fitness while taxa are under field conditions and interacting with other taxa.

While DNA-based sequencing techniques allow for the routine and sensitive detection of changes in the composition of microbial communities in response to altered precipitation, it has been difficult to link these changes to the physiological traits of the constituent taxa. Shifts in community composition under altered moisture regimes suggest differential sensitivity to moisture, but total DNA abundance of each taxa could reflect long-term legacies that precede a moisture manipulation. In addition, it is difficult to tease out whether the shifts in these studies are due to the direct effects of moisture, or to indirect responses to other variables that change with rainfall (e.g., primary production, root exudation, nitrogen availability, pH). Recent studies illustrate how molecular methods can be used to link community physiology to identity (Goldfarb et al. 2011, Placella et al. 2012) and describe the growth and functional response of individual taxa to variation in soil moisture within a community; in other words, to identify groups that occupy certain moisture niches. This information can, in turn, be used to predict how community structure might change as drought selects for dry-adapted species, similar to the way niche distribution models have been used to predict species distributions across space in larger organisms (Guisan and Thuiller 2005) and, more recently, in microbial communities (King et al. 2010, Larsen et al. 2012).

In this study, we took advantage of an 11-year field drought experiment whereby rainfall was excluded from plots within a semiarid grassland and added back after each rainfall event in amounts of 25%, 50%, and 100% of ambient rainfall. Water availability controls many of the processes in this ecosystem (Noy-Meir 1973) and much of the total biological activity occurring during periodic moisture pulses (Austin et al. 2004, Lauenroth et al. 2008). Our first objective was to determine whether bacterial community composition was altered by long-term drought. We then examined the mechanism through which this long-term pattern occurred by assessing whether these shifts were driven by the moisture niche of each taxa. We hypothesized that (1) antecedent drought alters bacterial activity and function in response to moisture, (2) distinct communities are active when exposed to pulses of varying moisture levels (demonstrating moisture niche partitioning), and (3) this moisture niche partitioning is a good predictor of the shifts in long-term communities under distinct moisture regimes in the field.

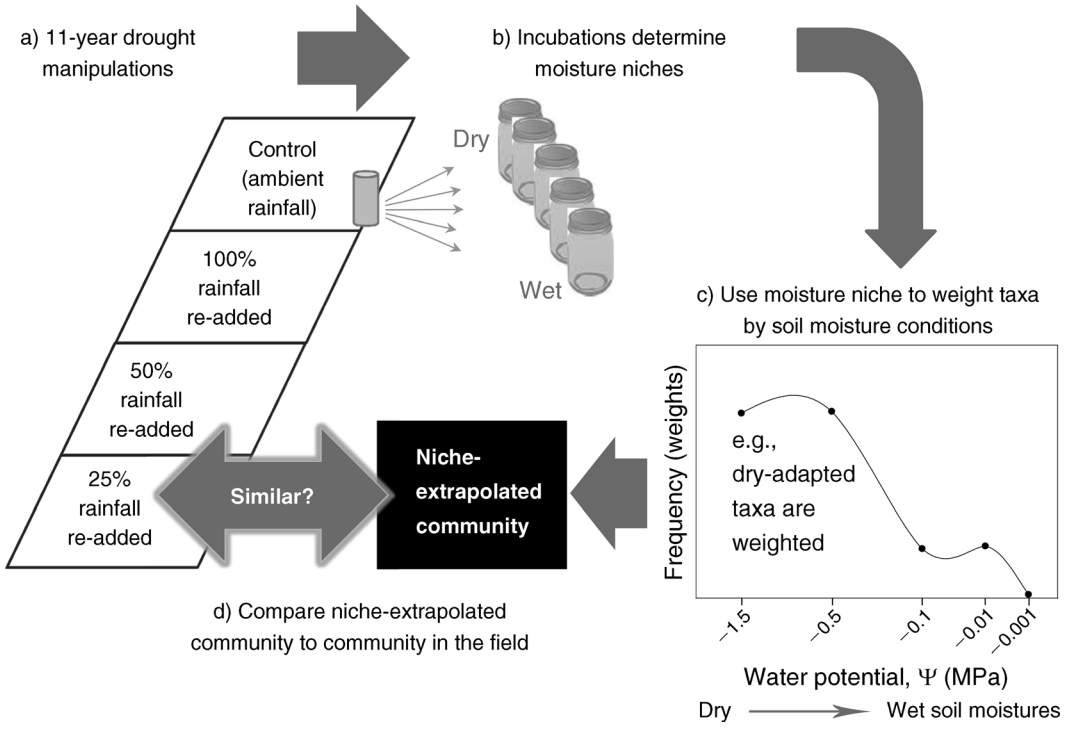

FIG. 1. Experimental approach. We asked whether (a) changes in the microbial community composition we observed under long-term drought could be reproduced by using (b) microbial moisture niches to (c) simulate communities we would expect to find under drought. (d) We compared the communities we would expect to emerge if moisture were driving changes in composition to gain insight into drivers of microbial community composition under long-term changes in rainfall.

METHODS

Our experimental approach consisted of four steps (Fig. 1). We first described bacterial community composition and other soil properties in a long-term drought manipulation study to address our first objective (Fig. 1a). To test our specific hypotheses (1, 2, and 3), we subjected soils from each drought treatment to a range of wet-up moisture levels and measured those species that grew in response to each moisture level, as well as soil respiration rate (Fig. 1b). We called the wet-up moisture level that a given species grew under its "moisture niche," and used these moisture niches to generate modeled communities we would expect to emerge in long-term droughts by weighting species abundance by the frequency with which a certain moisture level occurred in field manipulations (Fig. 1c). Finally, we compared this niche-extrapolated community to the community composition we observed in the field (Fig. 1d) to assess whether a direct effect of moisture was driving long-term changes.

Study site and rainfall manipulation

Our rainfall manipulation experiment (Fig. 1a) is located in the semiarid shortgrass steppe at the Central Plains Experimental Range (see Plate 1 and Appendix A: Fig. A1), 60 km northeast of Fort Collins, Colorado, USA (40°49′ N, 104°46′ W; Lauenroth et al. 2008). Mean annual temperature is 8.2°C, and mean annual precipitation is 341 mm (65-year average), with 83% of precipitation occurring between April and September (Sala et al. 1992). The site is dominated by short-stature C_4 grasses *Bouteloua gracilis* and *Bouteloua dactyloides* and soil types are Renohill and Ascalon fine sandy loam (USDA and Soil Conservation Service 1981).

In 1998, two blocks were divided into four 3.5×1.7 m plots: a control plot receiving ambient rainfall and three manipulated plots that were automatically covered by rainout shelters during rain events in the growing season (average dates 26 April–7 October) and received a proportion of ambient rainfall (25%, 50%, or 100% re-added) each week (one block shown in Fig. 1a; Evans et al. 2011, Evans and Burke 2013). Although field replication in this study is low ($N = 2$), these shelters gave us the unique opportunity to examine responses of bacterial communities that have experienced a shift in a single environmental factor (rainfall) for over a decade, and to test our novel laboratory approach in a system where we would expect moisture to be a primary driver of biological dynamics.

Four soil cores from each plot were collected in May of 2009 (11th year of drought). In sampling, we excluded a 25-cm buffer strip around the plot edges where water may have entered plots from the side above or belowground. After soils were sieved, we subsampled

TABLE 1. Mean (with standard error in parentheses) of environmental variables across precipitation treatments and correlations of these variables with short-term respiration rate and bacterial community composition in the field.

	Shifts in environmental properties under drought					Correlations		
							UniFrac distance	
Variable	25%	50%	100%	Control	ANOVA *P*	Resp†	Weighted	Unweighted
Field soil moisture§	4.05 (0.59)	3.81 (0.75)	4.07 (0.21)	3.77 (0.26)	0.23	0.37	0.21	0.34
Dried soil moisture	3.33 (0.16)	3.03 (0.27)	3.44 (0.08)	3.00 (0.14)	0.45	0.22	0.18	0.44
DOC (μg/g soil)	123.7 (13.3)	156.7 (31.1)	168.3 (31.6)	77.3 (7.5)	0.12	0.14	0.68#	0.57
DON (μg/g soil)	27.6 (1.1)	34.9 (6.2)	33.9 (0.3)	20.0 (0.9)	0.10	0.32	0.33	0.28
DOC : DON	4.51 (0.33)	4.55 (0.12)	5.15 (0.83)	4.07 (0.6)	0.28	0.32	0.62	0.82*
MBC (μg/g soil)	137.6 (8.5)	165.5 (29.0)	187.4 (32.9)	148.4 (8.2)	0.34	0.43	0.24	0.15
MBN (μg/g soil)	21.1 (7.9)	34.7 (12.4)	21.38 (3.6)	26.8 (5.9)	0.23	0.32	0.46	0.27
MBC : MBN	10.4 (4.0)	27.8 (17.0)	19.7 (10.1)	6.93 (1.6)	0.29	0.43	0.48	0.15
SOC (mg/g soil)	14.0 (0.7)	13.0 (0.4)	14.0 (0.1)	13.0 (0.2)	0.47	0.24	0.65	0.37
pH	7.6 (0.35)	7.7 (0.07)	7.7 (0.48)	6.2 (0.18)	0.10	0.12	0.27	0.43#
Plant community¶	···	···	···	···	0.03	···	0.18	0.19

Notes: Treatments represented a level of drought: a control plot received ambient rainfall and three manipulated plots that were automatically covered by rainout shelters during rain events in the growing season and received a proportion of ambient rainfall (25%, 50%, or 100% re-added) each week. Abbreviations are: comm abund, community abundance; P/A, presence/absence; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial biomass C; MBN, microbial biomass N; and SOC, soil organic carbon.

* $P < 0.05$; # $P < 0.1$.

† Correlations between environmental variables and short-term respiration measurements.

‡ Correlations with weighted (considers abundance) and unweighted (presence–absence) UniFrac distances (Lozupone and Knight 2005).

§ Gravimetric soil moisture: mL water/g dry soil (%).

¶ Mantel test correlation statistic (*r*) with plant community composition under drought (see Evans et al. 2011).

soils to characterize microbial community composition (see *Bacterial community pyrosequencing* below) under long-term drought (Fig. 1a). We also measured water-holding capacity, initial gravimetric soil moisture, as well as microbial biomass C and N (MBC, MBN), soil organic carbon (SOC), and pH for later correlation with community and functional response. MBC and MBN were determined using chloroform fumigation extractions (Brookes et al. 1985, Vance et al. 1987, Evans and Wallenstein 2012), by comparing carbon in 6-g non-fumigated subsamples to fumigated ones. We measured the percentage of organic carbon in soils by analyzing ground subsamples on a CHN-1000 analyzer (LECO, St. Joseph, Missouri, USA), and pH using a 1:1 mixture of soil and deionized H_2O and a pH meter (Sparks 1996). The remainder of the soil samples were stored at −10°C until initiation of the laboratory experiment in May of 2010.

Incubation of soils from drought treatments at a range of soil moistures

With the remaining soil from field manipulations, we set up laboratory incubations (Fig. 1b) that subjected soils from each field treatment to five levels of soil water potential (−0.001, −0.01, −0.1, −0.5, and −1.5 MPa) to examine (1) function (respiration rate) and (2) the species composition (active and total) of bacterial communities. Prior to bringing soils to different moisture levels, all samples were pre-incubated at 25°C and air-dried for 3 d, at which time soil moisture within treatment did not significantly differ (see Table 1). We then added the necessary water (with bromodeoxyuridine [BrdU], in the BrdU incubations) to bring soils to the desired water potential. To monitor the soil respiration of drought treatments under this range of moisture levels, we incubated 14–16 g of soil in 0.9-L (1-quart) glass Mason jars stored at 25°C. We measured soil respiration rates by analyzing the accumulation of CO_2 in the headspace of the jars with an infrared gas analyzer (IRGA; LI-COR, Lincoln, Nebraska, USA) every four hours for the first 48 h of the incubation, weekly for four months, and then biweekly for another two months.

To describe how active bacterial communities directly responded to contemporary moisture conditions, and to test for niche partitioning, we incubated 5-g soil subsamples from each core (four per plot) in sterile jars with bromodeoxyuridine (BrdU). BrdU is an analogue of thymidine, and therefore can be used to analyze the proliferation of living cells (Borneman 1999, Goldfarb et al. 2011, McMahon et al. 2011). We added BrdU to samples by dissolving a consistent amount (300 ng/g soil) in the water added to each incubation jar with a dropper. We incubated the vials at 25°C for 36 h, while growing microorganisms incorporated BrdU molecules into replicating DNA. We chose this time period (24–48 h) because it captures the period when much of the growth and activity of bacteria after rewetting occurs (Iovieno and Baath 2008, Placella et al. 2012, Goransson et al. 2013). We aimed to provide enough time for replication and BrdU incorporation to occur, and to capture most of the taxa that responded to a wet-up of

the particular moisture level, but not enough for significant turnover of the bacterial biomass or for BrdU to be used as a substrate for other microbes. BrdU-incubated samples were stored at −80°C until DNA was extracted.

We extracted DNA from incubated soils using MoBio PowerSoil Kits (MoBio Laboratories, Solano, California, USA) and performed an antibody immunocapture on a subsample of total DNA to isolate labeled DNA (containing the BrdU molecule) from inactive DNA (McMahon et al. 2009). To obtain enough BrdU-DNA for pyrosequencing preparation, we pooled separately incubated and extracted DNA from field cores (four from each plot) for a total of 40 "active" (BrdU-incorporated DNA) samples and 40 "total" (active + inactive, non BrdU-incorporated DNA) samples (5 water levels × 2 blocks × 4 treatments; Fig. 1a, b). We evaluated the efficacy of the BrdU immunocapture by visualizing DNA and amplified PCR product on agarose gels, and comparing bands to a control sample that we incubated with only water additions, without BrdU.

Bacterial community pyrosequencing

We analyzed the community structure of total and active communities from moisture incubations (Fig. 1b) and long-term field treatment communities (not subject to moisture incubations; Fig. 1a) using a pyro sequencing-based analysis of the 16S rRNA gene (Fierer et al. 2008). We amplified the 27 to 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 (Roche Applied Science, Branford, Connecticut, USA), connected with a TC linker, and reverse primer contained a 12-bp bar-coded sequence, Roche 454 "B" sequencing adapter, and a TC linker. Polymerase chain reactions (PCRs) 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, California, USA). We amplified samples in triplicate, pooled, and cleaned reactions using a PCR Cleanup Kit (MoBio Laboratories, Carlsbad, California, USA), andthen sequenced them on a Roche FLX 454 pyrosequencing machine at the EnGenCore (University of South Carolina, Columbia, South Carolina, USA).

We analyzed pyrosequencing data (Fierer et al. 2008, Hamady et al. 2008, Lauber et al. 2009) using QIIME (Quantitative Insights Into Microbial Ecology; Caporaso et al. 2010*b*). We first removed sequences <200 bp and with a quality score <25. We identified bacterial operational taxonomic units (OTUs) as those organisms whose 16S rRNA gene sequences were 97% similar, and used the most abundant sequence per OTU as the representative sequence for that OTU. We aligned sequences using PyNAST (Caporaso et al. 2010*a*) and assigned taxonomies to these representative OTU phylotypes using the RDP Classifier (Wang et al. 2007). We performed basic filtering on all data sets that excluded all OTU's that were only present in one sample.

Generation of "niche-extrapolated" communities

We generated modeled communities (based on moisture niche) that we might expect to emerge under drought treatments, where low-moisture conditions would occur more frequently (Fig. 1c) so that we could later compare these communities to actual field community composition (Fig. 1d) and gain insight into the mechanism through which these patterns emerged. To do this, we first assembled moisture frequency distributions for each field treatment (25%, 50%, 100%, control; Appendix A: Table A1) based on hourly soil moisture data collected during drought treatment in 2008 (see Appendix A: Table A1 for more details). We then used our moisture niche distribution (the abundance of species active at different moisture levels in control soils) to weight the abundances of certain species and generate what we call "niche-extrapolated" communities, just as niche distribution models are used to generate expected community composition across space (Guisan and Thuiller 2005, Larsen et al. 2012). If specific moisture conditions in the field favored the bacterial groups that were active under those moisture conditions, even in the short term, we would expect these species to outcompete other taxa under those conditions, and for niche-extrapolated communities to approximate field community composition.

Data analysis

We aimed to describe how bacterial communities responded to both long-term drought treatments and laboratory moisture incubations, and to develop an approach for testing whether microbial moisture sensitivity drives long-term shifts under rainfall manipulations. Although we examined a decadal-scale field drought manipulation and developed a novel laboratory approach, plots in the field had low replication. We analyzed univariate and multivariate (community composition) data from two types of designs: field plot measurements and measurements on soils from the field subject to a range of moistures. To describe the relative influence of treatment and moisture on respiration rate (univariate data), we used a mixed model in SAS that included treatment and moisture as fixed effects, and block as a random effect. We calculated partial r^2, indicating the relative explanatory power contributed by each factor by dividing sum of squares of a factor by the total model's corrected sum of squares. We also analyzed the correlation between respiration rate (at 36 hours and 6 months) and each environmental variable (transformed when not meeting normality assumptions) using linear regressions. We analyzed data from field treatments (not subject to moisture incubations, e.g., dissolved organic carbon [DOC]) with a similar model, but excluding moisture as a fixed effect.

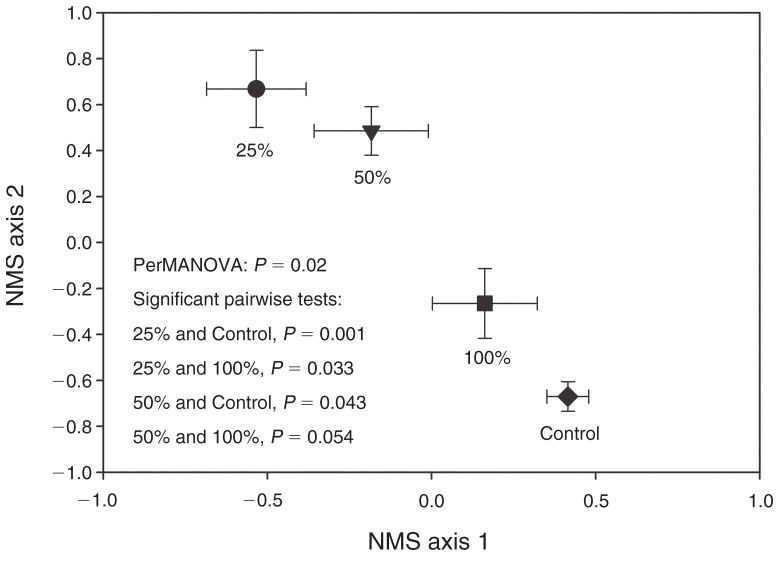

FIG. 2. Nonmetric multiple dimension scaling (NMS) ordination of microbial community similarity among long-term drought treatments in the field (see Fig. 1 for treatment descriptions). Error bars represent the standard error of mean coordinates ($N = 2$). Pairwise comparisons not listed had $P > 0.05$.

To analyze multivariate data, we examined variation in community structure among samples using the UniFrac distance metric (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 by using only presence or absence of phylotypes (unweighted UniFrac), and considering abundance (weighted UniFrac). Using these UniFrac distances, we created ordinations using nonmetric multidimensional scaling (NMDS). Then, using PerMANOVA analyses (Anderson 2001) in Primer v6, we tested for significant differences among communities in different treatments, across moisture levels, and also between those communities we observed in long-term treatments and niche-extrapolated communities. In addition, we tested these factors at different taxonomic scales. PerMANOVA is a permutation-based multivariate analysis that can accommodate many sampling designs, and allowed us to include "block" as a random effect and investigate the moisture by treatment interaction. This test calculates a pseudo F statistic (and P value) by comparing the total variance explained by sample identities (i.e., treatment, moisture) to that explained by random permutations of identities.

We also tested for correlations between long-term field community composition and environmental variables (DOC, pH, etc.) using Monte Carlo permutations (9999) and Nonmetric multiple dimension scaling (NMS) vector fitting, and for relationships between plant community composition and bacterial community composition by correlating plant and bacterial species distance matrices using a Mantel test (using R; R Development Core Team 2010).

RESULTS

Community composition under long-term drought treatments

Field community composition was significantly different among 11-year rainfall reduction treatments and control treatments at the species level ($P = 0.02$; Fig. 2). Pairwise results from PerMANOVA show that differences were not significant ($P > 0.1$) either between drought treatments (moderate vs. severe) or between types of "control" (unmodified plots vs. plots covered by the shelter, but receiving 100% of moisture re-added weekly). Although differences under drought were not statistically significant at the phyla level, trends indicate that Actinobacteria were less abundant under drought than control ($P = 0.12$), while abundance of Bacteroidetes was higher in the 25% treatment than in all other treatments (Appendix B: Fig. B1). The environmental variables that most strongly correlated to field community composition were DOC, DOC/N, and pH (Table 1).

Active and total community composition in moisture-manipulated laboratory incubations

We examined community composition of both the active and the total (active + nonactive) bacterial community from each drought treatment under five moisture levels (Fig. 1b) using BrdU and 454 pyrosequencing (summary in Appendix B: Table B1). Overall, active community composition was more variable under different wet-up moisture levels than total community composition (Fig. 3a), and more driven by contemporary moisture level than field drought treatment (Fig. 3a, b), although both factors were significant over several taxonomic levels (Appendix B: Fig. B2). Shifts

in community composition with moisture were primarily driven by changes in the relative abundance of active groups (as opposed to simply the presence or absence). Active communities from control plots were significantly different across moisture levels at both the phyla ($P = 0.01$; Fig. 3c) and species level ($P = 0.003$). Thus, we were able use this information as an indicator of moisture niche, and then use these moisture niches to generate niche-extrapolated communities.

Total community composition was only marginally affected by laboratory moisture conditions, but primarily determined by the soil's (antecedent) field treatment (25%, 50%, and so on; Fig. 3a, b). In addition, total communities, like communities characterized directly from the field, were more strongly affected by field treatment at coarser taxonomic levels (Appendix B: Fig. B2), especially class, while active communities were more responsive to both factors at the genus level. The distribution of phyla in total communities subject to moisture incubations (not shown) was very similar to that of the distribution of phyla in non-incubated communities from field treatments (Appendix B: Fig. B1).

Functional response of different drought treatments to moisture

We examined the relative influence of field treatment and lab moisture level on respiration rate. In both short-term (36-hour) and long-term (6-month) incubations, soil moisture was a stronger determinant of respiration rate than field treatment (Fig. 4). In short-term incubations, however, field treatment was also a significant driver of respiration rate, explaining 16% (partial r^2) of variation out of the 84% of total explanatory power. Specifically, respiration rates of control treatments were higher than those in 25% treatments at −0.001 MPa ($P = 0.08$) and significantly higher than both 50% and 25% treatments at −0.1 MPa ($P = 0.04$). Variation in short-term respiration rates among field treatments was not significantly correlated to variation in environmental factors among field treatments ($P > 0.1$; Table 1).

Overlap between moisture niche separation and community shifts under drought

In general, response to moisture in the laboratory (Fig. 3) was not a good predictor of changes in abundance under long-term drought treatments (Fig. 2; Appendix B: Fig. B1). For example, Actinobacteria, relative to other groups, were significantly more abundant in dry moisture incubations (Fig. 3c), and although this −1.5 MPa water potential did occur more frequently in the drought plots (Appendix A: Table A1), Actinobacteria were actually lower in drought plots than control plots in the field. Similarly, the abundance of Proteobacteria (specifically, alphaproteobacteria) taxa was higher under wet water potentials, but was not significantly different among drought and control plots in the field (Fig. 3c; Appendix B: Fig. B1).

Niche-extrapolated communities, generated by weighting the relative abundance of species active under certain moisture with soil moisture frequency distributions in the field (Appendix A: Table A1), were slightly more similar to communities from long-term drought plots than communities that had not been weighted (Fig. 5). However, the community composition was still quite distinct from long-term drought plots, and much more similar to the composition of the original active community that were not weighted based on field moistures (Fig. 5).

Discussion

In this study, we evaluated whether long-term drought alters bacterial community composition and whether variation in moisture sensitivities among bacterial groups could explain the shifts we observed. Overall, we found (1) significant shifts in bacterial community composition under 11-year drought treatments in the shortgrass steppe, (2) that drought history influenced short-term respiration rate and active community composition, (3) that specific taxa grew under certain moisture conditions, and could be partitioned by moisture niche, and (4) taxa moisture niche was not a good indicator of taxa that increased in abundance in field manipulations. Thus, although we show that moisture has the potential to drive bacterial community composition through moisture niche partitioning, we did not find evidence that differences in bacterial sensitivity to wet-up moisture level in the short term were driving the long-term shifts we observed under drought in this semiarid grassland.

Bacterial community composition shifts under long-term drought manipulations

Since rainfall was the only factor directly manipulated in this field study, the changes in bacterial community composition we observed (Fig. 2) provide evidence that rainfall is an important factor in structuring bacterial community composition in this system (either proximally or distally). Soil moisture within each plot explained finer patterns; the similarity of soil moisture in 25% and 50% treatments may explain why these communities were not significantly different (see Appendix A: Table A1 and Evans and Burke 2013). Similarly, community differences in different types of control (100% and control) were likely due to variation in moisture regime induced by differences in rainfall timing. These fine points highlight the importance of site-specific rainfall–soil moisture relationships for predicting the responses to changes in rainfall, but also suggest that our manipulation design may have affected soil moisture and other factors (Table 1).

Combined with low replication, these effects prevent us from making predictions about the effects of specific changes in rainfall (i.e., a 50% reduction in ambient

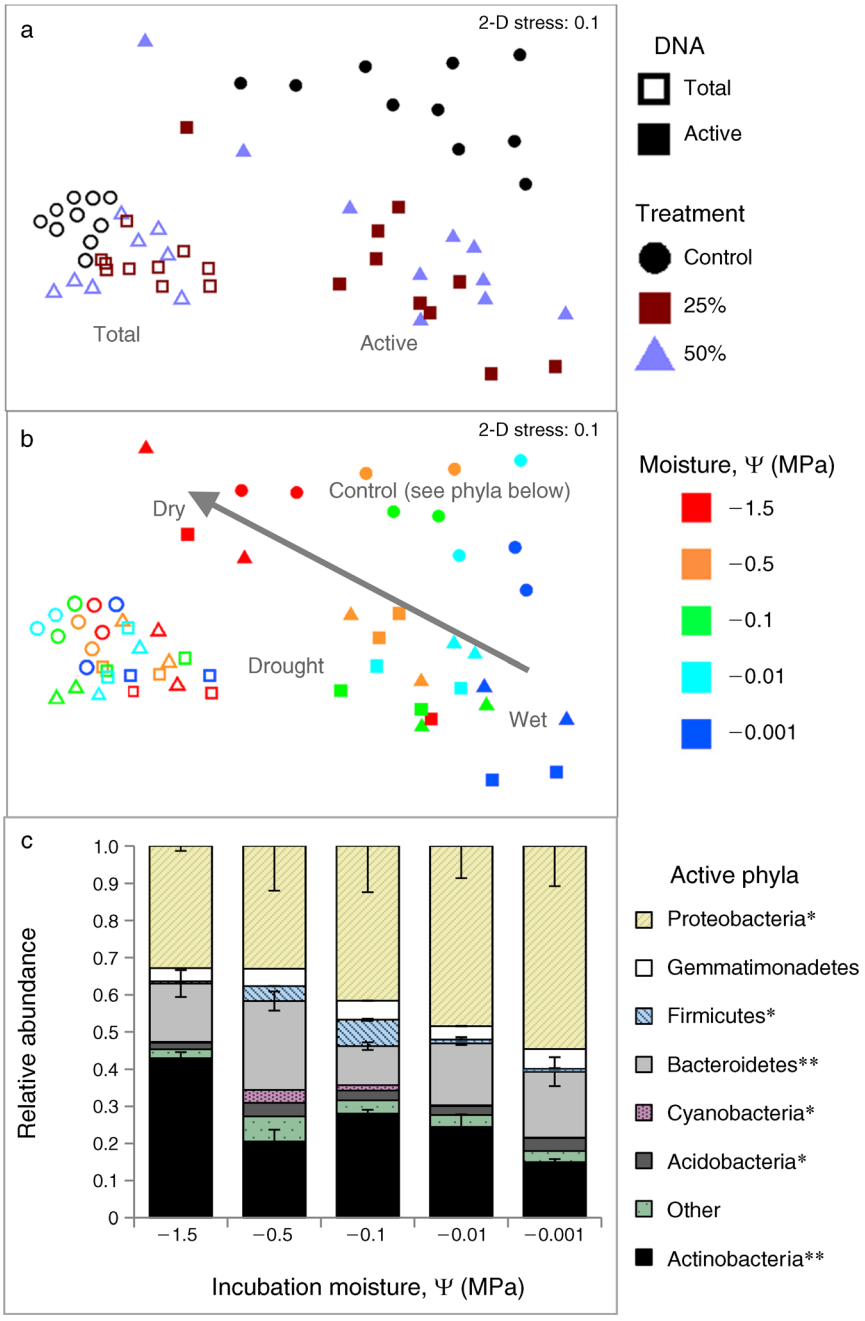

FIG. 3. Effect of (a) drought treatment and (b) laboratory moisture level on active and total species composition, and (c) relative abundance of active community phyla in the control treatment exposed to different moisture levels. DNA refers to either BrDU-associated DNA (active DNA) or total DNA (BrDU-associated and non-BrDU-associated, so active plus non-active, or Total). Panels (a) and (b) are identical except for the labels, and were analyzed by nonmetric multidimensional scaling using weighted UniFrac distances (Lozupone and Knight 2005). The 100% treatments were excluded for clarity, but were not significantly different from the control treatments. In panel (c), standard error bars are shown for dominant phyla only, for clarity. Asterisks indicate significant differences in phyla abundance across moistures.

* $P < 0.05$; ** $P < 0.01$.

precipitation). However, our monitoring does show that drought treatments effectively reduced soil moisture, and that this difference resulted in altered bacterial community composition under long-term drought in the field. Thus, we were able to test our hypotheses, which we discuss in the following three sections. If, as we hypothesized, variation in moisture sensitivity were driving these shifts in community composition, we

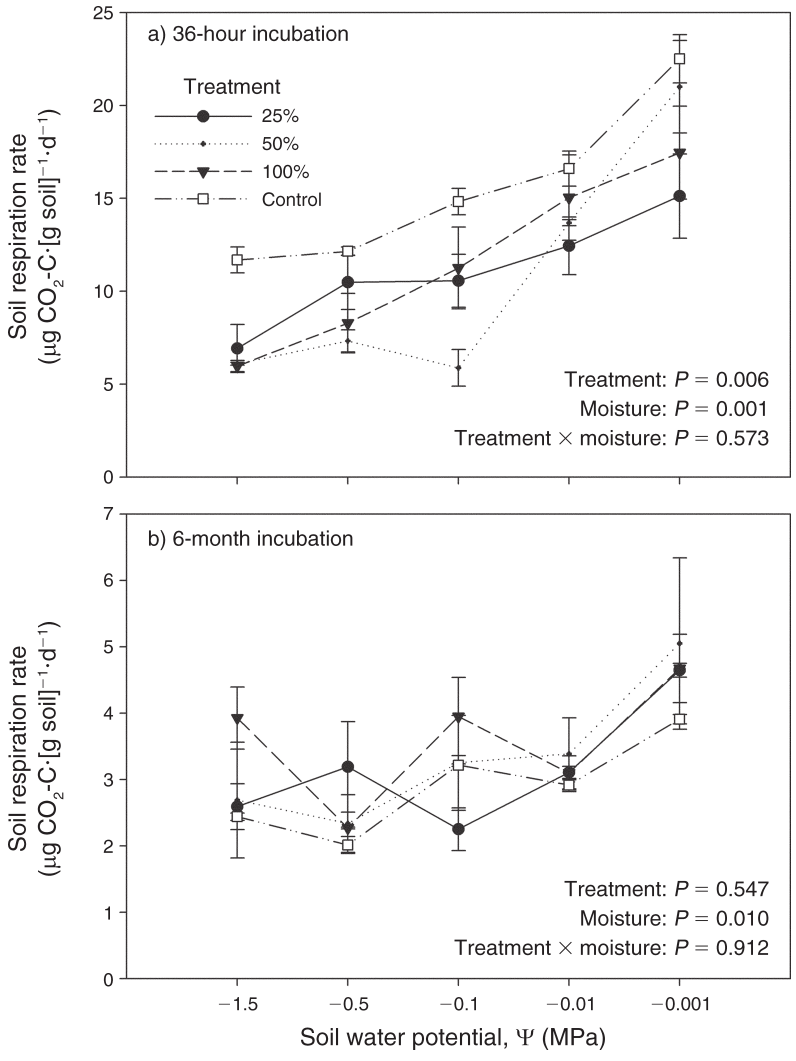

FIG. 4. Respiration rate of soils from rainfall manipulations (25%, 50%, 100% rainfall re-added, and control) in the shortgrass steppe incubated at five water potentials in the laboratory over (a) 36 hours and (b) 6 months.

would expect (1) antecedent drought to influence response of bacterial communities to moisture, (2) moisture niche partitioning of bacterial groups, and (3) species active at lower moistures to be those that emerged under reduced-moisture treatments.

Antecedent drought alters active community composition and short-term functional response

The composition of communities that were active under different moistures was most strongly affected by contemporary moisture (Figs. 3b and 4), but field drought treatment was also a significant factor (Figs. 3a and 4). The fact that communities did not completely (and immediately) converge in similarity, even when subject to the same moisture conditions in the laboratory, suggests that shifts in total community composition under drought may have affected community potential (i.e., the microbial "seed bank") influencing which species are active under certain conditions, at least in the short term (Fig. 3b). Interestingly, total community composition in these incubations was quite distinct from active communities, as other studies have observed (Edlund and Jansson 2006, Baldrian et al. 2011), and only weakly influenced by contemporary moisture level (Figs. 3b and 4). Our findings that drought history influences active community composition supports other studies suggesting that antecedent conditions are important for microbial responses to moisture (Fierer et al. 2003, Evans and Wallenstein 2012) and other environmental changes (Tobor-Kaplon et al. 2006, Ayres et al. 2009).

Drought also altered respiration rates of microbial communities, at least in the short term (Fig. 4). This may have been due to shifts in the functional potential of the microbial community that accompanied short-term shifts in activity that we observed, or shifts in abiotic

factors under drought, such as substrate quality, quantity, or accessibility (diffusion limitation; Or et al. 2007, Dungait et al. 2012). Although we could not capture all of these factors on a micro-scale, none of the environmental variables we measured across treatments explained the differences in short-term respiration (Table 1; correlations with long-term respiration not shown), suggesting that, in this case, the shifts in community potential that we observed may have been a proximal control on short-term respiration rates. A history of drought seems to reduce overall potential of the microbial community (i.e., respiration was lower than control at each moisture level, but there was no significant moisture × treatment interaction), instead of resulting in communities that can better take advantage of dry conditions. Although drought history did not affect long-term respiration rates, these findings may be important for explaining short-term CO_2 pulses following rewetting events that can constitute a large proportion of total carbon flux in semiarid systems (Huxman et al. 2004, Munson et al. 2010). Overall, our main goal was not to test the relationship between community composition and function. However, the influence of drought history on both compositional and functional response to wet-up level provides support for our hypothesis that moisture was the major driver of the long-term patterns we observed in the field.

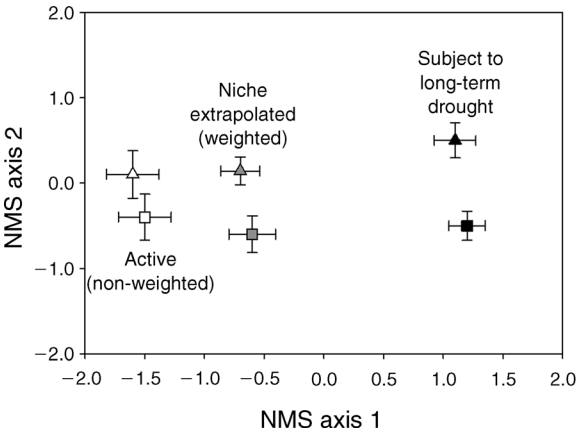

FIG. 5. Nonmetric multidimensional scaling (NMS) ordination of field treatments receiving 25% (triangles) and 50% (squares) of growing-season rainfall. We used the composition of microbial communities active in each treatment (open symbols) and weighted the taxa we would expect to grow in those conditions over time to generate niche-extrapolated communities (gray symbols). We expected these extrapolated communities to be similar to community composition we measured under 11-year drought (solid shapes). Pairwise distances were determined by PerMANOVA at the species level. Error bars represent the standard error of mean coordinates ($N = 2$).

Bacterial communities display niche partitioning

We extended previous work, which suggests that microorganisms vary in their sensitivity to moisture, by testing whether we can detect the partitioning of moisture niches amongst taxa in complex communities using soil mesocosms. We saw clear differentiation of active communities across a wet-up moisture gradient that was consistent among replicates, and fell along a primary niche axis, regardless of drought history (Fig. 3b, c). This suggests that microorganisms adopt different moisture niches (as a result of competitive or environmental pressures when a pulse occurs) that could facilitate coexistence and influence functional potential under certain moisture regimes.

This variation in moisture response may be due to traits related directly to moisture. Several traits that may constitute a microbial moisture niche were recently identified in culture, and, as we also found, conserved at a coarse phylogenetic level (Appendix B: Fig. B2; Lennon 2012). The way we measured moisture niche (by characterizing growth response) also would capture other traits not directly related to moisture, that are important for microbial fitness as moisture varies; for example, the ability to survive when soil pores are connected and predation may be higher (Wang and Or 2013), or the ability to outcompete other organisms when exposed to sudden increase in carbon or moisture availability (Schimel et al. 2007, Goldfarb et al. 2011). These traits would also be important in determining the fitness of these organisms in a field setting. Different experimental designs target different moisture traits, making it difficult to directly compare our findings to that of previous studies, but we find Actinobacteria were active at low moistures, and Proteobacteria at high moisture, supporting previous findings (Drenovsky et al. 2004, Castro et al. 2010).

One obvious limitation to this approach is that we have described the partitioning of moisture niches based only on short-term response to variation in wet-up moisture level, and by using a single response, growth, as indicative of niche occupation. Differences in growth under a certain moisture level for a longer time period, as well as death or reproduction, also contribute to that species' moisture niche and competitive ability under moisture regimes in the long term (as relevant to our hypothesis that moisture niche would predict how species composition would shift under drought). The groups we identified may reflect successional patterns, specific "wet-up moisture niches," or species that simply resuscitate quickly (Placella et al. 2012, Goransson et al. 2013). And even if short-term growth is a valid proxy for performance under long-term moisture regime, performance may not always correlate to abundance (McGill 2012).

Still, we saw in-soil niche differentiation in the short-term (indicating we did not just isolate fast-responders) in soils with the same properties. In addition, much of the dynamics in this system are drive by short-term pulses. Under drought, these pulses are likely to be smaller, but still represent a large proportion of total

microbial activity. Therefore, we would still have reason to expect the groups we identified (Fig. 3c) to have a competitive advantage in their specified moisture condition, and for that to contribute to its survival and abundance under long-term shifts in moisture conditions.

Moisture niche partitioning did not explain community shifts under long-term drought

We compared communities we observed in the field (Fig. 2) to those communities that we might expect to emerge under drought if changes in community composition were driven by moisture niche partitioning (Fig. 3c). We found that weighting species that we would expect to become more abundant under a given rainfall treatment generated communities that were *more* similar to those we observed in the field (compared to communities that were active but not weighted by moisture niche), but that these communities were still quite distinct (Fig. 5).

In addition to the way we represented moisture niche in this study (i.e., using a short-term response), methodological issues may have also affected community overlap. Specifically, although our depth of sequencing was comparable to other studies, many of the species active in dry conditions were simply not "present" (too rare to be detected by this depth of sequencing) in long-term field treatments. As a result, it was impossible for these species to be adequately "weighted" in simulated communities, and this made field and simulated communities more distinct. Considering this caveat, we could take the finding that communities did become more similar as an indicator that moisture dynamics are shaping long-term community composition, but the extent it "drives" niche-extrapolated communities is limited by the presence of important species. In addition, our taxonomic groupings (species at 97% similarity) could have affected species identification, and therefore, our detection of species overlap. As field and active communities differed in the taxonomic scale at which they were affected by moisture and long-term treatment (Appendix B: Fig. B2), more or less overlap could have occurred at different taxonomic levels that might integrate over possible errors at the species level.

An alternative explanation could be that other environmental factors that shifted under drought more strongly shaped community structure than the direct changes in soil moisture. Bacterial community composition in the field was also correlated to changes in pH and DOC/N, which shifted under drought (Table 1), though inference from correlation is limited by replication. Shifts in these factors can also result in changes in microbial community composition under field manipulations and across regions (Allison and Martiny 2008, Rousk et al. 2010), and aggregated effects of rainfall manipulations (e.g., change in rhizodeposition, frequent diffusion limitation) can drive community changes (Williams 2007). In this experiment, it is possible that other factors (DOC, pH) caused community compositions under drought to differ from the composition we would expect based on moisture niches, and shape the sensitivity of communities to rainfall manipulations.

Niche-characterization can clarify drivers of microbial community composition

A primary pursuit in microbial ecology has been to identify the drivers of microbial community composition. One fruitful approach to this pursuit has been to examine correlations between the abundance of microbial groups with abiotic factors such as soil pH and C content (Frey et al. 1999, Lauber et al. 2008). The limitation of this approach is that abiotic factors often co-vary and is not possible to elucidate the direct mechanisms that structure communities. Another approach has been to examine whether community composition changes in response to experimentally manipulated factors, which it often does (Allison and Martiny 2008). Like other studies, we observed shifts in microbial community composition under long-term drought manipulations in the shortgrass steppe. However, by linking these findings to an assessment of moisture response in the laboratory, we also found that these changes in the field may not necessarily be due only to the factors being manipulated.

The approach we developed in this study offers a way to link microbial physiology to community composition by characterizing niches for individual taxa. The same approach could be applied to other niche axes such as substrate utilization potential (Goldfarb et al. 2011), and allow us to more generally explore the relationships between microbial performance (growth rate, r) and abundance (N) in different environments (McGill 2012) as deeper sequencing captures more rare, yet active species. In turn, as data accumulates on the linkages between phylogeny and function, niche distribution models recently applied to microbial communities (King et al. 2010, Larsen et al. 2012) could be linked to trait-based approaches (Allison 2012) and contribute to predictions of shifts in community-level functions under changing environments.

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Literature Cited

Allison, S. D. 2012. A trait-based approach for modelling microbial litter decomposition. Ecology Letters 15:1058–1070.

Allison, S. D., and J. B. H. Martiny. 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences USA 105: 11512–11519.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46.

Austin, A. T., L. Yahdjian, J. M. Stark, J. Belnap, A. Porporato, U. Norton, D. A. Ravetta, and S. M. Schaeffer. 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141:221–235.

Ayres, E., et al. 2009. Home-field advantage accelerates leaf litter decomposition in forests. Soil Biology and Biochemistry 41:606–610.

Baldrian, P., et al. 2011. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISME Journal 6:248–258.

Borneman, J. 1999. Culture-independent identification of microorganisms that respond to specified stimuli. Applied and Environmental Microbiology 65:3398–3400.

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil-nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17: 837–842.

Caporaso, J. G., K. Bittinger, F. D. Bushman, T. Z. DeSantis, G. L. Andersen, and R. Knight. 2010*a*. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26:266–267.

Caporaso, J. G., et al. 2010*b*. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7: 335–336.

Castro, H. F., A. T. Classen, E. E. Austin, R. J. Norby, and C. W. Schadt. 2010. Soil microbial community responses to multiple experimental climate change drivers. Applied and Environmental Microbiology 76:999–1007.

Clark, J., J. Campbell, H. Grizzle, V. Acosta-Martìnez, and J. Zak. 2009. Soil microbial community response to drought and precipitation variability in the Chihuahuan Desert. Microbial Ecology 57:248–260.

Cruz-Martinez, K., K. B. Suttle, E. L. Brodie, M. E. Power, G. L. Andersen, and J. F. Banfield. 2009. Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. ISME Journal 3:738–744.

de Vries, F. T., M. E. Liiri, L. Bjornlund, M. A. Bowker, S. Christensen, H. M. Setala, and R. D. Bardgett. 2012. Land use alters the resistance and resilience of soil food webs to drought. Nature Climate Change 2:276–280.

Drenovsky, R. E., D. Vo, K. J. Graham, and K. M. Scow. 2004. Soil water content and organic carbon availability are major determinants of soil microbial community composition. Microbial Ecology 48:424–430.

Dungait, J. A. J., D. W. Hopkins, A. S. Gregory, and A. P. Whitmore. 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biology 18: 1781–1796.

Edlund, A., and J. K. Jansson. 2006. Changes in active bacterial communities before and after dredging of highly polluted Baltic Sea sediments. Applied and Environmental Microbiology 72:6800–6807.

Evans, S. E., and I. C. Burke. 2013. Carbon and nitrogen decoupling under an 11-year drought in the shortgrass steppe. Ecosystems 16:20–33.

Evans, S. E., K. M. Byrne, W. K. Lauenroth, and I. C. Burke. 2011. Defining the limit to resistance in a drought-tolerant grassland: long-term severe drought significantly reduces the dominant species and increases ruderals. Journal of Ecology 99:1500–1507.

Evans, S. E., and M. D. Wallenstein. 2012. Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? Biogeochemistry 109: 101–116.

Fierer, N., M. Hamady, C. L. Lauber, and R. Knight. 2008. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proceedings of the National Academy of Sciences USA 105:17994–17999.

Fierer, N., J. P. Schimel, and P. A. Holden. 2003. Influence of drying-rewetting frequency on soil bacterial community structure. Microbial Ecology 45:63–71.

Frey, S. D., E. T. Elliott, and K. Paustian. 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biology and Biochemistry 31:573–585.

Goldfarb, K. C., M. D. Wallenstein, K. K. Treseder, M. A. Bradford, C. A. Santee, C. A. Hanson, U. Karaoz, and E. L. Brodie. 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. Frontiers in Microbiology 2:1–10.

Goransson, H., D. L. Godbold, D. L. Jones, and J. Rousk. 2013. Bacterial growth and respiration responses upon rewetting dry forest soils: Impact of drought-legacy. Soil Biology and Biochemistry 57:477–486.

Griffiths, R. I., A. S. Whiteley, A. G. O'Donnell, and M. J. Bailey. 2003. Physiological and community responses of established grassland bacterial populations to water stress. Applied and Environmental Microbiology 69:6961–6968.

Guisan, A., and D. Thuiller. 2005. Predicting species distribution: offering more than simple habitat models. Ecology Letters 8:993–1009.

Hamady, M., J. J. Walker, J. K. Harris, N. J. Gold, and R. Knight. 2008. Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. Nature Methods 5:235–237.

Hawkes, C. V., S. N. Kivlin, J. D. Rocca, V. Huguet, M. A. Thomsen, and K. B. Suttle. 2011. Fungal community responses to precipitation. Global Change Biology 17:1637–1645.

Hueso, S., C. García, and T. Hernández. 2012. Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. Soil Biology and Biochemistry 50:167–173.

Huxman, T. E., K. A. Snyder, D. Tissue, A. J. Leffler, K. Ogle, W. T. Pockman, D. R. Sandquist, D. L. Potts, and S. Schwinning. 2004. Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. Oecologia 141:254–268.

Iovieno, P., and E. Baath. 2008. Effect of drying and rewetting on bacterial growth rates in soil. FEMS Microbiology Ecology 65:400–407.

King, A. J., K. R. Freeman, K. F. McCormick, R. C. Lynch, C. Lozupone, R. Knight, and S. K. Schmidt. 2010. Biogeography and habitat modelling of high-alpine bacteria. Nature Communications 1:1–10.

Landesman, W. J., and J. Dighton. 2010. Response of soil microbial communities and the production of plant-available nitrogen to a two-year rainfall manipulation in the New Jersey Pinelands. Soil Biology and Biochemistry 42:1751–1758.

Larsen, P. E., D. Field, and J. A. Gilbert. 2012. Predicting bacterial community assemblages using an artificial neural network approach. Nature Methods 9:91–98.

Lauber, C. L., M. Hamady, R. Knight, and N. Fierer. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75:5111–5120.

Lauber, C. L., M. S. Strickland, M. A. Bradford, and N. Fierer. 2008. The influence of soil properties on the structure of

bacterial and fungal communities across land-use types. Soil Biology and Biochemistry 40:2407–2415.
Lauenroth, W. K., I. C. Burke, and J. A. Morgan. 2008. The shortgrass steppe. Oxford University Press, New York, New York, USA.
Lennon, J. T. 2012. Mapping the niche space of soil microorganisms using taxonomy and traits. Ecology 93: 1867–1879.
Lozupone, C., and R. Knight. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Applied and Environmental Microbiology 71:8228–8235.
McGill, B. J. 2012. Trees are rarely most abundant where they grow best. Journal of Plant Ecology 5:46–51.
McMahon, S. K., M. D. Wallenstein, and J. P. Schimel. 2009. Microbial growth in Arctic tundra soil at −2 deg C. Environmental Microbiology Reports 1:162–166.
McMahon, S. K., M. D. Wallenstein, and J. P. Schimel. 2011. A cross-seasonal comparison of active and total bacterial community composition in Arctic tundra soil using bromodeoxyuridine labeling. Soil Biology and Biochemistry 43:287–295.
Munson, S. M., T. J. Benton, W. K. Lauenroth, and I. C. Burke. 2010. Soil carbon flux following pulse precipitation events in the shortgrass steppe. Ecological Research 25:205–211.
Noy-Meir, I. 1973. Desert ecosystems: Environment and producers. Annual Review of Ecology and Systematics 4: 25–51.
Or, D., B. F. Smets, J. M. Wraith, A. Dechesne, and S. P. Friedman. 2007. Physical constraints affecting bacterial habitats and activity in unsaturated porous media: a review. Advances in Water Resources 30:1505–1527.
Placella, S. A., E. L. Brodie, and M. K. Firestone. 2012. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. Proceedings of the National Academy of Sciences USA 109: 10931–10936.
R Development Core Team. 2010. R: a language and environment for statistical computing. Version 2.11.0. R Foundation for Statistical Computing, Vienna, Austria.
Rousk, J., P. C. Brookes, and E. Baath. 2010. The microbial PLFA composition as affected by pH in an arable soil. Soil Biology and Biochemistry 42:516–520.
Sala, O. E., W. K. Lauenroth, and W. J. Parton. 1992. Long-term soil–water dynamics in the shortgrass steppe. Ecology 73:1175–1181.
Schimel, J., T. C. Balser, and M. Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386–1394.
Sparks, D. L., editor. 1996. Methods of soil analysis. Part 3: Chemical and microbiological properties. American Society of Agronomy, Madison, Wisconsin, USA.
Tobor-Kaplon, M. A., J. Bloem, and P. C. De Ruiter. 2006. Functional stability of microbial communities from long-term stressed soils to additional disturbance. Environmental Toxicology and Chemistry 25:1993–1999.
USDA and Soil Conservation Service. 1981. Land resource regions and major land resource areas of the United States. USDA and Soil Conservation Service, Washington, D.C., USA.
Vance, E. D., P. C. Brookes, and D. S. Jenkinson. 1987. Microbial biomass measurements in forest soils: determination of Kc values and tests of hypotheses to explain the failure of the chloroform fumigation incubation method in acid soils. Soil Biology and Biochemistry 19:689–696.
Wang, G., and D. Or. 2013. Hydration dynamics promote bacterial coexistence on rough surfaces. ISME Journal 7: 395–404.
Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73:5261–5267.
Williams, M. A. 2007. Response of microbial communities to water stress in irrigated and drought-prone tallgrass prairie soils. Soil Biology and Biochemistry 39:2750–2757.
Williams, M. A., and C. W. Rice. 2007. Seven years of enhanced water availability influences the physiological, structural, and functional attributes of a soil microbial community. Applied Soil Ecology 35:535–545.

Supplemental Material

Appendix A

Full explanation on the method used to generate niche-extrapolated communities from field soil moisture profiles and moisture niches (*Ecological Archives* E095-010-A1).

Appendix B

Supplementary results from bacterial community 16S rRNA sequencing analysis (*Ecological Archives* E095-010-A2).