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Historical Drought Affects Microbial Population Dynamics and Activity During Soil Drying and Re-Wet

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Abstract

A history of drought exposure promoted by variable precipitation regimes can select for drought-tolerant soil microbial taxa, but the mechanisms of survival and death of microbial populations through the selective stresses of soil drying and re-wet are not well understood. We subjected soils collected from a 15-year field drought experiment ("Altered" precipitation history with extended dry periods, versus the "Ambient" field control) to a laboratory drying/re-wetting experiment, to learn whether selective population survival, death, or maintenance of protein synthesis potential and microbial respiration through variable soil water conditions was affected by field drought legacy. Microbial community composition, as measured by Illumina MiSeq sequencing of the 16S rRNA and 16S rRNA gene, shifted with laboratory drying/re-wet and field drought treatments. In Ambient soils, there was a higher proportion of reduced OTU abundance (indicative of mortality) during re-wet, whereas Altered soils had a greater proportion of stable OTU populations that did not change in abundance (indicative of survival) through drying/re-wet. Altered soils also had a lower proportion of rRNA:rRNA genes (lower protein synthesis potential) during dry-down, a greater weighted mean rRNA operon number (potential growth rate and r-selection) which was associated with higher abundance of *Firmicutes* (order *Bacillales*), and lower average microbial respiration rates. These data demonstrate that soils with a weaker historical drought legacy exhibit a higher prevalence of microbial water-stress mortality and differential survival and death at OTU levels following short-term dryingand re-wetting, concurrent with higher carbon loss potential. This work provides novel insight into the mechanisms and consequences of soil microbial changes resulting from extended drought conditions.

Keywords Drought · Soil · Microbial community · Drying/re-wet · Bacteria

Introduction

Soil water content is a controlling factor of microbial activity and biogeochemical cycling in terrestrial ecosystems [1]. Large proportions of carbon (C) budgets are linked to soil drying and re-wetting cycles due to the mineralization of CO_2 after precipitation events [2–4]. Conversely, dry soil conditions caused by reduced or less frequent precipitation decrease soil respiration and C efflux [5]. Given that precipitation patterns globally are predicted to have greater intra-annual variability, longer periods of drought, and more extreme rainfall events in certain regions [6–8], it is essential to understand the consequences of these heightened drought conditions on soil microbial populations and microbial C cycling activity.

Soil biota, including microorganisms, are sensitive to changes in soil water content and altered drought history, as demonstrated by known linkages between historical rainfall variability, microbial activity [9, 10], and total soil carbon efflux [5]. Extended drought and lack of water affect soil microbial contributions to carbon balance via reductions in soluble substrate supply and reductions in microbial activity and growth due to starvation, osmotic stress, and functional differentiation between microbial taxa [11, 12]. As soil water content decreases, biological activity does as well, with taxa exhibiting differential maintenance of metabolic activity at decreasing water potentials [13]. Subsequent re-wet of dried

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soils causes a flush of microbial respiration, which varies in magnitude depending on the amount of resolubilized organic substrate and microbial growth and activity rates [1, 14–16]. Furthermore, in both laboratory [17] and field experiments [18], historically drought-prone soils exhibit lower respiration and bacterial growth rates. These data suggest that drought history selects for microbiota with functional adaptations supporting lower C loss. Although it has been documented that more variable precipitation regimes support drought-adapted soil bacterial communities [19], the mechanisms underlying shifts in composition under this scenario, and possible consequences for C cycling, are not well understood.

Increased variability in precipitation causes more extreme environmental conditions for soil microbiota, including longer dry periods and larger re-wet events. These dynamics are physiologically stressful for microbial populations, particularly bacteria, which must respond to fluctuations in resource availability and maintain cell membrane and osmotic integrity or enter a dormant state to prevent cell lysis [11, 20, 21]. Cell death during drying or re-wetting reduces the relative abundance of populations that do not have the physiological capability of surviving drought, and conversely, populations that survive or thrive during drying or rapid re-wetting should become competitively dominant over repeated drought cycles. In this way, soil microbial population size fluctuations can accumulate over time, creating a legacy of greater drought history that is detectable as a change in community composition [15, 22–24]. Learning the mechanisms underlying these shifts in community composition is essential for understanding the drivers of variable microbial resistance or resilience to change [25] and predicting microbial functional responses to increasing drought conditions [26].

To understand the mechanisms underlying soil microbial community shift following greater drought history, it is necessary to detect and quantify ecological traits or drying/re-wetting tolerance strategies that allow for higher fitness under dynamic soil water conditions. Microbial communities previously exposed to drought stress are considerably less sensitive to drying [27] compared to those with less historical soil water variability, in both function and composition [15, 19, 23, 28]. Lennon et al. [29] demonstrated that soil microbes with low water potential niche optima have specific functional traits, such as biofilm production, which yield a higher fitness and competitive advantage under dry conditions. There are many functional traits related to greater fitness under resource limitation, such as cell size [30], dormancy or reduced protein synthesis potential [31, 32], and greater growth rate or growth efficiency [33]. Some of these traits are difficult to measure within complex microbial communities (e.g., biofilm production), while others (e.g., relative death or survival, growth rate potential, protein synthesis potential) can be inferred using standard 16S rRNA and rRNA gene-sequencing approaches. Despite being proxies of microbial trait distribution rather than direct measurement, these more accessible approaches can provide insightful information on microbial functional responses to environmental change. For example, microbial populations with higher potential growth rate and lower growth efficiency tend to have higher 16S rRNA gene copy number [33, 34], and a greater rRNA abundance per rRNA gene reflects greater protein synthesis potential and maintenance of cellular integrity through challenging conditions [32]. Thus, a higher prevalence of populations that can grow quickly following disturbance may be detected as higher weighted mean 16S rRNA gene copy number (relative abundance weighted metric), as estimated using the number of gene copies within the closest known relatives to taxa in a mixed soil community [35, 36]. Also, a population's capacity to maintain cell integrity and metabolic activity through greater protein production potential can be detected as higher rRNA abundance [37].

A key goal within the field of microbial ecology, and of this study, is to increase understanding of how long-term changes in the environment, such as increased drought stress, affect microbial community composition and affiliated functional attributes. Our overall hypothesis was that the mechanisms underlying microbial responses to soil drying and wetting would differ based on the historical drought legacy of the soil. Specifically, we predicted that soils with a history of greater drought would contain a higher proportion of microbial populations that display *survival* through laboratory drying and re-wet cycles, while microbial communities in soils with less historical drought would contain a higher proportion of taxa that decrease in abundance, displaying *mortality* during dynamic drying and re-wet treatments. Furthermore, we expected that the *maintenance* of protein synthesis potential during soil drying and re-wet, and the potential for higher growth rates upon re-wet, supporting enhanced survival and reproduction, respectively, would be greater in soil with a history of greater drought.

To evaluate these predictions, we collected soils from a 15-year field drought experiment and exposed these soils to a repeated series of extreme drying/re-wet events in the laboratory. Throughout this time-series incubation, we measured prokaryotic community composition via 16S rRNA and 16S rRNA gene sequencing, total 16S rRNA gene abundance, and soil microbial respiration (CO_2 production rate) to track concurrent community shifts, OTU population dynamics, and changes in integrated microbial C cycling function. Evidence for OTU population mortality was based on decreased abundance following drying or re-wet, OTU population survival was defined by stable abundance through drying/re-wet events, and the 16S rRNA:rRNA gene ratio was used to provide evidence for maintenance of protein synthesis potential [31, 32, 37]. Also, a life history trait indicative of r-selection and potential growth rate potential was estimated as the community weighted mean 16S rRNA gene copy

number [33, 36] calculated using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) [35] tool.

Materials and Methods

Site Description and Field Experiment

Soils used for incubations were collected in 2011 at the Rainfall Manipulation Plots (RaMPs) experiment, located in a large remnant tallgrass prairie ecosystem at Konza Prairie Biological Station (KPBS). KPBS is in northeastern Kansas, USA, in the Flint Hills Region, and recieves a mean annual precipitation (MAP) of 835 mm y^{-1}, with approximately 75% of precipitation occurring in late spring and summer [38]. The study soils are fine, mixed mesic Pachic Argiustolls (lowland Irwin silty clay loam, USDA NRCS), with a pH of 6.6 ± 0.05 (mean ± SE) in 1:2 soil:deionized water. The RaMPs is a long-term precipitation alteration experiment initiated in 1997 [39]. Twelve rainout shelters (14 × 9 m) were assigned to one of two rainfall treatments: ambient and altered. At each shelter, a clear, plastic roof collected all rainfall into two 4 m^3 reservoirs and then the water was reapplied to underlying experimental plots by an overhead irrigation system. In the ambient treatment plots, which represent a field control, the same amount of rainfall collected was applied each time a natural rainfall event occurred. In the altered treatments, all rain water was collected but was reapplied only during alternating natural rainfall events. Both treatments recieved the same cumulative rainfall, but the period between rain events was extended so that approximately 52% fewer in frequency, but 230% larger in amount, rainfall events occurred in the altered treatment [5]. This precipitation regime resulted in significantly drier soils [5, 40] over every growing season for the fifteen years prior to when soils were collected for our lab experiment. The historical legacy of deeper and more extended drought imposed by these long-term field experimental treatments is the first layer in our experimental design and is referred to throughout the manuscript as field "drought history" or altered "precipitation history."

Laboratory Experimental Setup

Bulk mineral soils (three 5.6 cm × 15 cm depth composite cores per plot) were collected in June 2011, 5 days after a rain event [23], sieved (4 mm), and stored at -20 °C. In January 2015, a subsample (~ 150 g soil) from 4 ambient and 4 altered plots was thawed and acclimated to room temperature for 7 days. A subset (~ 50 g dry mass) of soil from each plot was designated to a continuously wetted laboratory control (4 replicate controls for each field treatment, ambient and altered) or sequentially air-dried and re-wet treatment (4 replicate drying/re-wet laboratory treatments for each field (ambient and altered) treatment). The lab experiment was designed so that laboratory replicate units correspond directly to field plot replicate units. Each replicate mass of soil was incubated at room temperature in a ~ 470 mL mason jar with a septated lid (approx. 400 mL headspace at initiation of the experiment). Control soils were wet to equilibrate all replicates to the highest water content of the field soil replicates (38% volumetric water content (VWC) (approx. -0.005 MPa)) and maintained at this level for the duration of the experiment. Treatment soils were allowed to air dry at room temperature (~ 25 °C) over time from field water content to the level of minimal water retention in these soils (approx. 9% VWC (< -10 MPa)), then re-wet to 38% VWC, and this cycle was repeated once. Soil subsamples from each replicate jar were collected for molecular analysis at t_0, at the end of each drying period, and following each re-wet event (days 13 and 25; Fig. 1). Water potential was estimated from water volume using a water-release curve derived from empirical data on the study soils [23], and field data confirm that the laboratory drying point (9% VWC) represents dry soil conditions characteristic of the field experiment [5].

Data Collection

At day 0, and before and 4 h after re-wet on days 13 and 25, the headspace CO_2-C concentration accumulated over 2 h for all replicates of control and treatment soils was measured to estimate microbial respiration [41]. CO_2-C was measured using a Picarro G2101-i Analyzer (Picarro Inc., Santa Clara, CA, USA), and soil microbial respiration rate per soil dry mass was calculated using the Ideal Gas Law [41]. After CO_2-C concentration was measured, subsamples of soil from all control and treatment replicates were collected and stored at -20 °C for subsequent analysis of 16S rRNA gene abundance and community composition.

Total DNA and RNA was extracted from approximately 0.5 g of soil by lysing cells using bead-beating in a cetyltrimethylammonium bromide (CTAB) buffer with ammonium aluminum sulfate, followed by phenol:chloroform:isoamyl alcohol and chloroform extraction, and overnight precipitation at room temperature in polyethylene glycol 6000 [42]. Then, genomic DNA (gDNA) and RNA were isolated and separated using an AllPrep DNA/RNA kit (Qiagen, Venlo, Netherlands). This protocol includes a DNAse incubation step, and PCR was run on a subset of isolated RNA samples, using the 16S rRNA gene primers, to confirm the absence of DNA. Immediately after isolation, DNA and RNA were quantified (Quant-iT DNA and Ribogreen assay kits, ThermoFisher Scientific, Waltham, MA, USA) and cDNA was synthesized. First-strand cDNA synthesis on 8 μL (10–20 ng) of purified RNA using 50 ng of random primer mix and 200 U SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) was conducted

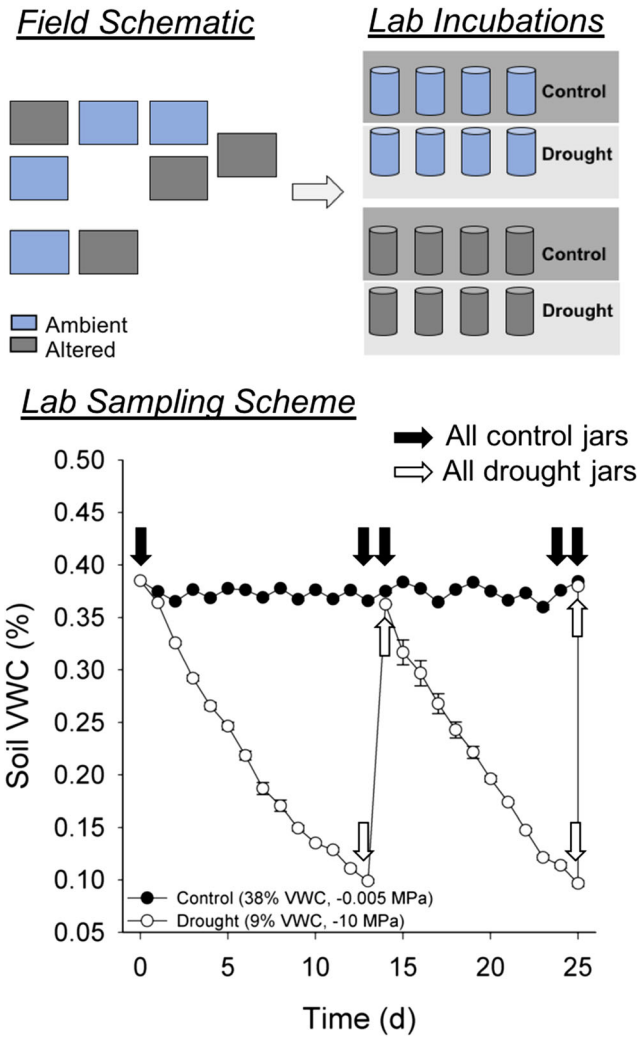

Fig. 1 A diagram of ambient (blue boxes and jars) and altered (gray boxes and jars) treatments within field settings, laboratory incubations, and experimental soil volumetric water content (VWC; mean ± SE) across the study period (25 d). Soils were collected from 4 ambient and altered field soil treatments (their placement reflective of their relative configuration within the field) and subsamples placed within control (soil VWC maintained at ~ 38% VWC, noted by dark gray blocks) and drought (dried to 9% VWC and re-wet, noted by light gray boxes) treatments. Black arrows indicate timing of sampling for all responses within laboratory control (closed circles; $n = 8$) and drought (open circles; $n = 8$) treatments.

using manufacturer's exact protocols in a 20 μL final reaction volume, and sscDNA and gDNA were stored at -80 °C until further analysis.

Bacterial and archaeal 16S rRNA and rRNA gene amplicons were prepared for Illumina MiSeq sequencing using primers 515F and 806R via the Earth Microbiome Project protocol [43], modified slightly: triplicate PCRs for gDNA libraries used 25 cycles and cDNA libraries used 28 cycles. PCR amplicons were cleaned using ExoSAP-IT (Affymetrix, Inc., Cleveland, OH), combined into one pooled library at equal molar concentration per sample and gel-purified (Qiagen, Venlo, Netherlands). Libraries were sequenced with a 25% PhiX spike using a 2 × 150 MiSeq v2 Reagent kit at the Kansas State University Integrated Genomics Facility (Manhattan, KS, USA). Demultiplexed .fastq files for 16S rRNA gene and 16S rRNA libraries are deposited on the National Center for Biotechnology Information's Sequence Read Archive under study accession number SRP118372.

Quantitative PCRs of the bacterial 16S rRNA gene (25 μL final reaction volume) were prepared with 10 ng template gDNA per sample and run in triplicate on a Bio-Rad CFX real-time PCR system using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) and established primers and protocols [44, 45]. Standard curves were generated with $1.24{\cdot}10^6$–1.24 copies of 16S rRNA genes from *Escherichia coli* ATCC 25922. Efficiencies ranged from 85 to 99%, and R^2 values from 0.99 to 1.0. All assays included a no-template control and melting curves to confirm amplification specificity.

Bioinformatics and Statistical Analysis

Raw .fastq files were processed in QIIME 1 (version 1.9.1) [46]. After joining .fastq files, sequences were demultiplexed, and operational taxonomic units (OTUs) were clustered at a 97% identity level using the pick closed reference workflow with the Greengenes reference database (version 13.5). Taxonomy was assigned via the Naïve Bayesian RDP Classifier [47], and chimeric OTUs (identified with ChimeraSlayer), singletons, and mitochondrial- and chloroplast-assigned OTUs were removed. Average sequencing depth for RNA data was ~ 30,000 sequences per sample, and for DNA data, was ~ 77,000 sequences per sample. However, due to several samples with relatively lower sequence yield and to maximize the number of samples retained, we rarefied all samples to 12,000 sequence reads for both RNA and DNA libraries (hereon termed "rRNA" and "rRNA gene"). The final dataset contained a total of 8736 OTUs and 2,652,000 sequences. OTU counts were corrected for variable 16S rRNA gene copy number using the web-based implementation of PiCRUST [35] for rRNA gene and rRNA datasets. After 16S rRNA gene copy number correction, the ratio of rRNA:rRNA gene counts for each OTU in each sample was calculated. Due to the "compositional" nature of sequencing data [48], after PiCRUST-implemented copy number correction, OTU absolute abundance within rRNA gene sequence read datasets was calculated by normalizing each OTU distribution count for the total number of 16S rRNA gene copies as measured by qPCR within each sample. This approach allows a more reliable estimate of absolute OTU abundances and differentiation of community composition among experimental units for rRNA gene data. Lastly, we used PiCRUST to calculate the mean weighted 16S rRNA operon number for each sample (the product of copy number

and OTU relative abundance summed for all OTUs in a sample [36, 49, 50]).

To evaluate differences in community composition, Bray–Curtis distance matrices of OTUs in rRNA gene and rRNA:rRNA gene datasets were calculated and visualized using non-metric dimensional scaling (NMDS) ordinations. The Bray–Curtis distances were also input to a permutational multivariate ANOVA model (perMANOVA) [51] to determine the percentage of community variation explained by laboratory drying/re-wet and precipitation history. Ordinations also indicated clustering of communities not represented by our experimental design; so, we included field plot as a fixed effect in the final perMANOVA model. A linear mixed-effects regression model was used to determine differences in microbial respiration rate, bacterial 16S rRNA gene copy number, weighted mean 16S rRNA operon copy number, and the relative abundance of dominant bacterial phyla across drying/re-wet events ("control," "drying," and "re-wet") and between soils with contrasting precipitation histories (ambient, altered). Notably, microbial respiration and 16S gene copy number did not differ between the two drying and re-wet cycles ($p > 0.13$). Therefore, we used statistical models that included a random effect term (replicate) to account for repeated temporal measurements from the same replicate unit and reported mean response values with data from both dry-down/re-wet events pooled. Because lab incubation replicates were explicitly designated to be the same as field replicate plots, this statistical model also accounts for field-scale variation within each laboratory treatment. We obtained F-values by calculating ANOVA tables of regression models and performed post-hoc tests for pairwise significance corrections for drying/re-wet treatment levels using Tukey's HSD contrasts. CO_2-C and 16S rRNA gene copy number data were log-transformed prior to analyses to fit assumptions of normality. All statistical analyses were performed in R (version 3.3.2, R Core Team 2015) using the nlme package (function lme) [52], multcomp package (function glht) [53], and vegan package (function metaMDS and adonis) [54].

A key goal of the laboratory experiment was to define drying and re-wet response strategies among archaeal and bacterial populations that coexist in each of the two contrasting field historical precipitation treatments. This was accomplished by categorizing how the abundance of each OTU changed in response to the dynamic water content phases of the lab experiment. OTUs that did not change in rRNA gene OTU abundance were classified as *survivors* of the drying or re-wetting treatment. OTUs that displayed a significant decrease in rRNA gene OTU abundance during the drying phase or following the re-wetting treatment were classified as experiencing *mortality*. OTUs that displayed a significant increase in the number of rRNA per rRNA genes (based on rRNA:rRNA gene counts as described previously) during the drying phase or following the re-wetting treatment were classified as investing in *maintenance* of protein synthesis potential [32] during these challenging environmental conditions. Note that a single OTU population could be defined as both a "survivor" and an activity potential "maintainer," if its gene abundance was stable and its rRNA abundance per rRNA gene copy increased. To identify response strategies, the DESeq2 package for R was used to identify OTUs that were differentially abundant (1) between wetted controls and the drought treatment at the end of the drying period and (2) after re-wet (Fig. 1). For rRNA:rRNA gene ratio, ratios within each sample were normalized using "1" as the lowest value to transforming DESeq2 input data to integer count values. Then, following the DESeq2 workflow [55], rRNA gene sequence read counts (used to estimate survivors and mortality) and rRNA:rRNA gene counts (used to estimate maintenance of protein synthesis potential) were log2 transformed and normalized to fit a negative-binomial distribution with equal variance among all samples. Difference in read count distribution between two groups of samples was evaluated using a Wald's test with Benjamini–Hochberg post-hoc false discovery rate-corrected p values, and OTUs for which p values were ≤ 0.05 were classified as differentially abundant. OTUs with no significant differential abundance were classified as survivors of the drying or re-wet periods. A linear mixed-effects regression model, analogous to other statistical analyses, was used to evaluate whether the proportional distribution of reads representative of mortality (differential decrease in the rRNA gene), survival (no change in rRNA gene), or maintenance (differential increase in rRNA:rRNA gene) was affected by precipitation history or varied between drying and re-wet periods.

Results

Microbial Respiration and Total 16S rRNA Gene Copies

Microbial respiration rate differed with drying/re-wet ($F_{2,46} = 226.02$, $p < 0.01$) and precipitation history ($F_{1,47} = 13.61$, $p < 0.01$; Fig. 2), with no significant interaction ($F_{2,46} = 2.31$, $p = 0.12$). Microbial respiration rates were lower in air-dried soils than control soils (Tukey's HSD $p < 0.01$), and re-wet soil microbial respiration rates (8.78 ± 0.61 μg CO_2-C g dry soil^{-1} h^{-1}1; mean ± SE) were greater than controls (3.52 ± 0.22 μg CO_2-C g dry soil^{-1} h^{-1}, 149% difference) and following drying (1.98 ± 0.11 μg CO_2-C g dry soil^{-1} h^{-1}, 343% increase; Tukey's HSD $p < 0.01$). Microbial respiration rates were 29% higher on average in ambient soils (5.35 ± 0.77 μg CO_2-C g dry soil^{-1} h^{-1}) compared to soils with altered precipitation history (4.15 ± 0.55 μg CO_2-C g dry soil^{-1} h^{-1}, $p < 0.01$; Fig. 2). In contrast, total 16S rRNA gene copies did not differ between drying/re-wet ($F_{2,46} = 1.76$, $p = 0.18$) or with different precipitation history ($F_{1,47} = 0.18$, $p = 0.68$; Fig. 2).

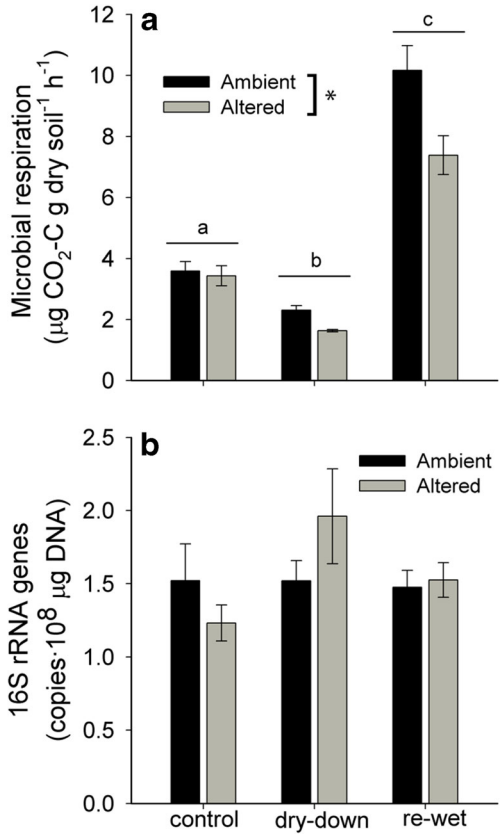

Fig. 2 Mean soil microbial respiration rates (**a**) and bacterial 16S rRNA gene copy numbers (**b**) of repeated drying and re-wet conditions and of soils with ambient and altered (greater drought) field precipitation history. Letters denote significant differences between drying/re-wet ($p < 0.01$); Tukey's HSD post-hoc was used for multiple comparison among water treatment. An asterisk denotes significant differences between precipitation history ($p < 0.01$). Bars represent means with standard error ($n = 8$).

Microbial Community Composition

A significant amount of variation in rRNA gene and rRNA:rRNA gene community composition was explained by the laboratory drying/re-wet treatment (perMANOVA, Table 1). Also, both rRNA gene and rRNA:rRNA gene communities were related to field drought history (Table 1). There were no statistically significant interactive effects, and 90–91% of variation in community composition remained unexplained by controlled drying/re-wet or precipitation history factors. With the identity of replicate samples explicitly represented in the statistical model, the experimental plot from which soils were collected explained the most variation in rRNA gene and rRNA:rRNA gene community composition (Table 1, SI Fig. 1).

Thirteen dominant (≥ 1.0% relative abundance in rRNA abundance or rRNA:rRNA ratio across experimental units) phyla, or subphyla within the *Proteobacteria*, were identified. Of these, nine phyla changed significantly through the drying/re-wet treatments based on rRNA gene abundance and

Table 1 Permutational multivariate ANOVA results for rRNA gene and rRNA:rRNA gene Bray–Curtis distance matrices. Values represent R^2 for each main effect (drying/re-wet and precipitation history) and their interaction terms and are denoted by significance level. Statistically significant effects for each model are in bold. The plot represents the experimental block location from where each of 8 RaMPs replicate soils was collected

perMANOVA model factors	SSE	MSE	Pseudo-F	R^2	p value
rRNA gene					
Drying/re-wet	**0.21**	**0.10**	**2.80**	**0.07**	**0.003**
Precipitation history	**0.08**	**0.08**	**2.08**	**0.02**	**0.03**
Drying × precipitation history	0.08	0.04	0.02	0.02	0.37
Plot	**1.18**	**0.20**	**5.27**	**0.37**	**0.001**
Residuals	1.61	0.04		0.51	
rRNA:rRNA gene					
Drying/re-wet	**0.65**	**0.33**	**2.07**	**0.07**	**0.001**
Precipitation history	**0.24**	**0.24**	**1.53**	**0.03**	**0.002**
Drying × precipitation history	0.26	0.13	0.83	0.03	0.99
Plot	**1.74**	**0.29**	**1.83**	**0.19**	**0.001**
Residuals	6.33	0.16		0.69	

rRNA:rRNA gene ratio (Table 2, SI Fig. 2). Based on rRNA gene abundances, *Firmicutes* ($p < 0.01$) and *Alphaproteobacteria* ($p = 0.02$) were less abundant, while *Acidobacteria* ($p < 0.01$) were more abundant, at the driest sampling time compared to following the re-wet. *Bacteroidetes* was more abundant in control soils compared to the dried soils ($p < 0.01$; Table 2, SI Fig. 2). Also, *Firmicutes* rRNA gene abundance was 47% greater in altered (0.40 ± 0.03%) than ambient (0.27 ± 0.02%) field history soils ($p < 0.01$). For the rRNA:rRNA gene data, *Actinobacteria* and *Planctomycetes* had highest rRNA:rRNA gene ratios in soils after the lab drying treatment (Tukey's HSD $p < 0.01$); *Bacteroidetes* and *Gammaproteobacteria* had the highest rRNA to rRNA gene ratios following re-wet (Tukey's HSD $p < 0.01$); *Alphaproteobacteria* had lowest rRNA to rRNA gene ratios in control soils ($p < 0.01$), and *Deltaproteobacteria* had highest rRNA to rRNA gene ratios in control soils ($p \leq 0.04$). Also, *Firmicutes* rRNA to rRNA ratio was 40% greater in altered (2.4 ± 0.2%) compared to ambient (1.7 ± 0.2%) history soils ($p = 0.04$). There were no significant drying/re-wet and precipitation history interactions in any of these statistical models ($p > 0.10$).

Proportions and Taxonomy of Different Drying/Re-Wet Responses

Survival through both drying and re-wet periods, defined as no significant change in DNA abundance of each OTU population, was the predominant strategy identified (mean 96.4–100%), while evidence for *mortality*, defined as a decrease in

Table 2 ANOVA table for linear mixed-model regressions for dominant Phyla, and Class for Proteobacteria, found in bacterial 16S rRNA gene and 16S rRNA:rRNA gene communities with drying/re-wet and precipitation history as explanatory, fixed variables. No interactions were significant; so, only ANOVA statistics for main effects are given. Statistically significant effects for each model are in bold. Phyla are listed based on percent relative abundance (abbreviated as Rel. abund.) in descending order.

			Drying/re-wet		Precipitation history	
Phylum	Rel. abund.	Response to treatments	F-statistic	*p* value	F-statistic	*p* value
rRNA gene						
Verrucomicrobia	32.7	No response	0.11	0.90	0.67	0.43
Actinobacteria	20.1	No response	2.08	0.15	0.50	0.50
Acidobacteria	14.8	↑ control, drying vs. re-wet	**22.38**	**< 0.01**	2.06	0.17
Alphaproteobacteria	9.9	↑ re-wet vs. drying	**4.47**	**0.02**	0.01	0.94
Deltaproteobacteria	3.6	No response	0.47	0.63	0.36	0.56
Gammaproteobacteria	3.3	No response	0.22	0.81	0.004	0.95
Planctomycetes	2.5	No response	2.67	0.09	0.63	0.44
Bacteroidetes	2.4	↑ control vs. drying	**5.65**	**< 0.01**	1.14	0.30
Chloroflexi	2.4	No response	0.62	0.55	0.09	0.77
Betaproteobacteria	2.2	No response	3.09	0.06	0.25	0.62
Nitrospirae	1.8	No response	1.40	0.26	1.47	0.25
Gemmatimonadetes	1.5	No response	2.02	0.15	1.76	0.21
Firmicutes	0.4	↑ control, re-wet vs. drying; ↑ altered vs. ambient	**22.14**	**< 0.01**	**17.81**	**< 0.01**
rRNA:rRNA gene						
Actinobacteria	24.6	↑ drying vs. re-wet, control	**11.15**	**< 0.01**	0.95	0.35
Deltaproteobacteria	18.7	↑ control vs. drying, re-wet	**65.67**	**< 0.01**	1.86	0.19
Alphaproteobacteria	16.6	↑ drying, re-wet vs. control	**9.1**	**< 0.01**	0.01	0.94
Acidobacteria	9.6	No response	1.61	0.22	1.19	0.29
Betaproteobacteria	7.9	↑ drying vs. re-wet	**7.45**	**< 0.01**	0.40	0.53
Bacteroidetes	5.7	↑ re-wet vs. drying, control	**7.34**	**< 0.01**	1.47	0.25
Verrucomicrobia	5.4	No response	2.53	0.10	1.71	0.21
Planctomycetes	3.1	↑ drying vs. re-wet, control	**13.37**	**< 0.01**	3.98	0.07
Gammaproteobacteria	2.8	↑ re-wet vs. drying, ontrol	**11.39**	**< 0.01**	0.001	0.98
Chloroflexi	2.1	No response	2.32	0.12	0.67	0.43
Firmicutes	2.0	↑ altered vs. ambient	0.29	0.75	**5.01**	**0.04**

DNA abundance of each OTU population following drying or re-wet, was less prevalent (mean 0–3.2%; Fig. 3a,b). OTUs exhibiting enriched rRNA per rRNA gene abundance, evidence for *maintenance* of protein synthesis potential, comprised 0.4–8.5% of the communities (Fig. 3c). *Mortality* and *ribosomal maintenance* during re-wet were more prevalent than during drying ($F_{1,22} = 117.01$, $p < 0.01$, $F_{1,21} = 57.12$, $p < 0.01$) whereas *survival* during drying was more prevalent than during re-wet ($F_{1,22} = 16.40$, $p < 0.01$). There was a significant drying/re-wet and precipitation history interaction for the proportion of *survival* ($F_{1,22} = 31.4$, $p < 0.01$), *mortality* ($F_{1,22} = 118.09$, $p < 0.01$), and *ribosome maintenance* ($F_{1,21} = 19.16$, $p < 0.01$). *Mortality* was significantly more prevalent in the ambient field treatment soils than in the altered field treatment soils following re-wet ($F_{1,22} = 118.09$, $p < 0.01$), whereas *survival* was more prevalent in the altered field treatment soils following both drying and re-wet. Furthermore, *survival* of drying was more prevalent than *survival* of re-wetting in the ambient field treatment soils ($p < 0.01$). *Ribosomal maintenance* was an order of magnitude less prevalent in the altered precipitation history soils during the drying phase than the re-wet phase or in the ambient field treatment ($p < 0.01$).

Membership from different archaeal and bacterial phyla comprised the putative *survivor* and *mortality*-prone groups (SI Fig. 3, SI Table 1). *Verrucomicrobia* OTUs dominated the

Fig. 3 The mean proportion of sequences categorized with strategy indicating survival (**a**), mortality (**b**), and ribosome maintenance (**c**) to repeated laboratory drying and re-wet events. Proportions for each category within the ambient and altered soils are given. OTUs were defined as surviving based on no difference in abundance between control and drying or re-wet events; as death-prone or "mortality" based on lower abundance at drying or re-wet compared to control; as maintenance based on higher differential rRNA:rRNA gene abundance at drying or re-wet compared to the control (DESeq2 analysis with false discovery rate-corrected significance at p value < 0.05). Bars represent means with standard error ($n = 8$).

drying *mortality* group in soils from the ambient field precipitation history, whereas the only OTU classified in this group from the altered field precipitation treatment was in *Acidobacteria*. Primarily Crenarchaeotal and Alphaproteobacterial OTUs exhibited *mortality* during re-wet only in the ambient soils (SI Fig. 3). No OTUs exhibited *mortality* from re-wet in the altered field treatment soils. Alphaproteobacterial, Deltaproteobacterial, and Actinobacterial OTUs were the most common drying and re-wet rRNA-enriched taxa from both the ambient and altered precipitation field history soils. There was a notably low abundance of rRNA-enriched phyla (0.4% total) in the altered drying soils compared to the ambient drying or re-wet: These were primarily Deltaproteobacterial and Actinobacterial OTUs. OTUs in several other phyla were found to respond significantly to drying or re-wet but were less common than those noted here (< 1.0% relative abundance across all survival, mortality, or maintenance OTU sequences, SI Fig. 3).

Weighted mean rRNA operon number, an index of r-selection within the community, responded to drying/re-wet ($F_{2,46} = 4.20$, $p = 0.03$; Fig. 4) in that it was higher following re-wet than before ($2.11 \pm 0.005 > 2.09 \pm 0.004$; Tukey's HSD $p = 0.01$). Weighted mean rRNA operon number also differed with precipitation history ($F_{1,47} = 11.60$, $p < 0.01$). It was significantly higher in the altered versus the ambient field precipitation treatment soils ($2.11 \pm 0.004 > 2.09 \pm 0.004$). There was no significant interaction between drying/re-wet and precipitation history ($F_{2,46} = 0.20$, $p = 0.78$).

Discussion

In this experiment, we found that 15 years of increased field drought conditions impacts soil microbial community structure and function by lowering carbon mineralization rates and shifting the prevalence of microbial mortality, survival,

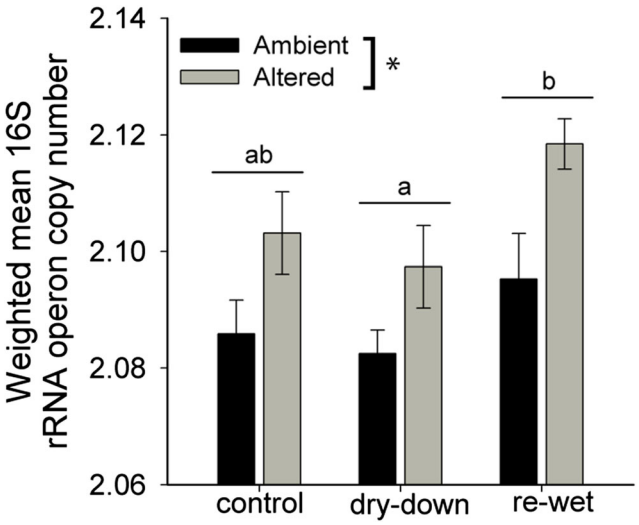

Fig. 4 PiCRUST-derived weighted mean 16S rRNA operon copy number for rRNA gene communities across drying/re-wet treatments in ambient and altered precipitation histories. Letters denote significant differences between groups ($p < 0.01$); Tukey's HSD post-hoc was used for multiple comparison among water treatments (control, drying, re-wet; $p = 0.03$). Bars represent means with standard error ($n = 8$). An asterisk denotes significant differences between precipitation history ($p < 0.01$).

maintenance, and growth potential during drying/re-wet cycles. Supporting our hypothesis, soils with a history of stronger drought had a greater prevalence of taxa that survived drought conditions (Fig. 3), higher weighted mean copy number (a proxy of r-selection and potential growth rate) as estimated by mean 16S rRNA operon number (Fig. 4), and lower average microbial respiration through drying and re-wetting (Fig. 2). In contrast, field control soils contained more taxa that experienced population decreases, or mortality, during drying and re-wetting periods and higher average microbial respiration through drying and re-wetting. Contrary to our prediction, field control soils had more OTUs that maintained greater ribosomal abundance through drying soil conditions and a similar proportion of OTUs exhibiting ribosomal maintenance at re-wet compared to the altered soils. In addition to the drying/re-wetting tolerance strategies identified by our combination of field drought history and proximate laboratory water content experiments, variation in total and rRNA-enriched microbial community composition was affected by field-scale heterogeneity, which indicates the importance of plant- (e.g., rhizodeposition) or soil-related (e.g., textural or nutrient pool differences) drivers not accounted for in our experimental design for mediating microbial responses in situ.

Microbial Respiration and Abundance

Microbial respiration decreased during drying and produced a flush of CO_2 immediately following re-wet in all soils. Further, the historical drought legacy affected soil microbial respiration, which was consistently higher in soils with ambient field precipitation history than soils that had experienced stronger field drought conditions (Fig. 2), supporting our prediction that microbial activity would be affected by historical drought. Numerous non-exclusive factors may contribute to greater soil C mineralization during drying/re-wet in the absence of concurrent plant activity, including an increase in total microbial biomass, DOC release from physically protected soil aggregates, increased extracellular enzyme activity, lower microbial C use efficiency, compatible solute release from live microbial cells, or greater DOC availability from microbial necromass. Previous work from this field drought experiment has established that total soil microbial biomass is actually lower in the ambient field soils and that extracellular enzyme activity does not differ based on precipitation history [22, 23]. In the current experiment, we did not estimate microbial C use efficiency or production or release of compatible solutes, mechanisms that may be linked to observed differences in microbial respiration [13, 15]. Our data showed no evidence that total bacterial population sizes differ between ambient and altered field precipitation history soils (Fig. 2); so, within this lab experimental context, it is unlikely that widespread bacterial cell death drives greater CO_2 loss, leaving differences in cellular efficiency, osmolyte turnover, or physical DOC release as possible mechanisms mediating the observed legacy effect. Furthermore, the activity of microbial groups that we did not measure, particularly fungi [56], may contribute significantly to soil microbial respiration rates, and the availability of root exudate-derived C may mediate soil microbial drought responses in situ [57].

Microbial Community Composition

The total 16S rRNA gene abundance did not differ between field precipitation history or laboratory drying/re-wet treatments (Fig. 2b), reinforcing that the differences in microbial community composition among rRNA gene libraries (SI Fig. 1) are a result of turnover in specific OTU populations. Given that greater soil water content supports both microbial cellular integrity and the availability of low-molecular weight substrates sustaining cellular activity [21], it is little surprise that short-term laboratory drying/re-wet treatment had a stronger effect on the variation in OTU-specific protein synthesis potential (as estimated via rRNA:rRNA gene ratios [32]) than on relative OTU population sizes (SI Fig. 1, Table 1). Overall, the laboratory drying/re-wet treatments, and the field precipitation history treatments, together explained 11–13% of the variation in both 16S rRNA gene and rRNA:rRNA gene community composition (Table 1). This supports previous work showing that drought history affects soil microbial community composition at this site [19, 23].

For both rRNA gene abundance and potential protein-synthesizing (rRNA:rRNA gene) microbial community turnover, the replicate field plot that a sample was collected from explained the highest amount of variation (19–37%; Table 1). This demonstrates that while soil water variability is asignificant driver of microbial structure and function at both weekly (lab experiment) and multi-annual (field experiment) time scales, other aspects of heterogeneity at the field plot scale are important drivers of community composition and function. While all field experiment plots are located within a < 100 m radius, plant community composition, exact slope position, and soil microtopography do vary among these plots [39]. Differences in organic matter input and quality via different plant species, and soil texture or slope-related water availability factors not accounted for in this study, may be proximate drivers of soil microbial community composition within a plot regardless of precipitation history manipulation. Our composite soil sampling approach also obscures processes at the micrometer to centimeter scale that are important for microbial community assembly [58–60]. By statistically defining populations that respond differentially to drying/re-wetting in soils with contrasting drought legacies (Table 1, Fig. 3), we differentiate the effects of soil water dynamics from other aspects of field-scale spatial heterogeneity. However, the among-plot replicate variation in this dataset highlights that only a subset of the diverse microbial taxa

known to respond to drought legacies [19] may occur at any particular small-scale location in situ, and also highlights the necessity of considering field heterogeneity to make valid inferences about the effects of microbial dynamics at ecosystem scales.

Many bacterial and archaeal Phyla responded to short-term drying and re-wet, including classic drought-tolerant groups, such as *Actinobacteria* and *Firmicutes*, and several other groups (Table 2). *Actinobacteria* are expected to maintain metabolic activity at low water potentials [11, 13, 61], and, in our experiments, this group did have higher rRNA:rRNA gene abundance during the drying period. More surprisingly, *Alphaproteobacteria, Betaproteobacteria*, *Deltaproteobacteria*, and *Planctomycetes* also showed evidence for higher protein synthesis potential during drying. Different microhabitats within the soil likely contained different microbial taxa due to differences in cell size, oxygen sensitivity, protection from predation or organic matter quality [62], and 9% of the soil volume still contained water during the drying period (Fig. 1). The physical protection of certain microbial cells in water-filled micropores within the soil may enhance tolerance to drought [21], or these groups might include populations that express high compatible solute production [11].

Microbial Mechanistic Responses to Drying and Re-Wet

In our experiments, most bacterial and archaeal OTUs were classified as drought *survivors* due to their stable population size through soil drying and re-wet (Fig. 3a), and membership in this group was higher in soils with greater drought history, as expected. This adds to a growing body of evidence that soil bacterial communities either naturally or experimentally exposed to previous drought can become less sensitive to drought [27, 63]. Hawkes et al. [18] demonstrated that soils with historically dryer versus wetter climates are functionally resistant (*e.g.*, carbon loss less variable) to soil moisture fluctuations. Drought resistance linked to legacy effects can emerge even after annual drought timescales [63], and traits of microbiota from more drought-prone, arid climates [18] may be indicative of local adaptation within microbial communities due to environmental selection and species-sorting processes. Even in our field control soil, microbial populations have likely experienced selective pressure over millennia due to highly variable regional precipitation [7] and are largely resistant to increased water availability [64]; so, it may not be surprising that many taxa can survive drying and re-wetting [15]. Indeed, the observed survival and resistance of this study's microbial populations to drying/re-wet is at least partially due to niche differentiation being driven primarily by some other field-scale factor (for example, plant composition, soil mineralogy, or soil texture). Overall, our experiments provide evidence that historical drought can reduce the sensitivity of microbial community composition to changes in soil water content. It is remarkable that just 15 years of more variable precipitation has created a legacy effect of more prevalent drought survival within the soil microbial community at this site [19, 22].

In complement, and as predicted, OTUs that showed evidence of *mortality* due to soil drying or re-wet were an order of magnitude more prevalent in the field control (ambient) than in soils with a history of increased drought (altered). Further, significant mortality due to re-wet was not detected in the altered field treatment soils (Fig. 3b), suggesting re-wet-sensitive populations have been extirpated from these field plots following 15 years of enhanced drought. The long-term field experiment altered rainfall patterns in the following two ways: the mean duration of the dry period between rainfall events was extended from 10 to 25 d and the mean size of each rainfall event was increased from 29 to 68 mm [5]. Our lab experiment suggests that this resulted in selection against microbial taxa that died during repeated drying and re-wet, and may further indicate that the rapid change in water potential during re-wet is a more selective stress than gradual drying [9, 19, 24]. We also note that our soils were frozen for an extended period prior to running the laboratory experiment; we recognize that this is not ideal due to prolonged storage reducing microbial viability and DNA yields [65, 66] and thus necessitates careful interpretation of our experimental results. It is normal for the study soil to freeze in the field over winter, and all soils were treated similarly after sample collection. So, it is most important to consider whether microbial populations from contrasting field historical drought treatments might be affected differently by freezing. Given that freezing conditions impose similar selective pressures on soil microbial populations as wetting and drying cycles [11], the most likely bias in the results is an underrepresentation of these drying/re-wet sensitive populations in the lab experiments, due to mortality resulting from freezing and thawing. While this consideration is speculative, it is important to acknowledge this caveat for interpretation of our results. Regardless, repeating this experiment in soils collected from a broader range of historical conditions is essential to assessing the generality of the results.

Populations with enriched ribosomal abundance per 16S rRNA gene, representing OTUs exhibiting *maintenance* of metabolic integrity through stressful conditions due to greater protein synthesis potential, were comprised primarily of *Actinobacteria*, as well as *Alpha-* and *Delta-proteobacteria* (SI Fig. 3). This fits the classic expectations for short-term responses to soil water fluctuation, since soil *Actinobacteria* can maintain cellular activity at much lower water potentials than most bacteria or fungi [13]. However, the ribosome maintenance strategy was substantially less common during the drying period in the soils with greater field drought history (Fig. 3c). We expected the opposite, that ribosome maintenance would be associated with the ability to thrive through drought periods and would thus be more abundant in the altered field treatment soils. Instead, it is possible that energetic

cost of maintaining protein synthesis through periods of extended drought might have reduced survival or reproductive potential of these OTU populations [67]. In a population with constitutively expressed mechanisms of drought tolerance, no new protein synthesis would be necessary to support survival through the drying period [11, 61], so constitutive drought tolerance mechanisms would not be detected using RNA-based methods, including the approach we used in this study. These results support our overall hypothesis that a legacy of greater drought affects microbial responses to dry-down and re-wet, and specific observations suggest that these response mechanisms could be driven by a loss of populations reliant on inefficient induction of protein synthesis potential for survival.

Finally, a higher proportion of r-selection and average potential *growth* rate within the altered precipitation history field soil was suggested by higher 16S rRNA weighted mean copy number (Fig. 4) [33]. This result should not be interpreted as a faster growth potential across all resident microbial populations, and we did not collect samples at an appropriate temporal resolution or use methods [9, 68] to directly infer growth of any OTU population through dynamic soil water conditions. Instead, this result indicates that certain taxa with higher potential growth rate are more abundant in soils with a history of greater drought stress. More specifically, the small difference in mean rRNA operon number ($2.11 \pm 0.004 > 2.09 \pm 0.004$) can be accounted for by the 52% greater *Firmicutes* (Order *Bacillales*) abundance in the altered soils ($0.41 \pm 0.03\% > 0.27 \pm 0.02\%$, Table 1), since *Bacillales* have a particularly high rRNA operon number (mean and median = 8) [38, 69]. This classically drought-tolerant group, the *Bacillales*, is characterized by the ability to form dormant spores through stressful periods and grow rapidly upon rewet [37, 70]. While spore formation is also associated with the capacity for rapid growth, growth rate is not the key trait that confers adaptation to extended dry conditions. Bacterial spore formation may be more analogous to a plant drought "escape" strategy, in which a life history adaptation allows the organism to grow and reproduce only when conditions are favorable [71].

Drought History Effects on Microbial Composition and Function

In sum, the results supported our hypothesis, in that the historical drought legacy of the study soil affected the distribution of mechanisms underlying microbial responses to soil drying and wetting. However, the effect of drought history on the survival, mortality, maintenance, and growth mechanisms that we evaluated did not fully fit our predictions. The most notable surprise was the lack of evidence for ribosomal maintenance during drying in drought-impacted soils, which emphasizes that tradeoffs in the energetic cost of survival strategies are likely to affect differential taxon survival within the soil community [29, 67]. Also, we detected high among-field-replicate variation, which emphasizes the challenges in applying microorganismal-scale mechanistic understanding of drought responses to the ecosystem scale. This experiment is a model study, designed to provide better understanding of the mechanisms underlying microbial responses to shifts in drought prevalence, at a field site where we already know that microbial communities have responded to altered precipitation history [19, 23]. To understand the broader distribution of these drought tolerance mechanisms, further work is necessary.

The consequences of these microbial shifts for integrated biogeochemical activity and C cycling also merit further investigation. Our results suggest that soils with an historical legacy of greater drought exhibit consistently lower microbial respiration rates, in conjunction with different microbial community composition and functional response distributions. While there is indication that proportion of mortality during the short-term re-wet event may be positively correlated with the concurrent flush of microbial respiration (SI Fig. 4), the dynamics of cell death, ribosomal maintenance, and cell growth, over the longer, more variable drying period [72] may confound quantitative identification of taxon abundance and linkage to their function. The coexistence of multiple drought response strategies makes it a challenge to understand the consequences of climate change on soil microbial community shifts and their concurrent functional properties. However, given the importance of soil microbial responses to drought for soil C and N cycling, thus soil fertility, global plant productivity, and carbon sequestration potential, it is essential to learn more about the functional mechanisms underlying the microbial drought tolerance strategies that may become more prevalent as the climate changes.

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