



Drought stress homogenizes maize growth responses to diverse natural soil microbiomes

Joel F. Swift · Matthew R. Kolp · Amanda Carmichael · Natalie E. Ford ·
Paige M. Hansen · Benjamin A. Sikes · Manuel Kleiner · Maggie R. Wagner

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Abstract

Background and Aims As the climate changes, plants and their associated microbiomes face greater water limitation and increased frequency of drought. Soil- and root-associated microbes perform important functions that affect plant drought resilience, but the dynamics of their interactions with the host are poorly understood. Plant growth responses to natural soil microbiomes, as well as patterns of root colonization by microbes, are challenging to predict.

Methods We collected soil microbiomes from four native prairies across a steep precipitation gradient in Kansas, USA. Seedlings of two *Zea mays* genotypes were inoculated with each soil microbiome in a factorial drought experiment. We investigated plant phenotypic and root microbiome responses to drought and compared the effects of natural microbiome variation on plant growth under water-limited and well-watered conditions.

Results Drought caused plants to accumulate shoot mass more slowly and achieve greater root/shoot mass ratios. Drought restructured the bacterial root-associated microbiome via depletion of *Pseudomonadota* and enrichment of *Actinomycetota*, whereas the fungal microbiome was largely unaffected. Taxonomically distinct soil microbiomes from the four

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J. F. Swift (✉) · M. R. Kolp · A. Carmichael · N. E. Ford ·
B. A. Sikes · M. R. Wagner (✉)
Department of Ecology and Evolutionary Biology,
University of Kansas, Lawrence, KS 66045, USA
e-mail: joel.swift@ku.edu

M. R. Wagner
e-mail: maggie.r.wagner@ku.edu

J. F. Swift · M. R. Kolp · P. M. Hansen · B. A. Sikes ·
M. R. Wagner
Kansas Biological Survey & Center for Ecological
Research, University of Kansas, Lawrence, KS 66045,
USA

Present Address:

M. R. Kolp
Center for Animal and Human Health in Appalachia,
College of Veterinary Medicine, Lincoln Memorial
University, Harrogate, TN 37752, USA

A. Carmichael
Department of Conservation and Research, Southeastern
Center for Conservation, Atlanta Botanical Garden,
Atlanta, GA 30309, USA

P. M. Hansen
Department of Soil and Crop Science, Colorado State
University, Fort Collins, CO 80521, USA

M. Kleiner
Department of Plant and Microbial Biology, North
Carolina State University, Raleigh, NC 27695, USA

contrasting environments affected plant growth under well-watered but not drought conditions.

Conclusion We demonstrated that the functional consequences of naturally-occurring soil microbiome variation are dependent on water availability, suggesting that future drying climates may dampen plants' responsiveness to beneficial and/or pathogenic microbes.

Keywords Root microbiome · Soil microbiome · Maize · Drought · Precipitation gradient

Introduction

Increasing drought frequency due to global climate change will challenge not only plants, but also soil- and plant-associated microbiomes (Chiang et al. 2021; Balting et al. 2021). Drought directly limits microbial growth, mobility, and access to resources, but also alters plant host traits (e.g., root physiology, morphology, and exudate composition) that comprise the habitat available to root-associated microorganisms (Naylor and Coleman-Derr 2018). Therefore, the availability of water to the host plant is a key environmental parameter that shifts patterns of root colonization by soil-dwelling microbes, as has been observed in several plant species (Fitzpatrick et al. 2018; Xu et al. 2018; Xu and Coleman-Derr 2019; Wang et al. 2020). Consequently, water availability is likely to also influence the effect of the root-associated microbiome on the phenotype and fitness of the host. Although the potential for microbes to alleviate host drought stress has been documented repeatedly, the immense complexity of natural soil microbiomes makes their functional potential challenging to predict and even more challenging to harness for applications in sustainable agriculture (Lau and Lennon 2012; Ngumbi and Kloepper 2016; Armanhi et al. 2021; Ali et al. 2022).

One major complicating factor is the great variation among microbiome members in their responses to both water limitation and host phenotype. Because plants recruit root microbes primarily from the soil (Zarraonaindia et al. 2015), we would expect drought-induced changes in the soil microbiome to be reflected in root-associated microbiomes. However, because many root-associated microbes are also sensitive to plant root traits, their responses to drought

may also reflect drought-induced changes in the host phenotype. Both soil- and root-associated microbiomes under drought exhibit consistent enrichment of gram-positive bacteria and depletion of gram-negative bacteria (Acosta-Martínez et al. 2014; Fuchssluger et al. 2016; Naylor et al. 2017). Within those categories, however, some bacterial phyla respond to drought differently in soil versus roots (Naylor and Coleman-Derr 2018). For example, enrichment of *Bacillota* (formerly Firmicutes) and *Actinomycetota* (formerly Actinobacteria) during drought are less pronounced in soil than in root-associated microbiomes, suggesting that these phyla respond to drought-induced changes in root physiology (Naylor et al. 2017; Fitzpatrick et al. 2018). Furthermore, the presence of a host plant can influence the drought responses of the taxa that comprise each phylum (Santos-Medellín et al. 2017). Overall, drought impacts both soil- and root-associated microbiomes, but in different ways, owing in part to the influence of the plant phenotype.

The extent of microbial diversity for direct and indirect (*i.e.*, plant-mediated) drought responses suggests that taxonomically distinct soil microbial communities are likely to differ in their ability to alleviate host drought stress. Biogeographic variation among soil microbiomes—which is the dominant factor shaping variation among root microbiomes (Coleman-Derr et al. 2016; Bell-Dereske et al. 2023)—reflects both contemporary and historical environmental conditions such as soil texture, pH, and nutrient availability (Fierer and Jackson 2006; Lauber et al. 2009; Xu et al. 2021). Additionally, both acute and chronic water limitation can leave lasting impacts, or legacy effects, on soil and root-associated microbiomes. For instance, geographic patterns of water availability constrain the functional potential of soil microbiomes (Hawkes and Keitt 2015; Averill et al. 2016; Canarini et al. 2021); however, the implications for host plants, including crops, are not well understood.

Variation in soil- and root-associated microbiomes—including variation caused by historical precipitation—may affect plant fitness under drought. For example, in *Arabidopsis*, a 4-member bacterial synthetic community improved survivorship and recovery after a 21-day drought via plant hormonal modification that enhanced chlorophyll content in the rosette and biofilm production, which

was hypothesized to improve root hydration (Yang et al. 2021). Fungi, particularly arbuscular mycorrhizal fungi, also have a beneficial role in plant tolerance to drought stress via a myriad of mechanisms, including soil structure alteration and increased water retention (Augé et al. 2001) and water transport to plant roots (Kakouridis et al. 2022). Drought-induced restructuring of root-associated microbiomes is hypothesized to be a conserved mechanism that supports survival of both the plant host and its symbionts (Naylor et al. 2017; Xu et al. 2018; Xu and Coleman-Derr 2019). Additional research suggests that exposure to short-term drought creates a microbial legacy effect that moderates drought stress in plants (Kaisermann et al. 2017; De Long et al. 2019) (and strengthens positive plant-soil feedbacks, although many such patterns were species-specific) (Buchenau et al. 2022). Together, these findings create the expectation that soil microbiomes from contrasting environments will vary in their effects on plant phenotype, but it is unclear whether these effects should be congruent between water-limited and well-watered conditions.

To address this open question about the congruence of microbiome effects on plant phenotype under water-stressed vs. unstressed conditions,

we designed an experiment to assess plant growth under a contemporary drought when inoculated with natural soil microbiomes from contrasting environments. To ensure that the inocula represented a meaningful degree of environmental variation, we collected soil microbiomes from native prairie remnants spanning a steep precipitation gradient in Kansas (Fig. 1). This precipitation gradient is coupled with multiple climatic metrics, with the environment becoming increasingly arid from east to west resulting in lower soil moisture and increased reference evapotranspiration (Fig. 1C-E). We inoculated *Zea mays* L. (maize) seedlings with these soil microbiomes and measured plant growth during our experimental drought. Our goals were to: 1) compare plant phenotype responses to these diverse microbiomes under drought-stressed and well-watered conditions, 2) characterize patterns of root colonization under both water availability conditions, and 3) identify microbial taxa that were associated with plant growth. Our results suggest that experimental drought treatment induced congruent shifts in the root colonization patterns of all four microbial communities. Furthermore, the contrasting soil microbiomes differentially affected

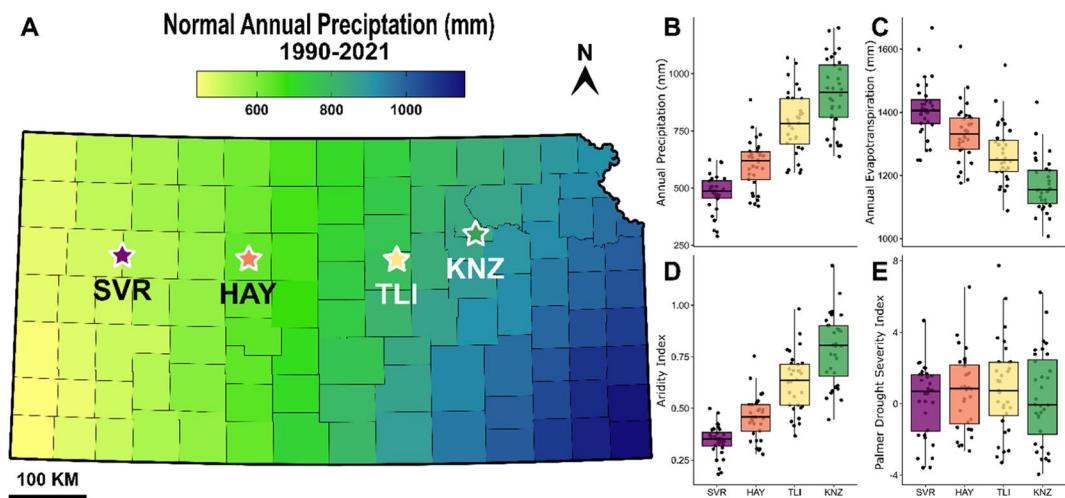


Fig. 1 Kansas precipitation gradient. **A** Soil samples were collected from four prairies in Kansas along an increasing precipitation gradient. Locations from east to west, Smoky Valley Ranch prairie (SVR; latitude 38.8665, longitude -100.9951), Hays prairie (HAY; 38.8355, -99.3033), The Land Institute prairie (TLI; 38.9698, -97.4690), and Konza native prairie (KNZ; 39.1056, -96.6099). Boxplots to the right of the map

depict annual climatic metrics extracted from the TerraClimate database for each collection site from 1990–2021: **B** precipitation, **C** reference evapotranspiration, **D** aridity index, and **E** Palmer Drought Severity index. Boxplot hinges represent the 1st and 3rd quartiles; whiskers represent 1.5 times the interquartile range

plant growth rate under well-watered but not drought-stressed conditions.

Material and methods

Soil inoculum sources and preparation

Soil samples were collected in May 2019 from four prairie sites across a steep precipitation gradient in Kansas, USA (Fig. 1). Prairies were selected at the same latitude and relative elevation such that they have very similar temperature profiles throughout the year. Western prairie sites (SVR and HAY) are predominantly short- to mixed-grass prairies (e.g., *Bouteloua* spp.), while eastern sites (TLI and KNZ) are dominated by tallgrass prairie species (e.g., *Andropogon gerardi*, *Schizachyrium scoparium*, *Panicum virgatum*). All collection sites were high-quality remnant prairies that have never experienced tillage or irrigation, which are strong perturbations that may obscure natural patterns of microbiome variation. Soil was collected from five evenly-spaced subsamples per site, sampling to an approximate depth of 60 cm. Soil sub-samples were homogenized and sieved to remove rocks and plant material and then stored in plastic bags at 4°C until use, approximately four months after collection.

Inocula were prepared by mixing 20 g of each soil into 100 mL 1× phosphate-buffered saline (PBS) and 0.0001% Triton X-100. After settling, the soil slurries were filtered through Miracloth (22–25 μm pore size; Calbiochem, San Diego, CA, USA) and then centrifuged for 30 min (3000×g). After discarding the supernatant, we resuspended the microbial pellets in 20 mL 1×PBS and kept them shaking for aeration until use, later that day. Each final inoculum consisted of 10 mL of the resuspended pellet in 1 L 0.5X Murashige and Skoog basal salt mixture (MS; Sigma-Aldrich, Darmstadt, Germany). A control treatment was made by adding 10 mL sterile 1×PBS into 1 L 0.5X MS.

Planting, inoculation, and greenhouse setup

To increase the generalizability of our results, two genotypes of *Zea mays* L. (maize) were used: B73 and Mo17. These genotypes have been shown to vary in their growth response to beneficial

endophytes (Schultz et al. 2022) and so may differ in response based on microbiome origin. In a laminar flow hood, seeds were surface sterilized by submerging in 70% ethanol, then 5% NaClO, followed by three rinses with sterile water. To avoid confounding effects of soil physicochemical properties, autoclaved calcined clay (“Pro’s Choice Rapid Dry”; Oil-Dri Corporation, Chicago, IL) was used as the growth medium. Seeds were planted one inch deep in 107 mL “cone-tainers” (Stuewe & Sons Inc., Tangent, OR, USA) with a sterilized cotton ball pushed to the bottom to prevent clay from passing through drainage holes. Approximately 100 mL of sterilized calcined clay was used per cone-tainer. The day after planting, cone-tainers were assigned randomly to treatments, inoculated with 25 mL of one of the four soil inocula (or the sterile control), and placed two inches apart in randomized complete blocks. Overall, each combination of treatments (drought treatment×soil inocula×genotype) was replicated 29 to 31 times across 27 randomized blocks (Figure S1A). Plants were grown in a temperature-controlled greenhouse (23/20 °C day/night, respectively) at the University of Kansas (Lawrence, KS, USA) arranged under growth lights (250 W Sun Systems Compact Cool fluorescent lamps) to supplement natural light for 13 h/day during the experiment (January–March 2020).

Monitoring and harvesting plants

All plants received 30 mL of reverse osmosis water twice weekly until emergence. After emergence, “well-watered” plants continued twice-weekly watering while “droughted” plants were watered every 8–12 days throughout the experiment. Plant height was measured weekly to the nearest 0.25 cm on the tallest fully expanded leaf held parallel to the stem. Plants were sequentially harvested to measure biomass at one of three time points during the experiment: 68 plants on day 24 and day 39, and the remainder (n=224) on day 50 (Figure S1B). Calcined clay was removed from roots by hand. Shoot and root fractions were separated and oven-dried at 65 °C to a constant weight. Before drying, one nodal root (from the uppermost soil-borne root whorl) per plant was collected and stored at -80 °C until DNA extraction.

Phenotypic analysis

All statistical analysis for phenotypic and microbiome data (see below) was conducted in R v4.2.2 (R Core Team 2021). Emergence was tracked daily, and this value was used, with harvest date, to convert dry shoot and root mass to accumulation rates (g/day) to account for the variable number of growing days plants experienced post-emergence. The ratio of root to shoot growth was calculated on a per-plant basis by dividing root dry mass by shoot dry mass. The lme4 package v1.1.29 (Bates et al. 2015) was used to fit a generalized linear mixed model for emergence (binary) and linear mixed models for shoot and root mass accumulation rates and root/shoot ratio (continuous). Models used the following formula (*growth response ~ Inoculum × Treatment × Genotype*) with all possible interactions included. Experimental block was treated as a random effect with time point included as an additional random effect in the root/shoot ratio model. Root/shoot ratio was natural log-transformed, and both shoot and root mass accumulation rates were square-root-transformed. Differences among group means were assessed using analysis of variance (ANOVA) with Type III sums of squares in the lmertest v3.1.3 package (Kuznetsova et al. 2017) with post-hoc comparisons made using the emmeans package v1.7.4.1 (Lenth et al. 2020). Finally, the estimated marginal mean growth rates of each genotype in each soil (derived from the above model) were modeled as a function of aridity (Annual Precipitation / Potential Evapotranspiration) and Palmer Drought Severity Index (PDSI) for the collection site of each soil using linear regression. Assumptions of all models were checked by inspecting model residuals (Harrison et al. 2018).

DNA extraction and library preparation

Nodal roots were freeze-dried for 48 h (FreeZone lyophilizer; Labconco, Kansas City, MO, USA) and ground to powder with a HT Lysing Homogenizer (OHAUS, Parsippany, NJ, USA). Each ground root sample was mixed into 800 μ L lysis buffer (1 M Tris pH=8.0; 0.5 mM NaCl; 10 mM EDTA) and transferred to 96-well plates containing 500 μ L of 2 mm garnet rocks per well (Biospec Products, Bartlesville, OK, USA), after which DNA purification proceeded

via bead-beating and chemical lysis as previously described (Wagner et al. 2020).

Amplicon sequencing libraries were prepared using established protocols and PCR conditions (Wagner et al. 2020). Briefly, we targeted the V4 region of the 16S SSU rRNA gene for bacteria/archaea using 515F-806R primers (Apprill et al. 2015; Parada et al. 2016) and the ITS1 region for fungi using ITS1f and ITS2 primers (White et al. 1990; Gardes and Bruns 1993). Both amplicons were amplified separately using an initial PCR and then barcoded via a second index PCR. Amplicons were sequenced (2×250 bp) on a NovaSeq 6000 SP flow cell (Illumina, San Diego, CA, USA). Each “batch” of 96 samples ($n=6$) remained together throughout DNA extraction, amplification, and sequencing; individual samples were randomized across batches.

To confirm that we inoculated the experimental plants with realistic microbial communities, we also sequenced the original soils and freshly derived inocula. From homogenized soil collected from each site, we created eight technical replicates which we used to test whether our protocol for creating microbial slurries preserved the original community composition. DNA from each soil replicate and from freshly generated inocula was extracted in December 2022 using the same protocol that we used for roots, except omitting the lyophilization step and placing 250 mg soil or 800 μ L inoculum directly into the garnet plate. From there, library preparation and sequencing proceeded as described above for roots.

Bioinformatic processing

Sequence processing and quality filtering were conducted using an established workflow (Wagner et al. 2020). Briefly, Cutadapt v2.3 (Martin 2011) was used to remove primers and demultiplex reads into 16S and ITS sequence files. DADA2 v1.14.1 (Callahan et al. 2016) was used to denoise reads, merge paired-end sequences, dereplicate, and remove chimeric sequences. The forward and reverse 16S reads were truncated to 220 and 170 bp, respectively and filtered based on the maximum number of expected errors of eight and nine bp per read, respectively. ITS reads were processed using DADA2 in an identical manner, except that reads were not truncated to preserve biologically relevant length variation. Forward and reverse 16S and ITS reads were then used separately

to estimate sequencing error rates for denoising prior to dereplication of reads into Amplicon Sequence Variants (ASVs) and merging of pair-end reads. Taxonomy was assigned to the resulting ASVs by a naïve Bayesian classifier (Wang et al. 2007) trained on the 11.5 release of the Ribosomal Database Project (Callahan 2017) for bacteria and the 01.12.2017 release of the UNITE database (UNITE Community 2017) for fungi. Phyloseq v1.40.0 (McMurdie and Holmes 2013) was used to remove host sequences (chloroplast and mitochondria; 0.35% of 16S reads) and ASVs unassigned to a kingdom. Chao1 richness and diversity indices (Shannon and Inverse Simpson) were calculated before the removal of ASVs not observed ≥ 25 times in ≥ 5 samples and samples with < 400 reads (200 bacterial and 133 fungal samples); 98.0% and 94.5% of reads remained after host filtering and thresholding (bacterial and fungal reads, respectively). Finally, ASV counts were normalized via a centered-log-ratio (CLR) transformation using the ALDEEx2 package v1.28.1 (Fernandes et al. 2014; Gloor et al. 2017). Due to low replication and low sequencing depth at the earlier timepoints, we analyzed only microbiome samples from the last time point. This retained 131 bacterial (mean read depth $123,554 \pm \text{SE } 18,276$) and 92 fungal samples ($41,081 \pm 10,129$).

Microbiome analysis

To test the similarity of inocula to their soils of origin, a linear model was fit for each alpha diversity metric with collection site, substrate (soil vs. inoculum), and sequencing depth (Z-transformed) as main effects. Community composition was assessed by permutational multivariate analysis of variance (PERMANOVA) and constrained partial redundancy analysis (RDA). For PERMANOVA, the *adonis2()* function from the vegan package v2.6.2 (Oksanen et al. 2019) was used to model the matrix of between-sample Aitchison distances (Gloor et al. 2017) with collection site and substrate as main effects. The RDA was fit with the same model, removing variance attributed to $\ln(\text{Sequencing Depth})$, and visualized using an ordination plot.

To investigate root microbiome responses, a model was fit for each alpha diversity metric ($\text{Metric} \sim \text{Soil} \quad \text{Inoculum} \times \text{Drought}$

$\text{Treatment} \times \text{Genotype}$), with *Block* and *Batch* as random effects. The CLR-transformed abundance of each family was modeled to test responses to experimental factors. Across all models and where appropriate, p-values were adjusted to correct for multiple comparisons using a Benjamini–Hochberg correction (Benjamini and Hochberg 1995). To explore changes in community composition, a PERMANOVA model was fit (*Aitchison distance matrix* $\sim \text{Soil Inoculum} \times \text{Drought Treatment} \times \text{Genotype}$) with permutations constrained by *Batch*. Main effects were tested for homogeneity of dispersions using the vegan package. Pairwise dispersions among factor levels were assessed using a permutation test with 999 permutations constrained by *Batch*. Constrained partial redundancy analysis of the Aitchison distance matrix was conducted to remove variance attributed to *Batch* and $\ln(\text{Sequencing Depth})$ and to visualize the variance in microbial composition explained by experimental factors.

The machine-learning package caret v6.0.92 (Kuhn 2008) with ranger's v0.14.1 (Wright and Ziegler 2017) implementation of the random forest algorithm was used to assess how accurately roots could be classified as droughted or well-watered based on microbiome community data (bacterial and fungal, separately). Samples were randomly split into a training set (80%) and a test set (20%), with the test set withheld from the model, and used to determine the final model's performance. Hyperparameters were optimized using a grid search over minimum node size (1, 5, 10) and the number of features available at each node (10–100% of the ASVs). For each combination in the grid, classifier accuracy was used to assess performance on out-of-bag samples with tenfold cross validation. Final predictions were made with the trained model on the withheld test set and visualized using confusion matrices. The contribution of each ASV to classification accuracy was assessed using permuted importance: mean decrease in accuracy (MDA) values were calculated for each ASV across the tenfold cross validation. The benefits of this strategy are twofold, in that we can assess whether drought induced a predictable shift in the microbiome as a whole and identify individual ASVs with the greatest differential abundance as a result of drought.

Connecting plant growth to microbial abundances

Linear models were used to test associations between taxon abundances and plant growth. To focus on the phenotypic variation that could not be explained by Genotype, Soil Inoculum, Block and Batch effects, we extracted residuals from a model that contained these terms and used them as the response variable for a second model (*Growth Response_{residual} ~ Taxon abundance × Drought Treatment*). The *Taxon abundance* coefficient described the association of the taxon (CLR-transformed counts of bacterial and fungal families) with plant growth rate; the interaction term described whether the taxon's effect on plant growth differed under droughted vs. well-watered conditions. We chose family and order as the taxonomic levels for testing bacteria and fungi, respectively, as a conservative measure to limit the number of tests. P-values were adjusted by term to account for multiple testing.

Results

Soil and inocula community composition differs across collection sites

We assessed the microbial community diversity and composition of soils and their derived inocula from the four collection sites ($n=8$ samples per site). Bacterial, but not fungal, alpha diversity of soils differed across collection sites (Tables S1 and S2); bacterial

diversity was highest in the westernmost prairie soil (Figure S2). Community composition of both bacterial and fungal microbiomes differed among sites (Table S3; $R^2=0.22-0.24$; p -value < 0.01).

Relative to the original soils, we observed that inocula generally had lower richness and diversity, but this difference was non-significant for most metrics (Figure S3; Tables S1 and S2). The composition of both bacterial and fungal communities also shifted between soils and their derived inocula (Figure S4-5; Table S3), indicating that this process may be selective to certain bacterial and fungal taxa. However, both soil and inocula samples clustered in ordination space by collection site (Fig. 2). Thus, the derived inocula largely retained the differences among the original soil microbial communities.

Plant growth rates vary across soil microbiomes, genotype, and drought treatment

We found no evidence that soil inocula impacted emergence rates (p -value = 0.19, Pearson's χ^2 test; Table S4), however, maize genotype B73 (81.3%) emerged more successfully than Mo17 (62.0%; p -value = 0.05, Pearson's χ^2 test). Post-emergence, both drought treatment and maize genotype affected plant growth (Table 1). Drought decreased both shoot and root mass accumulation rates (Fig. 3A-B; p -value = < 0.01) and increased root/shoot ratio (Fig. 3C; p -value < 0.01). These traits also differed between genotypes, with Mo17 investing more into root growth than B73 (Figure S6; p -value = < 0.01).

Fig. 2 Soil community composition is strongly differentiated across the soil sources and substrates. Redundancy analysis for (A) bacterial and (B) fungal communities from soil sources used to derive soil inocula. Soil source and substrate are represented by differing colors or shapes, respectively

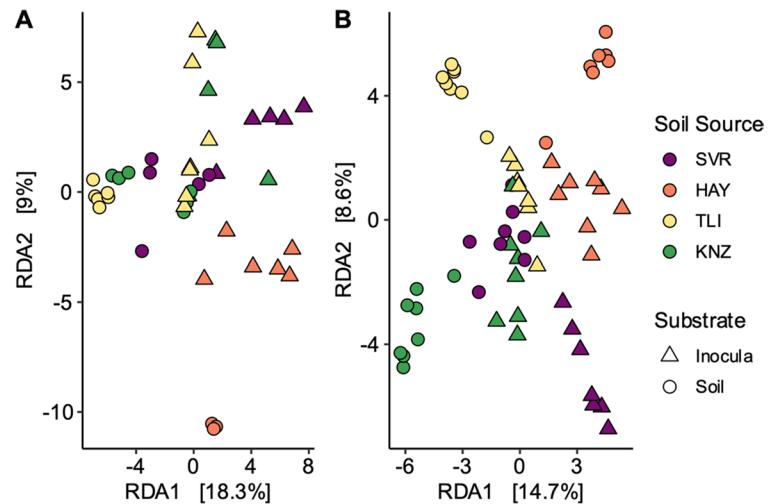


Table 1 Type three analysis of variance (ANOVA) for plant growth

Factor	DF	sqrt(Shoot Mass rate)		sqrt(Root Mass Rate)		log(Root/shoot Ratio)	
		F-value	p-value	F-value	p-value	F-value	p-value
Drought Treatment (DT)	1	335.67	<0.01	16.96	<0.01	161.21	<0.01
Genotype (G)	1	33.56	<0.01	22.74	<0.01	110.11	<0.01
Soil Inoculum (SI)	3	5.08	<0.01	0.67	0.57	3.30	0.02
DT x G	1	0.08	0.77	0.93	0.34	0.12	0.73
DT x SI	3	2.25	0.08	1.73	0.16	0.63	0.59
G x SI	3	2.13	0.10	0.61	0.61	0.44	0.73
DT x G x SI	3	0.25	0.86	0.09	0.96	0.13	0.94

p-value <0.05

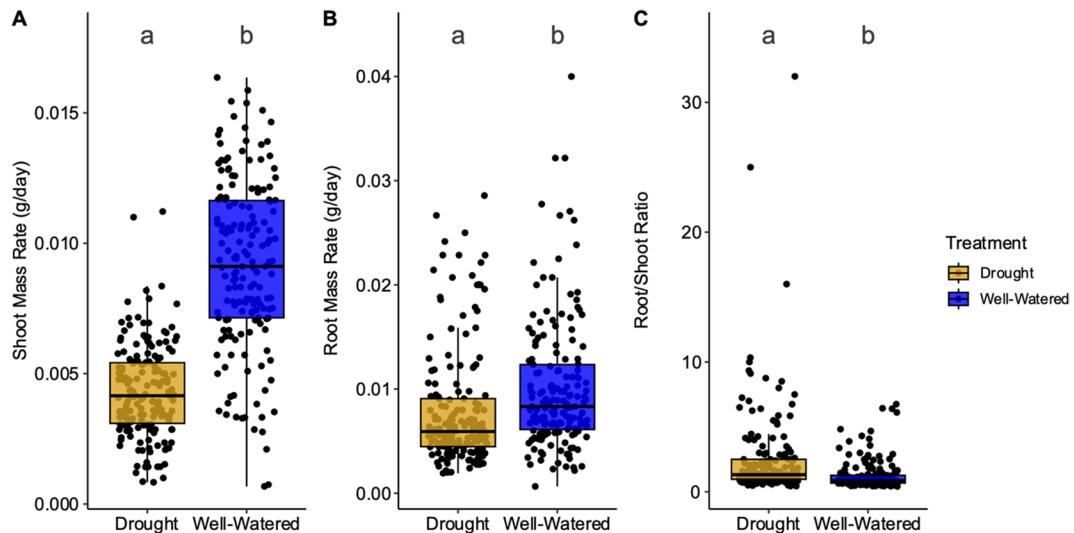


Fig. 3 Shoot and root growth vary across drought treatment. The effect of drought treatment on (A) shoot mass accumulation rate, (B) root mass accumulation rate, and (C) root/shoot ratio. Boxplot hinges represent the 1st and 3rd quartiles; whiskers represent 1.5 times the interquartile range; letters above box plots denote significant differences in estimated marginal means (*p*-value = <0.01; see Table 1 for full ANOVA table)

whiskers represent 1.5 times the interquartile range; letters above box plots denote significant differences in estimated marginal means (*p*-value = <0.01; see Table 1 for full ANOVA table)

Shoot mass accumulation rate and root/shoot ratio were both influenced by soil inoculum (Figure S7; Table 1; *p*-value = <0.01–0.02); this effect was driven by the TLI inoculum which caused the slowest accumulation of shoot mass and the lowest root/shoot ratio. We observed no evidence of interactions between drought treatment, genotype, and soil inoculum on plant growth.

Root microbiomes and community responses to drought differed between bacteria and fungi

We observed different patterns of richness and diversity for bacterial and fungal root microbiomes

exposed to prairie inoculum and drought (Tables S5 and S6). Bacterial Chao1 richness varied across soil inocula (Table S5; *p*-value = 0.03), with roots receiving the KNZ inoculum exhibiting the highest richness. Drought increased Shannon and Inverse Simpson's diversity of fungal communities (Table S6). We observed no difference in bacterial or fungal richness or diversity between the maize genotypes (Tables S5 and S6).

Root bacterial community composition was also structured by soil inoculum (Table 2; $R^2=0.05$, *p*-value = <0.01) and drought treatment (Fig. 4A; $R^2=0.01$, *p*-value = 0.01). We found no evidence that bacterial community dispersion differed among

Table 2 Permutational Multivariate ANOVA (PERMANOVA) table bacterial community composition of maize nodal roots

Factor	DF	SS	R ²	F-value	p-value
Genotype (G)	1	2687	0.01	0.88	0.47
Soil Inoculum (SI)	3	19091	0.05	2.10	<0.01
Drought Treatment (DT)	1	5484	0.01	1.81	0.01
G x SI	3	9849	0.02	1.08	0.47
G x DT	1	2289	0.01	0.75	0.72
SI x DT	3	10026	0.02	1.10	0.43
G x SI x DT	3	6352	0.02	0.70	0.95
Residual	115	349180	0.86		

p-value < 0.05

treatments, genotypes, or inocula (p -value > 0.05, Multivariate Levene's test). Redundancy analysis aligned with PERMANOVA results: soil inoculum and drought treatment explained the largest

proportions of variance (6.09% and 1.57%; Figure S8 and 4B, respectively), while genotype explained < 1% of variance (Figure S9). Among bacterial families, *Sphingomonadaceae* was responsive to drought treatment ($q = 7.1 \times 10^{-4}$), with this family decreasing from 1.11% relative abundance to 0.46% under drought conditions.

In contrast to bacteria, drought treatment did not alter relative abundances of any fungal families, nor overall root fungal community profiles (Fig. 5A). Variance in fungal community composition was mostly due to initial soil inoculum source (Figure S10; Table 3; $R^2 = 0.09$, p -value = < 0.01). As observed for bacterial communities, neither drought treatment, maize genotype, nor soil inoculum source affected fungal community dispersion (p -value = > 0.05, Multivariate Levene's test). Drought and maize genotype each explained only a small portion of the variance in fungal community composition (Fig. 5B and S11, respectively). Thus,

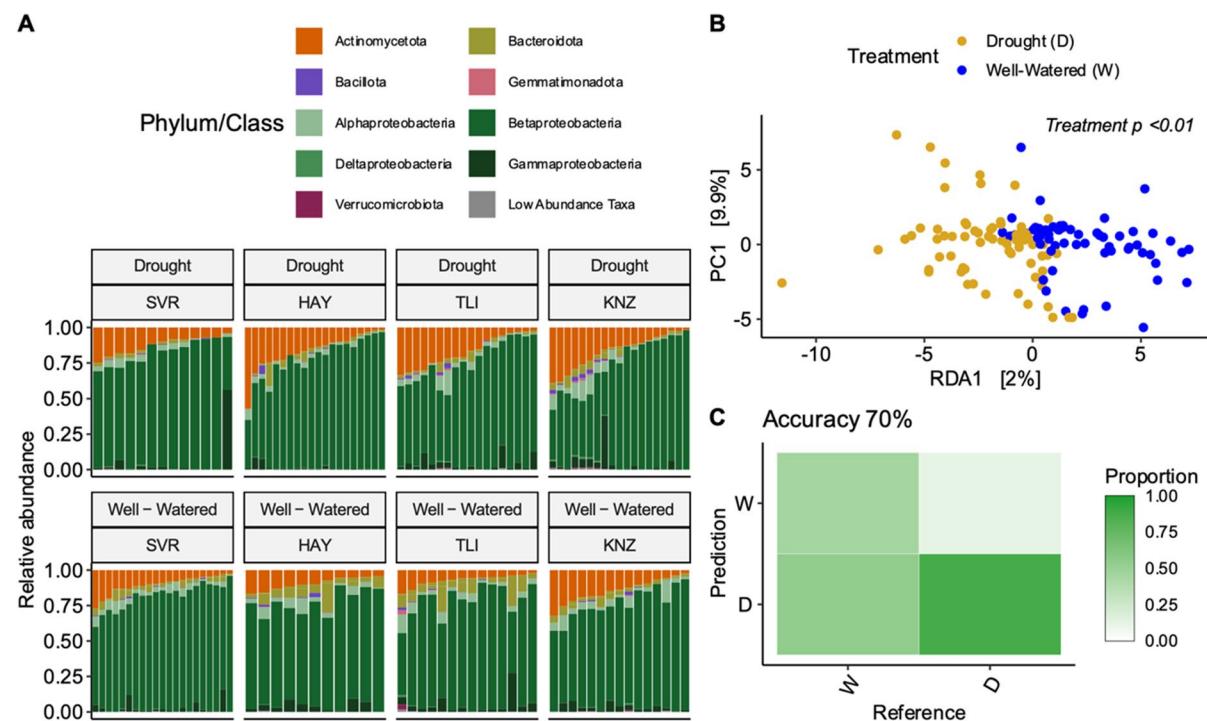


Fig. 4 Bacterial community composition across drought treatments. **A** Bacterial taxonomic barplots for drought-stressed and well-watered plants. Each site is represented by a facet with samples denoted by individual lines and the relative abundance (non CLR transformed data) of each phylum displayed by color. **B** Redundancy analysis on bacterial communities

constrained by drought treatment, conditioned on sequencing batch and ln-transformed usable reads per sample. **C** Confusion matrix depicting the classification accuracy of a random forest classifier predicting drought treatment trained on bacterial communities. Above, the confusion matrix balanced accuracy is shown; for additional classifier statistics, see Table S8

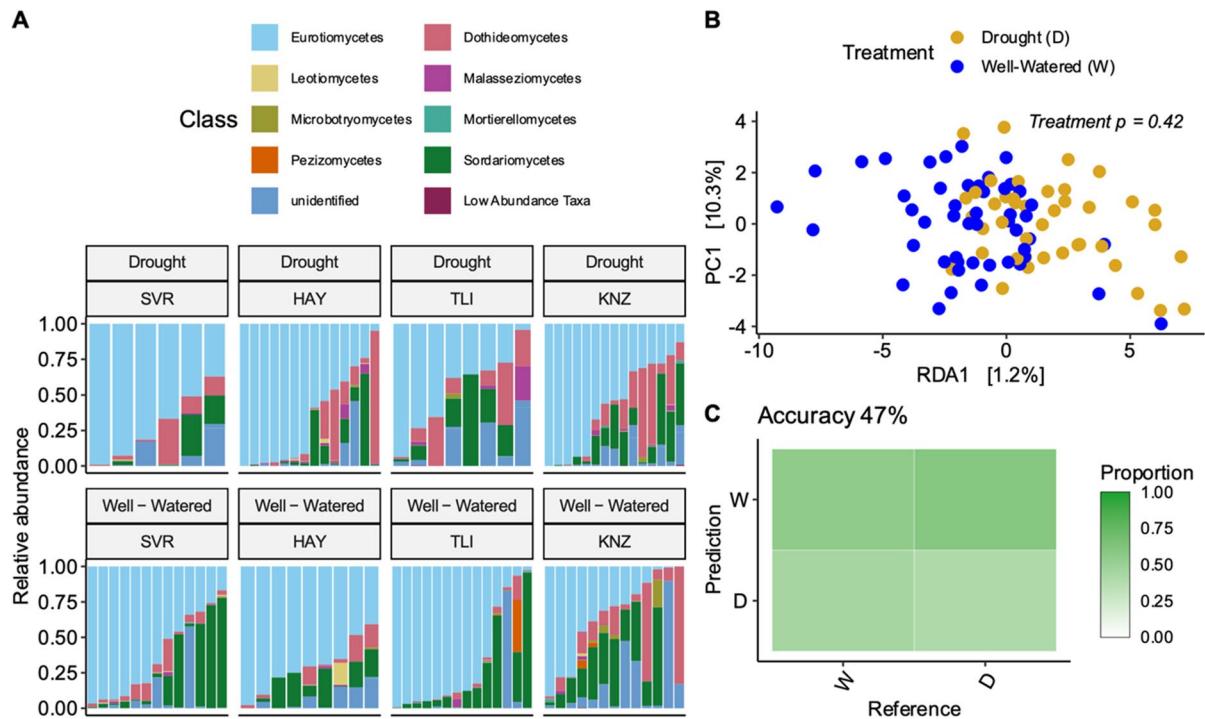


Fig. 5 Fungal community composition across drought treatment. **A** Fungal taxonomic barplots for drought-stressed and well-watered plants. Each site is represented by a facet with samples denoted by individual lines and the relative abundance (non CLR transformed data) of each phylum displayed by color. **B** Redundancy analysis on fungal communities con-

strained by drought treatment, conditioned on sequencing batch and ln-transformed usable reads per sample. **C** Confusion matrix depicting the classification accuracy of a random forest classifier predicting drought treatment trained on fungal communities. Above, the confusion matrix balanced accuracy is shown; for additional classifier statistics, see Table S9

while root-associated bacterial community composition responded to drought treatment, we observed no such trend for fungal community composition.

Identification of bacterial and fungal ASVs responding to drought

Our random forest models showed greater accuracy in predicting the drought treatment of a given sample when trained on bacterial communities than on fungal communities (Figs. 4C and 5C). Three bacterial ASVs greatly aided the prediction of treatment (Figs. 4C and S12-13): two from the family *Sphingomonadaceae* (Pseudomonadota) and one from the family *Sphingobacteriaceae* (Bacteroidota). These ASVs showed mean decrease in accuracy (MDA) values of 1.6%, 1.2%, and 1.2% when withheld from the model, respectively. In other words, these three bacterial ASVs contributed to ~ 4% increase of classifier accuracy in predicting. All these bacterial ASVs were depleted in drought-stressed plants relative to well-watered plants: the *Sphingomonadaceae* ASVs decreased in relative abundance by an order of magnitude (0.37% and 0.31% to 0.03% and 0.04%

Table 3 PERMANOVA table for fungal community composition of maize nodal roots

Factor	DF	SS	R ²	F-value	p-value
Genotype (G)	1	586	0.01	0.89	0.49
Soil Inoculum (SI)	3	5896	0.09	3.00	<0.01
Drought Treatment (DT)	1	541	0.01	0.83	0.63
G x SI	3	2181	0.03	1.11	0.60
G x DT	1	740	0.01	1.13	0.18
SI x DT	3	2335	0.04	1.19	0.17
G x SI x DT	3	1969	0.03	1.00	0.61
Residual	76	49822	0.78		

p-value < 0.05

relative abundance, respectively) and the *Sphingobacteriaceae* ASV responded similarly (0.9% to 0.1% relative abundance). We also detected two fungal ASVs that aided the prediction of treatment (Fig. 5C and S14–15), from the families *Aspergillaceae* (1.1% MDA; Eurotiomycetes) and *Sarcocladiaceae* (0.76% MDA; Sordariomycetes). These fungal ASVs showed different patterns in response to drought treatment: the *Aspergillaceae* ASV was enriched while the *Sarcocladiaceae* ASV was depleted in drought-stressed plants.

Connecting plant growth to bacterial and fungal communities

Soil inocula derived from collection sites across Kansas had only a small impact on plant growth on average, with no single inoculum causing significantly improved growth over other inocula or the control (Table 1; Fig. 6A and S7). However, the effects of the inocula on plant growth were more pronounced in the well-watered treatment than in the drought treatment (Fig. 6A). Although we lacked the statistical power to confirm it ($N=4$ soils; Table S10), our data suggested a negative relationship between mean annual

aridity and PDSI for the soil collection site and the inoculum's effect on plant growth under well-watered conditions (Fig. 6B–C). Of note is mean annual PDSI values do not follow a linear trend as does aridity, with the collection from Konza prairie (KNZ) having a higher propensity for drought stress than would be expected with aridity alone.

We identified bacterial and fungal taxa that were correlated with promotion or suppression of plant growth. Overall, 11 bacterial families showed a significant positive (3/11) or negative (8/11) association with shoot mass accumulation rate (Table 4). These represented only a few phyla (5 Actinomycetota, 4 Pseudomonadota, and 2 Bacillota) and were a mix of gram-positive (7) and gram-negative (5). One of the identified families, *Streptococcaceae*, interacted with drought treatment, indicating that its relationship with shoot mass accumulation was modulated by the amount of water the plant received. The abundance of *Streptococcaceae* was negatively correlated with shoot mass rate in well-watered plants but not in droughted plants (Figure S16). Relative to shoot mass accumulation rate, fewer bacterial families were associated with root mass accumulation rate (only *Acetobacteraceae* and *Corynebacteriaceae*, Table 4). This

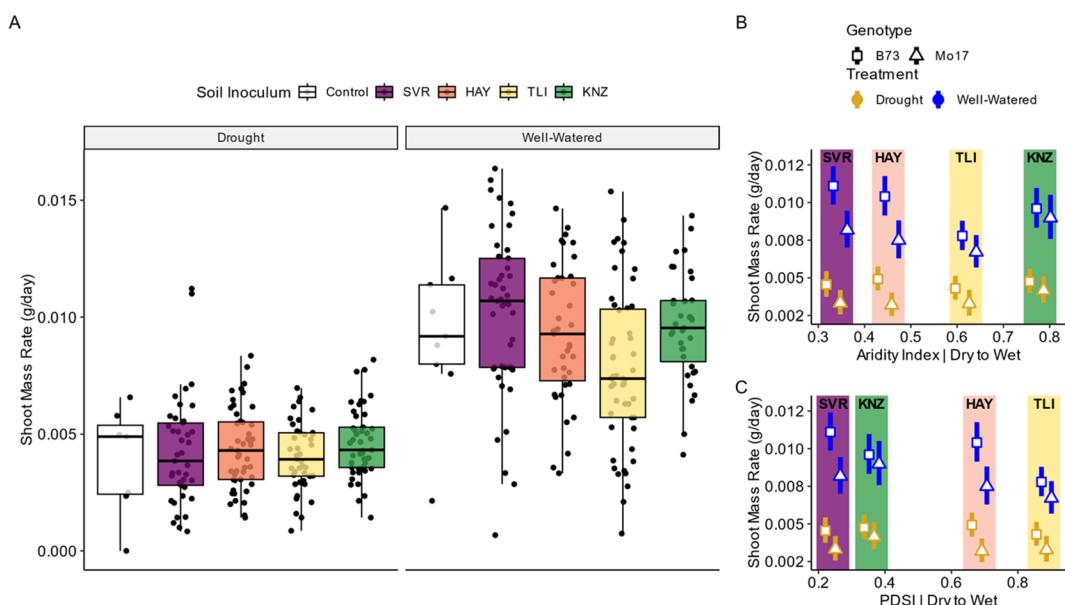


Fig. 6 Shoot mass is dependent on drought and soil inocula. Boxplots are colored by soil inoculum for (A) shoot mass rate with separate facets representing drought-stressed (D) and well-watered plants (W). Boxplot hinges represent the 1st and

3rd quartiles; whiskers represent 1.5 times the interquartile range. Estimated marginal means per site (shaded rectangles) and genotype (shape) for shoot mass rate vs. (B) mean annual aridity and (C) Palmer Drought Severity Index (PDSI)

Table 4 Bacterial and fungal families associated with plant traits; square root transformed shoot mass accumulation rate (SMR), square root transformed root mass accumulation rate (RMR), and log transformed root/shoot ratio (RSR). For each taxon the slope, intercept, residual percent variance explained (PVE_{Res}), relative abundance (Rel. Abund.), and corrected p value (*q*) are provided

**Streptococcaceae* shows a significant interaction between the family abundance and drought treatment, presented in Figure S11

Taxon	Trait	Slope	Intercept	PVE _{Res}	Rel. Abund	<i>q</i>
<i>Acetobacteraceae</i>	SMR	-1.25E-04	-1.33E-02	6.67	0.02	2.76E-03
<i>Corynebacteriaceae</i>	SMR	-5.46E-04	-1.29E-02	8.60	0.01	1.90E-04
<i>Dietziaceae</i>	SMR	-1.07E-03	-1.31E-02	4.94	0.00	6.48E-03
<i>Lactobacillaceae</i>	SMR	5.64E-04	-1.46E-02	4.26	0.09	1.55E-02
<i>Microbacteriaceae</i>	SMR	-1.07E-05	-1.32E-02	5.27	1.23	6.48E-03
<i>Micrococcaceae</i>	SMR	-8.43E-06	-1.31E-02	3.84	8.47	2.46E-02
<i>Nocardiaceae</i>	SMR	-3.71E-04	-1.33E-02	3.58	0.02	2.75E-02
<i>Rhodocyclaceae</i>	SMR	-4.92E-04	-1.14E-02	3.51	0.06	2.75E-02
<i>Sinobacteraceae</i>	SMR	2.12E-04	-1.38E-02	3.70	0.05	2.07E-02
<i>Sphingomonadaceae</i>	SMR	-1.03E-04	-1.24E-02	5.31	1.13	6.48E-03
<i>Streptococcaceae*</i>	SMR	1.38E-04	-1.34E-02	5.50	0.01	4.10E-03
<i>Acetobacteraceae</i>	RMR	2.32E-05	-5.94E-03	8.15	0.02	1.41E-02
<i>Corynebacteriaceae</i>	RMR	-2.65E-04	-5.74E-03	6.29	0.01	3.39E-02
<i>Polyangiaceae</i>	RSR	2.19E-02	1.37E-01	8.01	0.08	1.81E-02
<i>Sinobacteraceae</i>	RSR	-1.48E-03	1.67E-01	5.8	0.05	2.96E-02
<i>Sphingomonadaceae</i>	RSR	6.96E-04	1.58E-01	7.44	1.13	1.81E-02
<i>Sporidiobolaceae</i>	SMR	3.33E-04	-1.24E-02	6.17	0.48	2.95E-02

result may be due to variance in this trait decreasing over the course of the experiment as roots expanded to fill the entire container regardless of treatment or inoculum (Figure S17). Notably, *Acetobacteraceae* abundance was negatively associated with shoot mass accumulation rate but positively associated with root mass accumulation rate. We found that the families associated with root/shoot ratio, *Sinobacteraceae* and *Sphingomonadaceae* (Table 4), were a subset of families associated with shoot mass accumulation rate, suggesting that a change in shoot mass was responsible for this relationship. For fungi, only a single family, *Sporidiobolaceae*, was correlated with shoot mass accumulation rate (Table 4). Similar results were found at lower taxonomic levels for both bacteria and fungi (Table S11-12).

Discussion

Plant and microbiome responses to drought

Understanding plant-microbiome interactions under drought stress is critical to safeguard agricultural systems in the face of global climate change. In our experiment, maize displayed phenotypic tradeoffs in response to drought. Drought-treated plants increased root growth relative to shoot growth, resulting in

shorter plants over the course of the experiment. This supports our understanding of plant responses to drought, as plants prioritize root over shoot growth (Sharp and Davies 1979; Lynch et al. 2014), favoring deeper and often denser root systems in order to obtain sufficient water (Zhan et al. 2015; Gao and Lynch 2016; Sebastian et al. 2016). The maize genotypes, B73 and Mo17, showed significant differences between root/shoot allocation patterns, but in general both genotypes responded to drought in a similar manner. These phenotypic changes in root growth, along with direct effects of water limitation in the soil, contributed to the responses of the root microbiomes in our study.

Drought altered the bacterial communities of maize roots, although the magnitude of compositional change within our study was small ($R^2=0.01$) relative to other studies ($R^2=0.04\text{--}0.10$) (Santos-Medellín et al. 2017; Naylor et al. 2017; Fitzpatrick et al. 2018; Xu et al. 2018). Similar to previous studies (Xu and Coleman-Derr 2019), we observed a depletion of gram-negative and an enrichment of gram-positive bacteria in response to drought, likely relating to the desiccation resistance provided by the thick peptidoglycan cell wall of gram-positive bacteria (Manzoni et al. 2012). Enrichment of Actinomycetota during drought tends to be more pronounced in root-associated microbiomes than in soil, suggesting that shifts in plant root physiology also play

a role (Xu et al. 2018; Xu and Coleman-Derr 2019); however, the specific root traits driving this effect are unknown. During drought, root exudate production is generally maintained or increased (Henry et al. 2007; Preece and Peñuelas 2016; Karlowsky et al. 2018), while the exudate composition is typically altered (Svenningsson et al. 1990; Canarini et al. 2016; Calvo et al. 2017) which can contribute to the root microbiome drought response (Zhalnina et al. 2018). In *Sorghum*, for instance, drought-induced changes in root exudate composition and iron homeostasis contribute to Actinomycetota enrichment in the rhizosphere (Xu et al. 2018, 2021).

Fungal communities of maize roots were less sensitive to drought relative to their bacterial neighbors. While drought increased root fungal diversity in the plants inoculated with the TLI prairie soil inoculum, it did not significantly affect overall fungal community composition. Machine learning classification by drought treatment further illustrated this difference: when trained on bacteria, we achieved 70% balanced accuracy, while for fungi, we achieved only 47% accuracy. This discrepancy indicates that bacterial communities responded in a predictable manner to drought, whereas fungal communities were less predictable between well-watered and drought plants. This cross-kingdom difference agrees with previous reports, as both free living and root-associated fungal communities tend to tolerate desiccation (de Vries et al. 2012; Furze et al. 2017). The observed difference in survival between bacteria and fungi under drought may relate to both the vegetative and dormant growth forms of fungi: compared to bacterial cells, fungal hyphae comprise most vegetative growth and can cross air-gaps within dry soil in search of resources (Griffin 1985; Manzoni et al. 2012), and dormant spores are generally desiccation-resistant (Wyatt et al. 2013). Finally, the process of generating the soil inocula led to a small decrease in fungal richness (i.e., soil *vs.* inocula comparison; Table S2). We note that while unlikely, this could impact fungal community responsiveness to drought, if drought-sensitive fungi were excluded.

Impacts of soil microbiomes on plant phenotype under low and high water availability

We observed that although the four soil microbiomes in our study were shaped by contrasting environments

and were taxonomically distinct (Figs. 1 and 2), none of them improved or reduced plant performance under drought conditions, relative to the others or to the control. In contrast, the variation in plant growth caused by these inocula was apparent only under well-watered conditions (Fig. 6A). In other words, plant performance responded to soil microbiome variation under stressful but not more permissive conditions. Our result is opposite to one recent study in maize, which found that two microbiomes from contrasting environments (a grassland and an organic farm) differentially affected shoot and root fresh weight under drought-stressed but not well-watered conditions (Zhang et al. 2022). Another recent maize study, however, found that both natural microbiome differences (between a forest and an unirrigated dryland farm soil) and experimentally-induced microbiome differences (in response to a drought conditioning phase) had consistent effects on root growth and stem diameter regardless of the amount of water available to the plants (Carter et al. 2023). A related study reported very similar results: consistent growth stimulation by a forest-derived inoculum relative to an agricultural inoculum, regardless of contemporary water availability (Moore et al. 2023). Altogether, these studies provide mixed support for the stress gradient hypothesis (Bertness and Callaway 1994), which predicts that drought-stressed plants should perform better in the presence of microorganisms from historically dry soils, where chronic drought stress would have fostered positive interactions with neighboring plants.

The reason for our varied results among inocula sources is not immediately clear, but there are several possible explanations. The availability of water may relax the fitness consequences of expression of microbial traits that are critical under drier environments; for instance, microorganisms counteract the physiologic stress of drought by accumulating solutes, which reduces their internal water potential as a mechanism to avoid desiccation (Harris 1981). The accumulation of solutes requires considerable energy expenditure and constitutes a large sink of C and N resources (Killham and Firestone 1984; Schimel et al. 1989, 2007). The contemporary well-watered conditions relax these costly solute needs, perhaps allowing drought-adapted microbes to reallocate resources to functions mutually beneficial to plants. Another possibility is that soils from historically wetter sites generally possess greater microbial biomass

and diversity (Neilson et al. 2017; Araya et al. 2020; Wan et al. 2021), which is predicted to promote positive interactions and improved community function (Hooper et al. 2005). We found this to be the case for fungi, where our wettest prairie site showed the highest diversity, but this was less consistent for bacterial communities (Figure S2-3). A third potential explanation involves the balance of beneficial and pathogenic microbes in the soil community. Pathogen richness in soil, root, and shoot microbiomes is often positively associated with mean annual temperature and precipitation (Větrovský et al. 2019; Delgado-Baquerizo et al. 2020; Chen et al. 2021). Thus, soil communities from historically dry sites may have relatively fewer plant pathogens, resulting in greater plant performance under contemporary well-watered conditions. We cannot distinguish between these possible explanations using the data in hand; however, a future experiment that leverages rain exclusion structures to manipulate precipitation for a given site could effectively monitor changes in diversity and composition over time. In addition, this experimental manipulation strategy paired with a technique to quantify microbial biomass (e.g. quantitative stable-isotope probing) (Greenlon et al. 2022) could enable the exploration of the balance between beneficial, neutral, and pathogenic microbes.

In addition to these ecological and evolutionary mechanisms that may have shaped the soil microbiome differences observed across the Kansas precipitation gradient, real-time changes in microbial physiology could also explain our finding that microbiome effects on plant growth were more pronounced under well-watered conditions. Microbiome members that have the potential to influence plant growth under optimal conditions may instead invest their energy into survival mechanisms such as solute regulation and water retention, as has been recently demonstrated for communities of microbial decomposers (Malik et al. 2020). Additionally, in the absence of water an unknown portion of the microbial community goes dormant or dies (Salazar et al. 2018; Schimel 2018), suspending or ending their normal metabolic activities that could influence plant growth. In the rhizosphere of field-grown maize, for instance, drought and heat stress depleted levels of ammonium and nitrate, the bioavailable forms of nitrogen produced by soil bacteria via the processes of mineralization and nitrification, respectively (Yuan et al. 2024).

Another study used metatranscriptomics and metabolomics to characterize the effects of experimental drought on the function of three soil microbiomes collected from Konza Prairie, which was also the source of one of our soil inocula (Fig. 1). Across communities, bacterial genes involved in both primary and secondary metabolism were differentially expressed under dry conditions, indicating a shift from growth to stress mitigation (Roy Chowdhury et al. 2019). Notably, however, despite their close proximity and shared historical climate, the three microbiomes each showed a unique metabolic response to 15 days of water limitation, indicating that functionally relevant, naturally-occurring soil microbiome variation exists even on local scales and interacts with real-time water availability.

Microbial taxa associated with plant growth

Multiple bacteria and fungi had abundances correlated with plant responses to drought, with more taxa associated with shoot growth than root growth. This is likely the result of space limitation in the pots, as root mass accumulation rates tended to decrease across the experiment regardless of the drought treatment (Figure S17). Even so, we identified two families that were associated with both shoot and root mass, *Acetobacteraceae* and *Corynebacteriaceae*. *Acetobacteraceae* was associated with suppressed shoot growth but increased root growth. Although in our dataset *Acetobacteraceae* was represented mainly by the poorly-studied genus *Rhodopila*, this family contains many known diazotrophs, which may improve mineral nutrition of the plant (Reis and Teixeira 2015). Members of *Acetobacteraceae*, including the particularly well-studied *Gluconacetobacter diazotrophicus*, have been observed to colonize internal root tissues under both natural conditions and inoculation experiments (Luna et al. 2010; Rouws et al. 2010). This root colonization proficiency may help to explain the increased root mass we observed from this association; however, given the decreased shoot mass we also observed, *Acetobacteraceae* may have compartment-specific dynamics that warrant further investigation. In contrast, *Corynebacteriaceae* was associated with suppressed growth of both roots and shoots. Members of this family have been identified as root endophytes of multiple plant species (Stafford et al. 2005; Vik et al. 2013), with some showing

phytopathogenic properties and specificity to maize (Vidaver and Mandel 1974; Dye and Kemp 1977). Additionally, during a multi-generation experimental evolution study, *Corynebacteriaceae* (among other bacterial families) was consistently associated with delayed flowering in multiple plant species (Panke-Buisse et al. 2015). *Streptococcaceae* was the only family that showed an association with plant growth that was modulated by the drought treatment, with this family's relative abundance negatively correlated with shoot mass accumulation rate in well-watered plants but not in droughted plants. Less is known about the relevance of *Streptococcaceae* to plant growth. In *Zingiber officinale*, this family was significantly enriched in rhizomes with advanced stages of soft rot (Huang et al. 2022). However, *Streptococcaceae* is prevalent in soil and plants (Chuah et al. 2016; Wang et al. 2021), typically at higher abundance in aerial plant compartments than in the rhizosphere (Minervini et al. 2015; Yu et al. 2020). These families represent targets for direct manipulation and *in-planta* testing to better understand the mechanism responsible for the growth suppression we observed under well-watered conditions.

Conclusions

Water is a critical and limiting resource for plant growth as well as a beneficial microbiome. As maize is cultivated widely across the United States, production will have to contend with more frequent episodes of drought as the global climate warms. Engineering plant microbiomes to assist in plant resilience under drought remains an ambitious, but worthwhile goal. Our work illustrates the differential responses of root and shoot growth allocation to drought in two well-studied maize genotypes and shows that bacterial, but not fungal, microbiota undergo restructuring with drought, with decreases in the relative abundance of drought-intolerant taxa. In contrast to predictions based on the stress gradient hypothesis, soil microbiomes from perennially dry environments along a natural precipitation gradient did not enhance plant growth under drought. Instead, the effects of contrasting soil microbiomes on plant phenotype were only apparent under well-watered conditions. The bacterial and fungal groups associated with biomass can serve

to direct future cultivation and *in-planta* testing on maize growth.

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Author contributions BAS and PMH collected soil samples across Kansas. MRK, MK, and MRW designed the experiment and collection methods. MRK performed sample collection and processing. AC and NEF performed DNA extractions and library preparations. NEF and JFS performed the data analysis. JFS wrote the first draft of the manuscript. All authors contributed to data interpretation, the writing and reviewing of the manuscript, and approved the final draft.

Data availability Raw sequencing data is available on NCBI SRA under BioProject ID PRJNA913622 and SRA accessions SAMN32302538-SAMN32303072 for root-associated samples and SAMN33863891-SAMN33864082 for soil and inoculum samples. Code to reproduce the analysis and figures are available on GitHub at https://github.com/Kenizzer/Maize_Drought_Microbiome.

Declarations

Competing interests The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the United States Department of Agriculture or the National Science Foundation.

References

Acosta-Martínez V, Cotton J, Gardner T et al (2014) Predominant bacterial and fungal assemblages in agricultural soils during a record drought/heat wave and linkages to enzyme activities of biogeochemical cycling. *Appl Soil*

Ecol 84:69–82. <https://doi.org/10.1016/j.apsoil.2014.06.005>

Ali S, Tyagi A, Park S et al (2022) Deciphering the plant microbiome to improve drought tolerance: Mechanisms and perspectives. Environ Exp Bot 201:104933. <https://doi.org/10.1016/j.envexpbot.2022.104933>

Apprill A, McNally S, Parsons R, Weber L (2015) Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol 75:129–137. <https://doi.org/10.3354/ame01753>

Araya JP, González M, Cardinale M et al (2020) Microbiome dynamics associated with the Atacama Flowering Desert. Front Microbiol 10:1–13. <https://doi.org/10.3389/fmicb.2019.03160>

Armanhi JSL, de Souza RSC, Biazotti BB et al (2021) Modulating drought stress response of maize by a synthetic bacterial community. Front Microbiol 12. <https://doi.org/10.3389/fmicb.2021.747541>

Augé RM, Stodola AJW, Tims JE, Saxton AM (2001) Moisture retention properties of a mycorrhizal soil. Plant Soil 230:87–97. <https://doi.org/10.1023/A:1004891210871>

Averill C, Waring BG, Hawkes CV (2016) Historical precipitation predictably alters the shape and magnitude of microbial functional response to soil moisture. Glob Change Biol 22:1957–1964. <https://doi.org/10.1111/gcb.13219>

Balting DF, AghaKouchak A, Lohmann G, Ionita M (2021) Northern Hemisphere drought risk in a warming climate. npj Clim Atmos Sci 4:1–13. <https://doi.org/10.1038/s41612-021-00218-2>

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67(1):1–48. <https://doi.org/10.18637/jss.v067.i01>

Bell-Dereske LP, Benucci GMN, da Costa PB et al (2023) Regional biogeography versus intra-annual dynamics of the root and soil microbiome. Environmental Microbiome 18:50. <https://doi.org/10.1186/s40793-023-00504-x>

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc 57:289–300. <https://www.jstor.org/stable/2346101>

Bertness MD, Callaway R (1994) Positive interactions in communities. Trends Ecol Evol 9:191–193. [https://doi.org/10.1016/0169-5347\(94\)90088-4](https://doi.org/10.1016/0169-5347(94)90088-4)

Buchenau N, van Kleunen M, Wilschut RA (2022) Direct and legacy-mediated drought effects on plant performance are species-specific and depend on soil community composition. Oikos 2022:e08959. <https://doi.org/10.1111/oik.08959>

Callahan BJ, McMurdie PJ, Rosen MJ et al (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. <https://doi.org/10.1038/nmeth.3869>

Callahan BJ (2017) RDP taxonomic training data formatted for DADA2 (RDP trainset 16/release 11.5). <https://doi.org/10.5281/zenodo.4310150>

Calvo OC, Franzaring J, Schmid I et al (2017) Atmospheric CO₂ enrichment and drought stress modify root exudation of barley. Glob Change Biol 23:1292–1304. <https://doi.org/10.1111/gcb.13503>

Canarini A, Merchant A, Dijkstra FA (2016) Drought effects on *Helianthus annuus* and *Glycine max* metabolites: from phloem to root exudates. Rhizosphere 2:85–97. <https://doi.org/10.1016/j.rhisph.2016.06.003>

Canarini A, Schmidt H, Fuchsleger L et al (2021) Ecological memory of recurrent drought modifies soil processes via changes in soil microbial community. Nat Commun 12:5308. <https://doi.org/10.1038/s41467-021-25675-4>

Carter KR, Nachtsheim AC, Dickman LT et al (2023) Drought conditioning of rhizosphere microbiome influences maize water use traits. Plant Soil 492:587–604. <https://doi.org/10.1007/s11104-023-06204-2>

Chen Q-L, Hu H-W, Yan Z-Z et al (2021) Precipitation increases the abundance of fungal plant pathogens in Eucalyptus phyllosphere. Environ Microbiol 23:7688–7700. <https://doi.org/10.1111/1462-2920.15728>

Chiang F, Mazdiyasi O, AghaKouchak A (2021) Evidence of anthropogenic impacts on global drought frequency, duration, and intensity. Nat Commun 12:2754. <https://doi.org/10.1038/s41467-021-22314-w>

Chuah L-O, Yap K-P, Thong KL et al (2016) Genome sequence of “*Anthococcus*,” a novel genus of the family Streptococcaceae isolated from flowers. Genome Announcements 4. <https://doi.org/10.1128/genomeA.01410-16>

Coleman-Derr D, Desgarnes D, Fonseca-Garcia C et al (2016) Plant compartment and biogeography affect microbiome composition in cultivated and native *Agave* species. New Phytol 209:798–811. <https://doi.org/10.1111/nph.13697>

De Long JR, Semchenko M, Pritchard WJ et al (2019) Drought soil legacy overrides maternal effects on plant growth. Funct Ecol 33:1400–1410. <https://doi.org/10.1111/1365-2435.13341>

de Vries FT, Liiri ME, Björnlund L et al (2012) Land use alters the resistance and resilience of soil food webs to drought. Nat Clim Chang 2:276–280. <https://doi.org/10.1038/nclimate1368>

Delgado-Baquerizo M, Guerra CA, Cano-Díaz C et al (2020) The proportion of soil-borne pathogens increases with warming at the global scale. Nat Clim Chang 10:550–554. <https://doi.org/10.1038/s41558-020-0759-3>

Dye DW, Kemp WJ (1977) A taxonomic study of plant pathogenic *Corynebacterium* species. N Z J Agric Res 20:563–582. <https://doi.org/10.1080/00288233.1977.10427375>

Fernandes AD, Reid JNS, Macklaim JM et al (2014) Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. Microbiome 2:15. <https://doi.org/10.1186/2049-2618-2-15>

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci 103:626–631. <https://doi.org/10.1073/pnas.0507535103>

Fitzpatrick CR, Copeland J, Wang PW et al (2018) Assembly and ecological function of the root microbiome across angiosperm plant species. Proc Natl Acad Sci 115:E1157–E1165. <https://doi.org/10.1073/pnas.1717617115>

Fuchsleger L, Bahn M, Hasibeder R et al (2016) Drought history affects grassland plant and microbial carbon turnover during and after a subsequent drought event. J Ecol

104:1453–1465. <https://doi.org/10.1111/1365-2745.12593>

Furze JR, Martin AR, Nasielski J et al (2017) Resistance and resilience of root fungal communities to water limitation in a temperate agroecosystem. *Ecol Evol* 7:3443–3454. <https://doi.org/10.1002/ece3.2900>

Gao Y, Lynch JP (2016) Reduced crown root number improves water acquisition under water deficit stress in maize (*Zea mays* L.). *J Exp Bot* 67:4545–4557. <https://doi.org/10.1093/jxb/erw243>

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Mol Ecol* 2:113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>

Gloster GB, Macklain JM, Pawlowsky-Glahn V, Egoscue JJ (2017) Microbiome Datasets Are Compositional: And This Is Not Optional. *Front Microbiol* 8:1–6. <https://doi.org/10.3389/fmicb.2017.02224>

Greenlon A, Sieradzki E, Zablocki O et al (2022) Quantitative Stable-Isotope Probing (qSIP) with metagenomics links microbial physiology and activity to soil moisture in mediterranean-climate grassland ecosystems. *mSystems* 7:e00417-22. <https://doi.org/10.1128/msystems.00417-22>

Griffin DM (1985) A comparison of the roles of bacteria and fungi. Springer US, Boston

Harris R (1981) Effect of Water Potential on Microbial Growth and Activity. Water Potential Relations in Soil Microbiology, 9th edn. Soil Science Society of America, Madison, pp 23–95

Harrison XA, Donaldson L, Correa-Cano ME et al (2018) A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ* 6:e4794. <https://doi.org/10.7717/peerj.4794>

Hawkes CV, Keitt TH (2015) Resilience vs. historical contingency in microbial responses to environmental change. *Ecol Lett* 18:612–625. <https://doi.org/10.1111/ele.12451>

Henry A, Doucette W, Norton J, Bugbee B (2007) Changes in crested wheatgrass root exudation caused by flood, drought, and nutrient stress. *J Environ Qual* 36:904–912. <https://doi.org/10.2134/jeq2006.0425sc>

Hooper DU, Chapin FS III, Ewel JJ et al (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol Monogr* 75:3–35. <https://doi.org/10.1890/04-0922>

Huang K, Sun X, Zou Y et al (2022) Comparison of the endophytic bacterial microbiota of asymptomatic and symptomatic ginger rhizomes during the activation of adventitious bud development. *Plant Dis* 106:2470–2479. <https://doi.org/10.1094/PDIS-09-21-2069-RE>

Kaisermann A, de Vries FT, Griffiths RI, Bardgett RD (2017) Legacy effects of drought on plant–soil feedbacks and plant–plant interactions. *New Phytol* 215:1413–1424. <https://doi.org/10.1111/nph.14661>

Kakouridis A, Hagen JA, Kan MP et al (2022) Routes to roots: direct evidence of water transport by arbuscular mycorrhizal fungi to host plants. *New Phytol* 236:210–221. <https://doi.org/10.1111/nph.18281>

Karlowsky S, Augusti A, Ingrisch J et al (2018) Drought-induced accumulation of root exudates supports post-drought recovery of microbes in mountain grassland. *Front Plant Sci* 9:1–16. <https://doi.org/10.3389/fpls.2018.01593>

Killham K, Firestone MK (1984) Salt stress control of intracellular solutes in streptomycetes indigenous to saline soils. *Appl Environ Microbiol* 47:301–306. <https://doi.org/10.1128/aem.47.2.301-306.1984>

Kuhn M (2008) Building predictive models in R using the caret Package. *J Stat Softw* 28:159–160. <https://doi.org/10.18637/jss.v028.i05>

Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest package: Tests in Linear mixed effects models. *J Stat Softw* 82. <https://doi.org/10.18637/jss.v082.i13>

Lau JA, Lennon JT (2012) Rapid responses of soil microorganisms improve plant fitness in novel environments. *Proc Natl Acad Sci* 109:14058–14062. <https://doi.org/10.1073/pnas.1202319109>

Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol* 75:5111–5120. <https://doi.org/10.1128/AEM.00335-09>

Lenth R, Love J, Herve M (2020) Emmeans: estimated marginal means, aka least-squares means. <https://cran.r-project.org/package=emmeans>

Luna MF, Galar ML, Aprea J et al (2010) Colonization of sorghum and wheat by seed inoculation with *Gluconacetobacter diazotrophicus*. *Biotechnol Lett* 32:1071–1076. <https://doi.org/10.1007/s10529-010-0256-2>

Lynch JP, Chimungu JG, Brown KM (2014) Root anatomical phenes associated with water acquisition from drying soil: Targets for crop improvement. *J Exp Bot* 65:6155–6166. <https://doi.org/10.1093/jxb/eru162>

Malik AA, Swenson T, Weihe C et al (2020) Drought and plant litter chemistry alter microbial gene expression and metabolite production. *ISME J* 14:2236–2247. <https://doi.org/10.1038/s41396-020-0683-6>

Manzoni S, Schimel JP, Porporato A (2012) Responses of soil microbial communities to water stress: results from a meta-analysis. *Ecology* 93:930–938. <https://doi.org/10.1890/11-0026.1>

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17:10. <https://doi.org/10.14806/ej.17.1.200>

McMurdie PJ, Holmes S (2013) phyloseq: An R Package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* 8:e61217. <https://doi.org/10.1371/journal.pone.0061217>

Minervini F, Celano G, Lattanzi A et al (2015) Lactic acid bacteria in durum wheat flour are endophytic components of the plant during its entire life cycle. *Appl Environ Microbiol* 81:6736–6748. <https://doi.org/10.1128/AEM.01852-15>

Moore ER, Carter KR, Heneghan JP et al (2023) Microbial drivers of plant performance during drought depend upon community composition and the greater soil environment. *Microbiol Spectrum* 11:e01476-e1522. <https://doi.org/10.1128/spectrum.01476-22>

Naylor D, Coleman-Derr D (2018) Drought Stress and Root-Associated Bacterial Communities. *Front Plant Sci* 8:1–16. <https://doi.org/10.3389/fpls.2017.02223>

Naylor D, DeGraaf S, Purdom E, Coleman-Derr D (2017) Drought and host selection influence bacterial community dynamics in the grass root microbiome. *ISME J* 11:2691–2704. <https://doi.org/10.1038/ismej.2017.118>

Neilson JW, Califf K, Cardona C et al (2017) Significant impacts of increasing aridity on the arid soil microbiome. *mSystems* 2. <https://doi.org/10.1128/mSystems.00195-16>

Ngumbi E, Kloepper J (2016) Bacterial-mediated drought tolerance: Current and future prospects. *Appl Soil Ecol* 105:109–125. <https://doi.org/10.1016/j.apsoil.2016.04.009>

Oksanen J, Guillaume Blanchet F, Friendly M et al (2019) vegan: Community Ecology Package. <https://doi.org/10.32614/CRAN.package.vegan>

Panke-Buisse K, Poole AC, Goodrich JK et al (2015) Selection on soil microbiomes reveals reproducible impacts on plant function. *ISME J* 9:980–989. <https://doi.org/10.1038/ismej.2014.196>

Parada AE, Needham DM, Fuhrman JA (2016) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ Microbiol* 18:1403–1414. <https://doi.org/10.1111/1462-2920.13023>

Preece C, Peñuelas J (2016) Rhizodeposition under drought and consequences for soil communities and ecosystem resilience. *Plant Soil* 409:1–17. <https://doi.org/10.1007/s11104-016-3090-z>

R Core Team (2021) R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. <https://www.R-project.org/>

Reis VM, dos Santos Teixeira KR (2015) Nitrogen fixing bacteria in the family Acetobacteraceae and their role in agriculture. *J Basic Microbiol* 55:931–949. <https://doi.org/10.1002/jobm.201400898>

Rouws LFM, Meneses CHSG, Guedes HV et al (2010) Monitoring the colonization of sugarcane and rice plants by the endophytic diazotrophic bacterium *Gluconacetobacter diazotrophicus* marked with gfp and gusA reporter genes. *Lett Appl Microbiol* 51:325–330. <https://doi.org/10.1111/j.1472-765X.2010.02899.x>

Roy Chowdhury T, Lee J-Y, Bottos EM et al (2019) Metaphenomic responses of a native prairie soil microbiome to moisture perturbations. *mSystems* 4. <https://doi.org/10.1128/msystems.00061-19>. <https://doi.org/10.1128/msystems.00061-19>

Salazar A, Sulman BN, Dukes JS (2018) Microbial dormancy promotes microbial biomass and respiration across pulses of drying-wetting stress. *Soil Biol Biochem* 116:237–244. <https://doi.org/10.1016/j.soilbio.2017.10.017>

Santos-Medellín C, Edwards J, Liechty Z et al (2017) Drought stress results in a compartment-specific restructuring of the rice root-associated microbiomes. *mBio* 8:1–15. <https://doi.org/10.1128/mBio.00764-17>

Schimel JP (2018) Life in dry soils: effects of drought on soil microbial communities and processes. *Annu Rev Ecol Evol Syst* 49:409–432. <https://doi.org/10.1146/annurev-ecolysys-110617-062614>

Schimel JP, Scott WJ, Killham K (1989) Changes in cytoplasmic carbon and nitrogen pools in a soil bacterium and a fungus in response to salt stress. *Appl Environ Microbiol* 55:1635–1637. <https://doi.org/10.1128/aem.55.6.1635-1637.1989>

Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88:1386–1394. <https://doi.org/10.1890/06-0219>

Schultz CR, Brantley KM, Wallace JG (2022) The role of genetic variation in *zea mays* response to beneficial endophytes. *Plant Growth Regul* 98:167–177. <https://doi.org/10.1007/s10725-022-00842-9>

Sebastian J, Yee M-C, Goudinho Viana W et al (2016) Grasses suppress shoot-borne roots to conserve water during drought. *Proc Natl Acad Sci* 113:8861–8866. <https://doi.org/10.1073/pnas.1604021113>

Sharp RE, Davies WJ (1979) Solute regulation and growth by roots and shoots of water-stressed maize plants. *Planta* 147:43–49. <https://doi.org/10.1007/BF00384589>

Stafford WHL, Baker GC, Brown SA et al (2005) Bacterial diversity in the rhizosphere of Proteaceae species. *Environ Microbiol* 7:1755–1768. <https://doi.org/10.1111/j.1462-2920.2005.00929.x>

Svenningsson H, Sundin P, Liljenberg C (1990) Lipids, carbohydrates and amino acids exuded from the axenic roots of rape seedlings exposed to water-deficit stress. *Plant Cell Environ* 13:155–162. <https://doi.org/10.1111/j.1365-3040.1990.tb01287.x>

UNITE Community (2017) UNITE general FASTA release. <https://doi.org/10.15156/BIO/587475>

Větrovský T, Kohout P, Kopecký M et al (2019) A meta-analysis of global fungal distribution reveals climate-driven patterns. *Nat Commun* 10:5142. <https://doi.org/10.1038/s41467-019-13164-8>

Vidaver AK, Mandel M (1974) *Corynebacterium nebraskense*, a new, orange-pigmented phytopathogenic species. *Int J Syst Bacteriol* 24:482–485. <https://doi.org/10.1099/00207713-24-4-482>

Vik U, Logares R, Blaaidal R et al (2013) Different bacterial communities in ectomycorrhizae and surrounding soil. *Sci Rep* 3:3471. <https://doi.org/10.1038/srep03471>

Wagner MR, Roberts JH, Balint-Kurti P, Holland JB (2020) Heterosis of leaf and rhizosphere microbiomes in field-grown maize. *New Phytol* 228:1055–1069. <https://doi.org/10.1111/nph.16730>

Wan X, Chen X, Huang Z, Chen HYH (2021) Global soil microbial biomass decreases with aridity and land-use intensification. *Glob Ecol Biogeogr* 30:1056–1069. <https://doi.org/10.1111/geb.13282>

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73:5261–5267. <https://doi.org/10.1128/AEM.00062-07>

Wang P, Marsh EL, Kruger G et al (2020) Belowground microbial communities respond to water deficit and are shaped by decades of maize hybrid breeding. *Environ Microbiol* 22:889–904. <https://doi.org/10.1111/1462-2920.14701>

Wang X, Shi W, Wang J et al (2021) Control of southern root-knot nematodes on tomato and regulation of soil bacterial community by biofumigation with *zanthoxylum bungeanum* seed. *Hortic Plant J* 7:49–58. <https://doi.org/10.1016/j.hpj.2020.12.005>

White T, Burns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322

Wright MN, Ziegler A (2017) ranger : A fast implementation of random forests for high dimensional data in C++ and R. *J Stat Softw* 77. <https://doi.org/10.18637/jss.v077.i01>

Wyatt TT, Wösten HAB, Dijksterhuis J (2013) Chapter Two - Fungal Spores for Dispersion in Space and Time. In: Sariaslani S, Gadd GM (eds) Advances in Applied Microbiology. Academic Press, Cambridge, pp 43–91

Xu L, Coleman-Derr D (2019) Causes and consequences of a conserved bacterial root microbiome response to drought stress. *Curr Opin Microbiol* 49:1–6. <https://doi.org/10.1016/j.mib.2019.07.003>

Xu L, Naylor D, Dong Z et al (2018) Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. *Proc Natl Acad Sci* 115:E4284–E4293. <https://doi.org/10.1073/pnas.1717308115>

Xu L, Dong Z, Chiniquy D et al (2021) Genome-resolved metagenomics reveals role of iron metabolism in drought-induced rhizosphere microbiome dynamics. *Nat Commun* 12:3209. <https://doi.org/10.1038/s41467-021-23553-7>

Yang N, Nesme J, Röder HL et al (2021) Emergent bacterial community properties induce enhanced drought tolerance in *Arabidopsis*. *npj Biofilms Microbiomes* 7:1–11. <https://doi.org/10.1038/s41522-021-00253-0>

Yu AO, Leveau JHJ, Marco ML (2020) Abundance, diversity and plant-specific adaptations of plant-associated lactic acid bacteria. *Environ Microbiol Rep* 12:16–29. <https://doi.org/10.1111/1758-2229.12794>

Yuan A, Kumar SD, Wang H et al (2024) Dynamic interplay among soil nutrients, rhizosphere metabolites, and microbes shape drought and heat stress responses in summer maize. *Soil Biol Biochem* 191:109357. <https://doi.org/10.1016/j.soilbio.2024.109357>

Zarraonaindia I, Owens SM, Weisenhorn P et al (2015) The soil microbiome influences grapevine-associated microbiota. *mBio* 6:e02527-14. <https://doi.org/10.1128/mBio.02527-14>

Zhelnina K, Louie KB, Hao Z et al (2018) Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nat Microbiol* 3:470–480. <https://doi.org/10.1038/s41564-018-0129-3>

Zhan A, Schneider H, Lynch JP (2015) Reduced lateral root branching density improves drought tolerance in maize. *Plant Physiol* 168:1603–1615. <https://doi.org/10.1104/pp.15.00187>

Zhang Z, Jatana BS, Campbell BJ et al (2022) Cross-inoculation of rhizobiome from a congeneric ruderal plant imparts drought tolerance in maize (*Zea mays*) through changes in root morphology and proteome. *Plant J* 111:54–71. <https://doi.org/10.1111/tpj.15775>

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