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RESEARCH ARTICLE

Microbial Drivers of Plant Performance during Drought Depend upon Community Composition and the Greater Soil Environment

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ABSTRACT The increasing occurrence of drought is a global challenge that threatens food security through direct impacts to both plants and their interacting soil microorganisms. Plant growth promoting microbes are increasingly being harnessed to improve plant performance under stress. However, the magnitude of microbiome impacts on both structural and physiological plant traits under water limited and water replete conditions are not well-characterized. Using two microbiomes sourced from a ponderosa pine forest and an agricultural field, we performed a greenhouse experiment that used a crossed design to test the individual and combined effects of the water availability and the soil microbiome composition on plant performance. Specifically, we studied the structural and leaf functional traits of maize that are relevant to drought tolerance. We further examined how microbial relationships with plant phenotypes varied under different combinations of microbial composition and water availability. We found that water availability and microbial composition affected plant structural traits. Surprisingly, they did not alter leaf function. Maize grown in the forest-soil microbiome produced larger plants under well-watered and water-limited conditions, compared to an agricultural soil community. Although leaf functional traits were not significantly different between the watering and microbiome treatments, the bacterial composition and abundance explained significant variability in both plant structure and leaf function within individual treatments, especially water-limited plants. Our results suggest that bacteria-plant interactions that promote plant performance under stress depend upon the greater community composition and the abiotic environment.

IMPORTANCE Globally, drought is an increasingly common and severe stress that causes significant damage to agricultural and wild plants, thereby threatening food security. Despite growing evidence of the potential benefits of soil microorganisms on plant performance under stress, decoupling the effects of the microbiome composition versus the water availability on plant growth and performance remains a challenge. We used a highly controlled and replicated greenhouse experiment to understand the impacts of microbial community composition and water limitation on corn growth and drought-relevant functions. We found that both factors affected corn growth, and, interestingly, that individual microbial relationships with corn growth and leaf function were unique to specific watering/microbiome treatment combinations. This finding may help explain the inconsistent success of previously identified microbial inocula in improving plant performance in the face of drought, outside controlled environments.

KEYWORDS Plant microbiome, drought, microbial composition, plant-microbe interactions, plant growth, soil ecology

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Croplands are increasingly likely to be subjected to drought conditions that threaten crop productivity and food security worldwide. Drought substantially alters the physical and chemical properties of the soil, leading to increased salinity, decreased nutrient availability, greater spatial heterogeneity, and reduced chemical diffusion (1–5). These changes in soil properties, in addition to location, seasonal, and environmental drivers of variability in microbiome composition, directly impact both plants and bacterial communities living in the soil (6). Given the potential compounding effects of biotic and abiotic conditions on plant performance, it is essential to better understand the individual and combined influences of water limitation and microbiome composition to predict plant responses in different environments. Using this knowledge, we can identify novel, drought-relevant plant-microbe interactions, and we can use microbiology-based approaches to improve agricultural productivity.

Plants, especially those living in dryland environments, have evolved numerous mechanisms by which to survive and respond to periods of extended water limitation. Water limitation primarily decreases plant turgor pressure and induces stomatal closure to limit transpiration, thereby inhibiting CO_2 exchange and primary production (growth) (7–10). Plants optimize productivity and reduce stress during droughts by altering structural and functional traits (11). Decreased productivity as a result of water limitation often manifests as stunted above-ground growth and reduced yield in agricultural settings (12); however, these changes also promote plant survival under water limitation. For example, a high leaf mass per area (LMA) is typical in plants that are adapted to more arid habitats, whereas thicker cell walls and decreased photosynthesis may contribute to greater drought tolerance (13, 14). Additional structural modifications of leaf tissues can alter the water holding capacity by adjusting the leaf water content (LWC) to prevent damage and desiccation (11). Stimulating or modifying root growth patterns may also allow for greater access to water and nutrients, thereby resulting in increased survival, as well as in optimized above-ground biomass and yield (15–17). In addition to structural adaptations, such as stunted growth and increased investment in roots, changes in plant function have been linked to improved stress tolerance. For example, leaf physiological plasticity allows plants to optimize carbon uptake to maximize intrinsic water use efficiency (WUEi; carbon uptake over water loss) under drought conditions (18). Thus, the effect of water limitation on plant physiology and function is fairly well-established. Water limitation also induces changes in plant root exudate quantity and composition, and this directly impacts the carbon availability in the soil on which microbes depend (19). Therefore, plant stress responses to water limitation can directly modify the soil microbial community composition. This may be an adaptive mechanism that plants use to recruit beneficial microorganisms that modify specific plant traits during drought stress (20, 21).

Water limitation substantially alters soil and rhizosphere bacterial community composition and activity. Desiccated soils tend to decrease in species richness and biomass (22–24), and they undergo broad changes in composition that are conserved across different environments and plant hosts (23, 25–27). For instance, the phyla Actinobacteriota, Firmicutes, and Chloroflexi are typically more abundant in dry soils (23, 26, 28, 29). This is partially explained by traits that increase fitness under dry conditions, including sporulation and monoderm morphology (26), but in the plant rhizosphere, where community shifts in response to drought tend to be large (29), this may also be a consequence of plant responses to drought that alter root exudate quality and select for taxa that promote plant drought tolerance (19, 23, 30). Bacterial communities from arid and historically droughted environments often show less-extreme responses to drought due to adaptations or historical compositional legacies from prior droughts (25, 30–33), which may also increase plant fitness during periods of water limitation (29–32). Further, drought-induced shifts in microbial diversity and activity lead to a major functional reorganization of microbe-microbe and specific plant-microbe interactions (23, 24). Thus, changes in interactions between plants and microbes under drought conditions are likely to modify plant performance, with the

outcomes being dependent on the context of the local community composition, plant host, and drought history.

Some microorganisms are known to improve plant performance under various types of stress. Numerous studies have focused on the effects of single bacterial isolates on the performance of a variety of plants that are grown in sterile soil under water limitation. These inoculations have been shown to increase growth, nutrient content, and water content, decrease oxidative stress, salt content, and wilting, and change stomatal conductance and photophysiology (e.g., [34–39]). Further, some specific mechanisms have been described. For instance, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity reduces ethylene concentrations in stressed plants (40, 41). Reductionist approaches targeting individual taxa have been highly valuable in identifying organisms and mechanisms that improve plant performance under stress, but, unfortunately, positive results that are identified under controlled conditions are not always replicable in a field environment. One reason for this may be that microbial activities can vary under different biotic and abiotic contexts (42, 43). Studies utilizing more realistic experimental conditions will complement isolate-based studies to identify and confirm plant-microbe interactions in the context of complex natural environments.

In this study, our main goal was to assess the contribution of the soil bacterial community (microbiome) influence on plant productivity under different levels of water availability. Maize (B73 inbred) seeds were cultivated in the presence of two distinct soil microbiomes and under two watering regimes in a balanced 2×2 factorial designed experiment. The microbiomes were derived from an agricultural soil and ponderosa pine forest to obtain complex communities with distinct compositions. Two watering regimes provided different amounts of water to plants: well-watered (irrigated to 65% volumetric water content [near the water-holding capacity of the soil] three times a week) and water-limited (irrigated to 45% volumetric water content [5% greater than the wilting point] three times a week). Using this design, we aimed to compare the individual and combined effects of microbiome composition and water availability on several metrics of plant performance that are relevant to drought tolerance and to examine how microbial relationships to plant phenotypes may be context-dependent and vary under different combinations of microbial composition and water availability. Our findings show that the bacterial community composition affected the plant structure (height, stem diameter, and root biomass), independent of the watering regime, but did not drive differences in the leaf functional traits (leaf mass per area [LMA], leaf water content [LWC] or water use efficiency [WUEi]). However, whereas leaf functional traits were not different across treatments, we found significant relationships between microbial features and variability in leaf functional traits within individual treatments, particularly within the water-limited regime. These findings reveal new links between microbiome composition and plant performance under water limitation and suggest that important plant-bacterial interactions are influenced by the greater community composition and abiotic environment.

RESULTS

Microbiome and watering effects on plant growth and physiology. We observed significant microbiome-driven changes in plant structure under both watering regimes. Microbiome treatment explained 6.0%, 25.0%, and 7.1% of the variability in height, stem diameter, and root dry biomass, respectively, and it explained no variation in the leaf functional traits, namely, LMA, LWC, and WUEi (Fig. 1; Table 1). Plant height and stem diameter were consistently higher in plants grown with the forest microbiome. After the emergence of the tenth leaf (growth period of 7 to 9 weeks), maize plants grown in the presence of the forest soil microbiome were an average of 3.64 ± 2.64 cm taller than were those grown in the agricultural microbiome, regardless of watering treatment (Fig. 1A; Table 1) (ANOVA, $P = 0.008$). Similarly, the stem diameter was 2.28 ± 0.87 cm larger, on average, in plants grown with the forest community (Fig. 1B; Table 1) (ANOVA, $P = 3.1 \times 10^{-6}$). The forest microbiome marginally increased the dry root mass by an average of 2.27 ± 2.46 g (Fig. 1C; Table 1) (ANOVA, $P = 0.082$), relative to plants grown with the agricultural

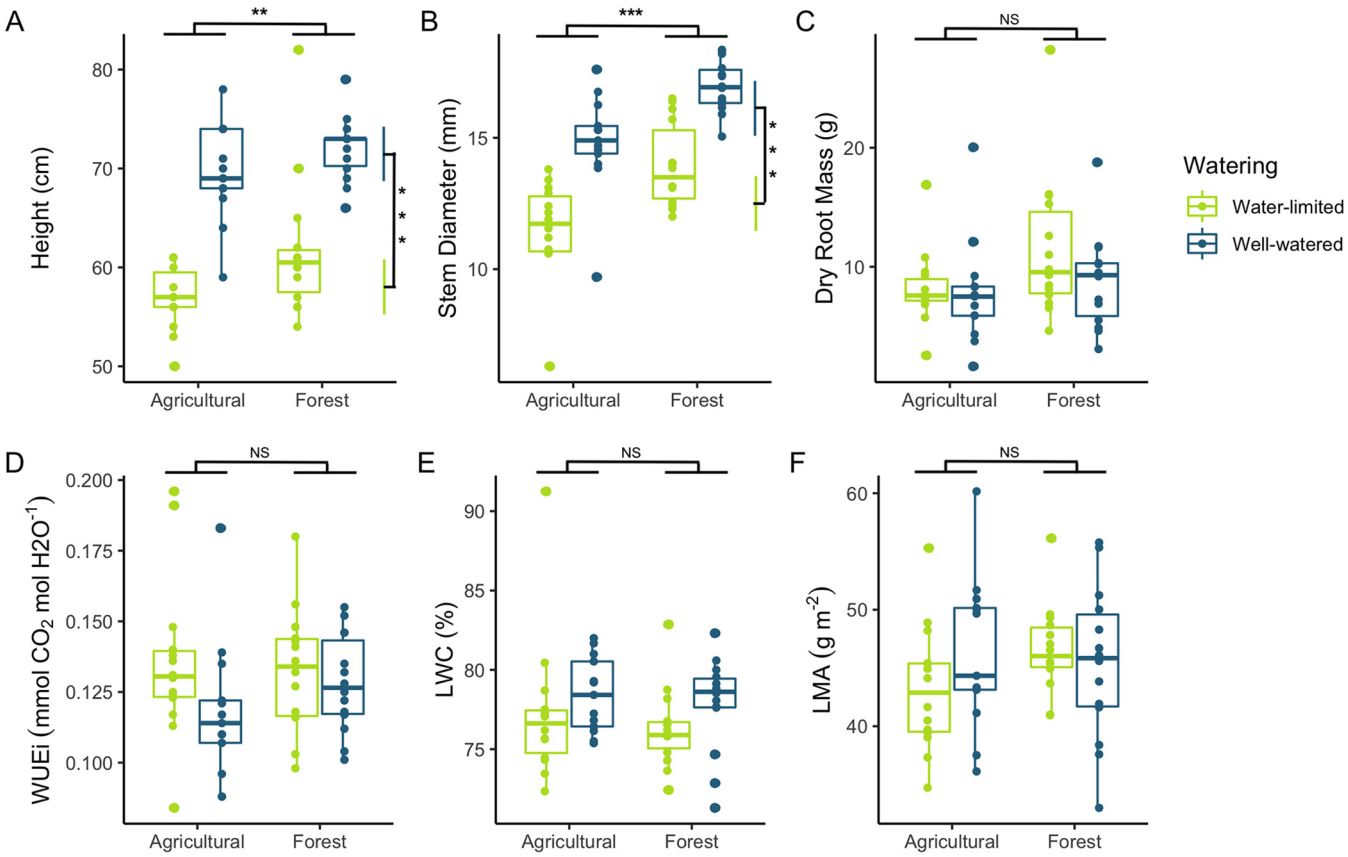

FIG 1 Maize structural and functional measurements. Watering regimes are shown in different colors: water-limited (green) and well-watered (blue). (A) Plant height. (B) Stem diameter. (C) Dry root biomass. (D) Intrinsic water use efficiency (WUEi). (E) Leaf water content (LWC). (F) Leaf mass per area (LMA). Boxplots show the mean, the 25th and 75th percentiles, and the min and max values. The measurements of individual plants are shown as points. A two-way ANOVA was used to determine the statistical significance of different treatments for each of the plant metrics. Plant height and stem diameter showed significant microbiome and watering effects (shown as dendrograms) but no interaction effects. All other traits showed no significant microbiome, watering, or interaction effects at α=0.05. Detailed ANOVA results are shown in Table 1.

microbiome. No statistically significant microbiome-driven differences were observed for the leaf functional traits WUEi, LMA, or LWC for plants grown in either microbiome (Fig. 1D–F; Table 1).

Expectedly, water limitation also modified the plant structure under both microbial treatments and altered the plant structure more substantially than the microbiome treatment. The watering regime accounted for 69.1% and 50.2% of the variability in the height and the stem diameter, respectively. The plant height and stem diameter were 11.56 $\pm$ 2.63 cm and 3.80 $\pm$ 0.87 cm smaller, respectively, in the water-limited plants (Fig. 1A and B; Table 1). Comparisons of root mass between watering regimes could only be tested after normalizing root mass to plant height to account for asynchronous measurements of the root system (see Materials and Methods). The root mass per plant height was 0.05 $\pm$ 0.04 g cm^{-1} larger

TABLE 1 ANOVA results comparing treatment effects on plant traits of interest[a]

Trait	Watering	Microbiome	Interaction
Height	F = 77.66, V = 69.1%, P = 7.99 $\times$ 10^{-12}[b]	F = 7.70, V = 6.0%, P = 0.008[b]	F = 0.79, V = 0.0%, P = 0.379
Stem diam	F = 53.97, V = 50.2%, P = 1.55 $\times$ 10^{-9}[b]	F = 27.44, V = 25.0%, P = 3.11 $\times$ 10^{-6}[b]	F = 0.11, V = 0.0%, P = 0.736
Dry root mass	F = 1.51, V = 1.7%, P = 0.224	F = 3.15, V = 7.1%, P = 0.082	F = 0.90, V = 0.0%, P = 0.347
Root mass per ht	F = 6.40, V = 16.0%, P = 0.015[b]	F = 1.91, V = 2.7%, P = 0.173	F = 0.72, V = 0.0%, P = 0.399
WUEi	F = 2.98, V = 6.7%, P = 0.090	F = 0.34, V = 0.0%, P = 0.563	F = 1.06, V = 0.0%, P = 0.309
LWC	F = 2.45, V = 5.3%, P = 0.124	F = 0.81, V = 0.0%, P = 0.373	F = 0.05, V = 0.0%, P = 0.828
LMA	F = 0.36, V = 0.0%, P = 0.550	F = 0.71, V = 0.0%, P = 0.403	F = 1.92, V = 6.4%, P = 0.171

[a]All tests were based on one degree of freedom.V represents the % of variance explained by each factor (87).
[b]Significant value at the α = 0.05 level.

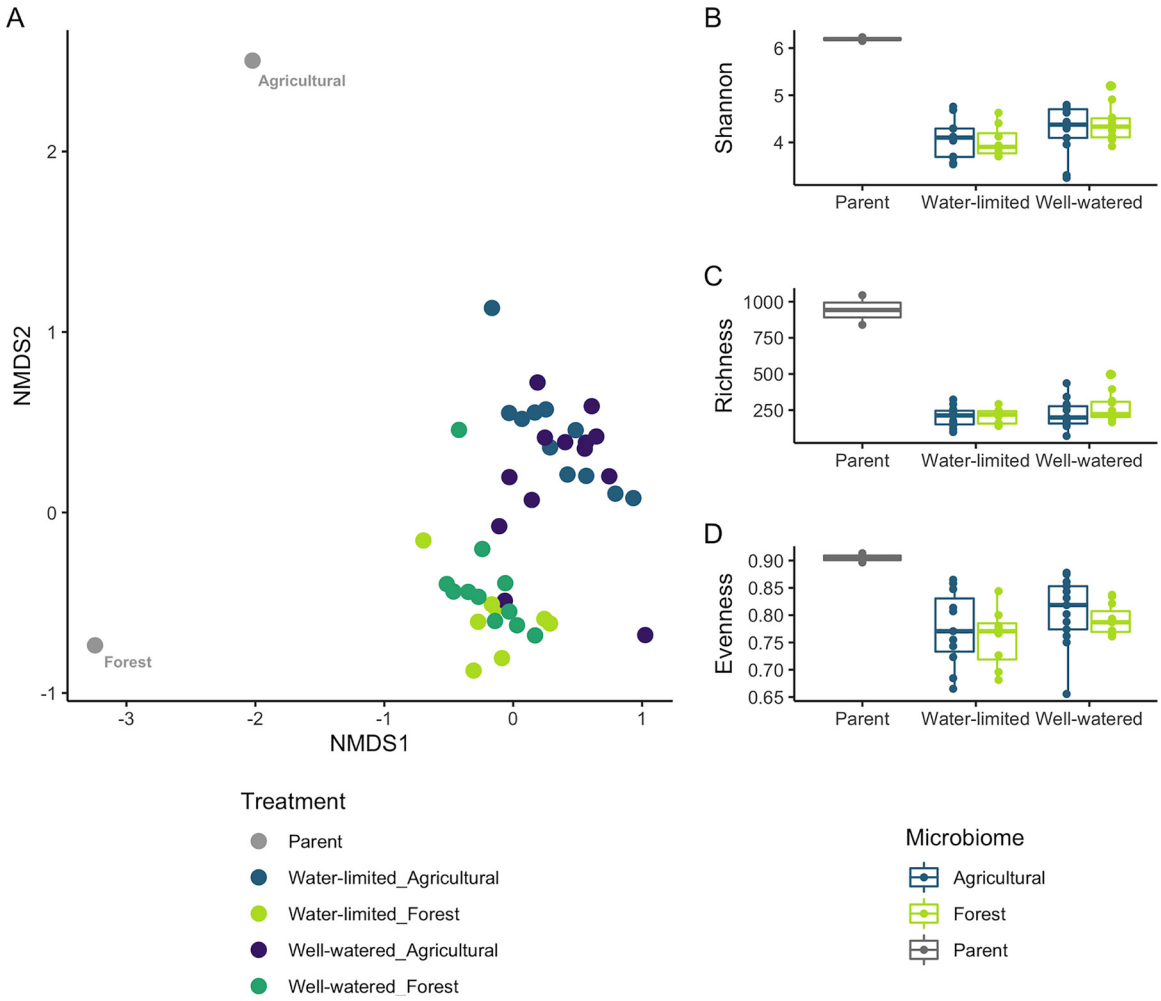

FIG 2 Microbial community composition and alpha diversity. (A) Nonmetric multidimensional scaled plot representing relative differences in overall community composition in the four treatments: well-watered, agricultural-derived community (violet); water-limited, agricultural-derived community (blue); well-watered, forest-derived community (dark green); and water-limited, forest-derived community (light green). An NMDS plot was generated based on the Bray-Curtis dissimilarity and was plotted using the ordinate function in Phyloseq. The NMDS stress was 0.139. A PERMANOVA analysis using the adonis function indicated significant differences in composition between the microbiome (R^2 = 0.121, P = 0.001) and watering treatments (R^2 = 0.118, P = 0.001) but not the interaction (R^2 = 0.021, P = 0.336). A pairwise PERMANOVA indicated significant differences between all treatment combinations ($P_{adj} < 0.05$). (B–D) Alpha diversity metrics for each microbiome source are shown by color: forest (green), agricultural (blue), and parent microbiome (gray). A two-way ANOVA indicated a significant watering effect for Shannon diversity only (F = 4.53, df =1, P = 0.039). For all other comparisons, $P > 0.05$.

in water-limited plants than in well-watered plants (ANOVA, F = 6.40, df = 1, P = 0.015). As observed with microbiome treatments, the watering regime did not significantly affect WUEi, LMA, or LWC in the presence of either microbiome (Fig. 1D–F; Table 1).

No statistically significant interaction among microbiome and watering regime was apparent for any of the six plant measurements (Fig. 1; Table 1).

Microbiome variability across treatments. Soil microbial community composition varied by watering regime and soil source. Microbial communities showed distinct clustering, based on the origin of the soil microbiome (PERMANOVA: R^2 = 0.121, P = 0.001), and composition changed substantially, following inoculation into the experimental environment. Microbial communities from both soil sources also showed significant differences in composition across watering regimes (Fig. 2A) (PERMANOVA: R^2 = 0.119, P = 0.001); however, no interaction effect of watering treatment and microbiome was evident (PERMANOVA: R^2 = 0.021, P = 0.336), suggesting that both microbiomes had similar responses and sensitivities to water limitation. A pairwise PERMANOVA revealed significant compositional differences between all individual microbiome and watering

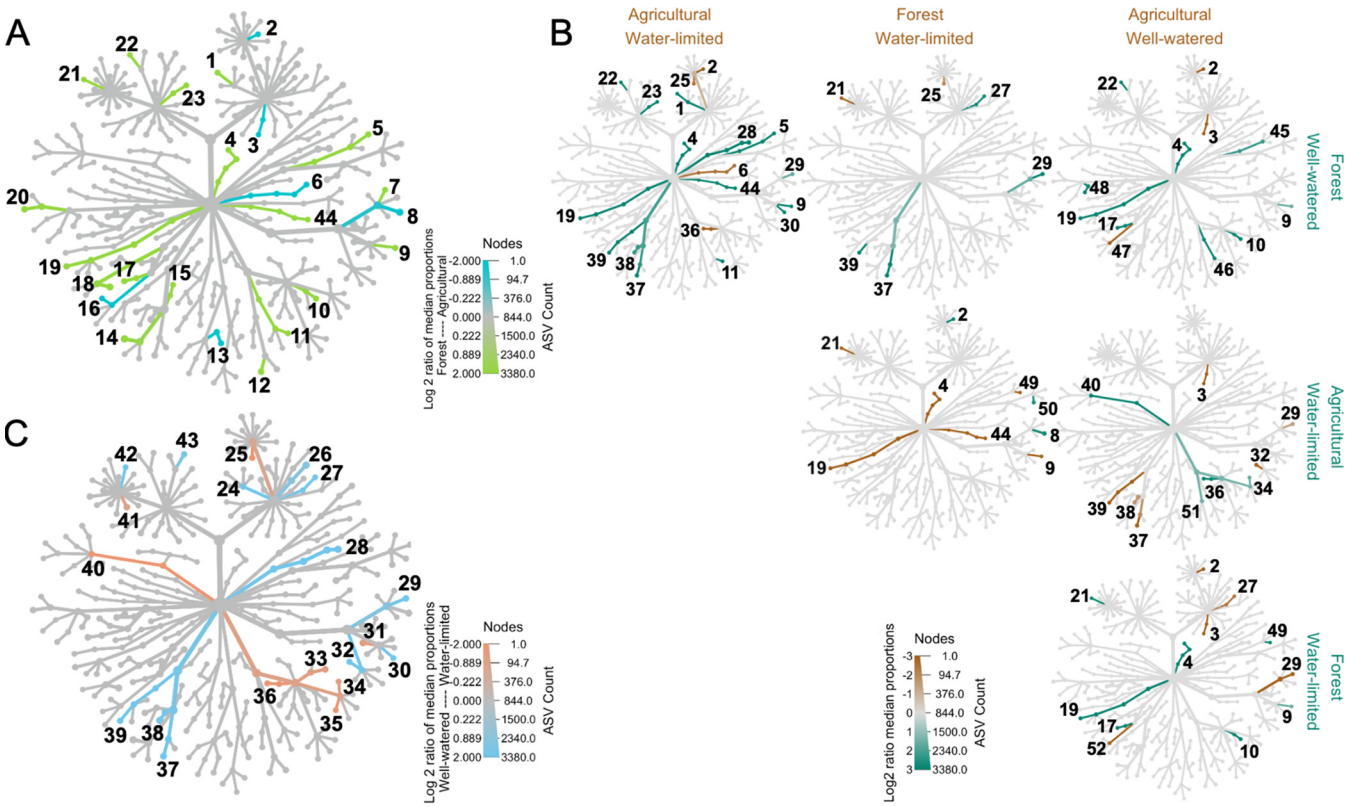

FIG 3 Differential abundance of taxa across treatments. Each number represents a significantly differentially abundant branch. The specific taxa names corresponding to each numbered branch are listed in Table S1 for reference. (A) Differential abundance of soil taxa between pots inoculated with communities originating from forest and agricultural soils. The taxa highlighted in green were significantly more abundant in the forest-derived community, whereas those in blue were more abundant in the agricultural community. (B) Pairwise comparisons of taxa abundance between specific treatment combinations. Taxa colors matching the row or column names indicate the treatment in which the taxon was more abundant. (C) The differential abundance of taxa between watering treatments. Those highlighted in orange and blue were significantly more abundant in the water-limited and well-watered treatments, respectively. For all plots, the color indicates the $\log_2$ ratio of the abundance proportions (differential abundance), and the node/branch size indicates the relative number of ASVs for each taxon.

treatment combinations: agricultural/water-limited versus forest/water-limited ($R^2 = 0.18$, $P_{adj.} = 0.006$), agricultural/water-limited versus forest/well-watered ($R^2 = 0.26$, $P_{adj.} = 0.006$), agricultural/water-limited versus agricultural/well-watered ($R^2 = 0.17$, $P_{adj.} = 0.006$), forest/water-limited versus forest/well-watered ($R^2 = 0.14$, $P_{adj.} = 0.030$), forest/water-limited versus agricultural/well-watered ($R^2 = 0.22$, $P_{adj.} = 0.006$), and forest/well-watered versus agricultural/well-watered ($R^2 = 0.14$, $P_{adj.} = 0.006$). Despite the microbiome source and the watering regime driving compositional differences of similar magnitudes, only Shannon diversity was significantly lower in the water-limited treatment (ANOVA: $P = 0.039$) (Fig. 2B). Species richness and community evenness were not significantly different between microbiomes or watering treatments, and their interaction was likewise not statistically significant (Fig. 2C and D).

Numerous bacterial taxa significantly differed in abundance between soil sources and watering treatments (Fig. 3; Table S1). When watering treatment was not considered, 18 taxa were significantly more abundant in the forest-derived community, whereas 6 were enriched in the agricultural community. Differentially abundant taxa ranged from the family to phylum level, but differentially abundant families and orders were the most common. When considering watering treatment, some taxa were only found to be differentially abundant between the forest and agricultural communities in one of the two watering treatments (Fig. 3B; Table S1). Across both microbiomes, watering regime also significantly impacted microbial abundances from the family to the phylum level (Fig. 3C; Table S1), with 8 taxa being found to be enriched in the water-limited communities and 12 taxa being found to be enriched in well-watered communities. Notably, the phyla Actinobacteriota and

TABLE 2 Mantel tests using correlated sample pairwise Bray-Curtis dissimilarity in the community composition to sample the pairwise differences in plant traits[a]

	WUEi		LWC		LMA		ht		Stem diam		Root mass	
Community	R	P	R	P	R	P	R	P	R	P	R	P
Forest	−0.090	0.802	0.014	0.447	0.184	0.090	0.338[b]	0.003[b]	0.182[b]	0.038[b]	0.383[b]	0.014[b]
Agricultural	0.302[b]	0.01[b]	−0.032	0.583	0.081	0.190	0.028	0.422	0.032	0.346	−0.066	0.700
Well-watered	0.012	0.437	−0.010	0.522	−0.034	0.623	0.053	0.306	0.194	0.064	0.181	0.053
Water-limited	0.300[b]	0.048[b]	−0.142	0.762	0.214	0.077	0.207	0.094	−0.092	0.646	0.464[b]	0.008[b]

[a]R represents the correlation coefficient, and *P* indicates the *P* value calculated by the Mantel test.
[b]Significant correlation ($P < 0.05$).

Firmicutes, as well as some subtaxa, were more abundant in the water-limited communities, whereas the phyla Verrucomicrobiota and Gemmatimonadota, as well as some subtaxa, were more abundant in well-watered communities, overall (Fig. 3C; Table S1). When the microbiome group was considered, distinct taxa were differentially abundant between the well-watered and water-limited treatments for each microbiome source (Fig. 3B; Table S1).

Microbiome associations with plant traits. At the community scale, Mantel tests showed that compositional distances between pairs of samples significantly correlated with sample-pairwise differences in some plant metrics. Composition explained 30.0% of the variability ($P = 0.048$) in WUEi for plants grown under water-limitation (across both microbiome treatments) and 30.2% of the variability in WUEi ($P = 0.01$) for plants grown in the presence of the agricultural microbiome (across both watering treatments) (Table 2; Fig. S1). Community composition explained 33.8, 18.2, and 38.3% of the variability in height, stem diameter, and root mass, respectively, for the forest-derived community (Table 2; Fig. S1) ($P < 0.05$). Composition also explained 46.4% of the variability ($P = 0.008$) in the root mass of plants subjected to water limitation (Table 2; Fig. S1). No statistically significant associations were observed between composition and LMA or LWC for either the forest-derived or the agriculture-derived microbial communities.

Pearson's correlations were used to investigate the relationships between plant traits and the abundances of specific taxa. Seven taxa in the agricultural microbiome showed positive correlations with plant WUEi (Fig. 4; Table S2). In contrast, in the forest microbiome, the bacterial family LWQ8 (phylum Patescibacteria, class Saccharimonadia) indicated a strong negative correlation with WUEi. ASV57 (*Devosia* sp., but not the greater *Devosia* genus) and the genus *Duganella* were positively correlated with root mass, and ASV24 (family Rhizobiaceae) were negatively correlated with root mass in the forest-derived community only (Fig. 4; Table S2). Additionally, the abundance of family Oxalobacteraceae showed a negative relationship to LMA in the agricultural community, whereas *Brevindimonas* (family Caulobacteraceae) indicated a positive correlation with LWC in the forest community (Fig. 4; Table S2). Notably, all of the correlations identified were specific to the water-limited treatments, except for *Duganella* and root mass, which were statistically significant only under well-watered conditions.

DISCUSSION

Growing maize inoculated with microbial communities sourced from a forest and agricultural soil under two levels of water availability revealed that microbiome and watering treatments both modified above-ground and below-ground growth. Regardless of water availability, plants grown in the presence of the forest community had larger root systems, thicker stems, and were taller than those grown with the agricultural community. Expectedly, water-limitation reduced growth overall, but water-limited plants had larger root systems when normalized to plant height. This is consistent with previous studies that have shown that root growth and hydrotropism increase under mild to moderate water stress in attempt to increase access to water (15); however, root responses to water-limitation can vary by species and water stress severity (44).

Surprisingly, microbiome or watering treatment effects were not observed for the drought-relevant functional traits, namely, WUEi, LMA, and LWC. Plants growing under water limitation typically have greater LMA and WUEi values and lower LWC values

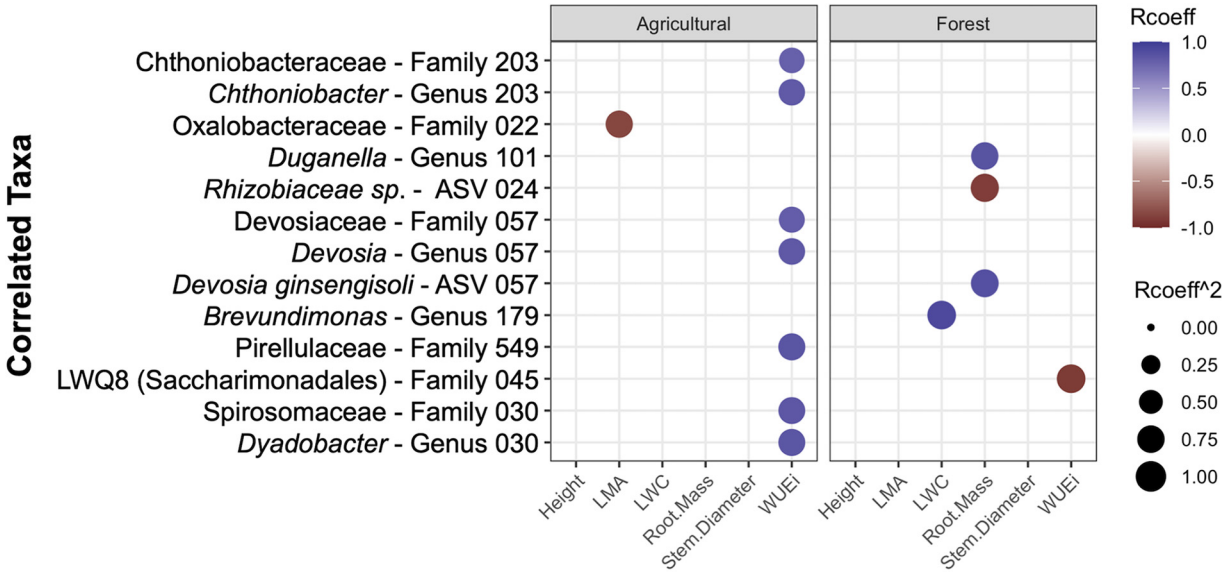

FIG 4 Significant Pearson's correlations between plant traits and individual taxa abundances at the ASV, genus, or family level. Only significant ($P < 0.05$) correlations are displayed. The points are sized and colored according to the magnitude and direction of Pearson's *r*. Taxonomic names are provided at the finest resolution available.

(13, 45–47), and they often lower primary productivity. Thus, our finding was unexpected. However, the water-limitation treatment (45% VWC) was chosen to avoid severe disruptions to plant growth. Thus, the treatment may have been too mild to induce the expected responses. Although sampling occurred during the late vegetative growth stage, and although no signs of reproductive development were observed, plant functional activity may have already been declining as the plants approached reproductive maturity, and this may provide an additional explanation for the absence of microbiome effects on WUEi, LMA, and LWC. Alternatively, the lack of response in LMA, LWC, and WUEi during water limitation could be a function of the reduced water demand that is associated with decreased growth and ample nutrient availability, which allows these traits to remain constant across treatments (11). The microbiome-driven modulation of plant growth through altered auxin production, namely, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, is one mechanism that has been shown to optimize plant growth and water demand by the plant, (41, 48, 49), thereby facilitating homeostasis in leaf functional traits under mild to moderate stress (32, 38, 50). However, future work is needed to test specific mechanisms impacting plant performance during droughts. Nevertheless, compositional variability between the forest and agricultural microbiomes suggests that growth is dependent on the soil microbial community composition.

Statistical interactions among the microbiome and the watering treatment were not observed for any of the plant traits that were examined. This result suggests that water limitation affected plant growth similarly in each microbiome, that growth improvements driven by the forest-derived microbiome were not limited to a particular watering regime, and that water-limitation impacts on plants were not compounded by the microbiome. The historical exposure of microbial communities to drought has been shown to improve plant performance and mitigate microbial composition shifts under drought, compared to communities with no history of drought (25, 30–33). In this experiment, both microbiomes originated from semiarid environments with similar mean annual precipitation (51, 52), and they may have already been primed for water limitation, which would have potentially mitigated the physiological drought responses in the plants. Although the mean annual precipitation at each microbiome source is similar, the agricultural field soil was minimally irrigated and was previously cultivated with corn, whereas the forest soil only received natural precipitation and was in close proximity to native plants that were adapted to the semiarid

environment. The lack of irrigation and the potential for native plants to more effectively recruit beneficial microbes (50) may have further primed the forest soil microbiome to respond to drought more effectively and serve as possible explanations for the observed differences in plant structure. However, soil microbial communities from substantially wetter or drier climates were not tested in this experiment; thus, we cannot confirm whether plant growth and functional traits would respond substantially differently to water limitation when plants are grown in the presence of soil communities originating from wet climates (25, 30–33).

Given the microbiome treatment effects on plant growth, differentially abundant taxa between microbiome treatments (Fig. 3A; Table S1) may provide some explanation for the observed differences in plant growth. For example, the forest-microbiome could have higher abundances of some growth-promoting taxa or lower abundances of inhibitory taxa than the agriculture microbiome that directly contribute to the differences in plant growth. Additional taxa were differentially abundant between soil communities within specific watering regimes (Fig. 3B; Table S1), indicating that water availability affected community compositional differences between the forest and agricultural microbiomes and that the taxa that are potentially driving the improved growth in the forest microbiome may be different, depending on water availability. Two mechanisms may explain how differentially abundant taxa alter plant growth. The enrichment of beneficial taxa (or the depletion of inhibitory taxa) directly facilitates an advantage (or disadvantage) to the growing plant. Alternatively, the differential abundance of certain taxa modifies the community structure, function, and plant-microbe interactions (53) as well as indirectly affects plant performance. Indeed, many studies investigating the performance of plants inoculated with specific taxa demonstrate the advantages conferred by particular organisms (39, 41, 50, 54–56), but their effects may vary in different community and environmental contexts (53). Thus, the importance of the community component in plant-microbe interactions should not be overlooked. However, taxa that are differentially abundant as a result of the environmental differences between each microbiome source may also be inert in their specific relationships to plant growth. Additional data are necessary to confirm the direct impacts that these differentially abundant taxa have on plant growth. Nevertheless, taxa differential abundance is one factor that drives compositional distinctions between the forest and agricultural microbiomes, and it may contribute to microbiome growth effects in the plants.

The differential abundance of taxa between watering treatments was consistent with generalized shifts in composition in response to water limitation in both microbiomes, specifically, a broad enrichment of the phyla Actinobacteriota and Firmicutes under water-limited conditions as well as a greater abundance of the phyla Gemmatimonadota and Verrucomicrobiota under well-watered conditions (1, 28, 57). Actinobacteriota and Firmicutes are typically found at higher relative abundance and activity during droughts across disparate ecosystems and plant hosts (24, 28, 29, 57). However, when considering each microbiome source separately, different bacterial taxa varied in abundance between each watering condition (Fig. 3B; Table S1), In the forest microbiome, only Gallionellaceae was enriched under drought, whereas the complete phyla Firmicutes and Actinobacteria, as well as the subtaxa Streptomycetaceae, Micrococcaceae, Thermoleophilia, and Bacilli, were more abundant in the agricultural community. The unique response of each community to water-limitation is consistent with the lack of convergence in overall composition (Fig. 2). In other words, both communities showed a similar magnitude of generalized response to drought at coarse community resolution, but they did not converge toward a common composition. The legacy of compositional and ecological differences between the pine forest and the agricultural field from where each microbiome was obtained likely drove the unique responses due to different adaptations to water limitation (58). Thus, changes in the abundance and activity of certain taxa in response to drought will modify which taxa actively interact with their plant hosts, thereby potentially altering plant performance. In particular, the higher abundance of Streptomycetaceae, Micrococcaceae, Thermoleophilia, and Bacilli in the agricultural community under drought is highly

reflective of the compositional changes that were observed in the rhizosphere communities of diverse plants experiencing water-limitation (24, 29, 31). Isolate-based studies have shown that many taxa belonging to these groups are capable of improving plant performance under water limitation or salt stress, including in maize (24, 38, 59–63). The enrichment of these families in the water-limited agricultural community suggests that they may function beneficially for the plant under drought, and this may partially explain why the mean WUEi was slightly higher under water limitation than under well-watered conditions for the agricultural community, although this difference was not statistically significant. Alternatively, interacting with certain bacteria to decrease growth may also be a strategy to decrease water demand and increase fitness under dry conditions. Preconditioning microbial communities to drought has been shown to improve plant performance during experimental drought (29–33). Therefore, historical precipitation and management differences between the forest and agricultural microbiomes used in this experiment could be reasons why each community showed different fine-scale compositional responses to water-limitation; and they are likely important factors that influence plant performance.

Despite the absence of significant microbiome or watering treatment effects on the maize leaf functional traits (WUEi, LMA, and LWC), microbial features (taxa and community level characteristics) correlated with the considerable variability that was observed for each plant trait within individual treatments. In some cases, plant trait variability within treatments was nearly twofold, and it was sometimes as high as the between treatment variability (Fig. 1). Examining each treatment separately allowed for a robust assessment of the plant-microbe functional relationships within individual species pools while avoiding the challenges associated with aggregating diverse communities from disparate soil sources and environmental conditions. We identified a number of microbial features that showed statistically significant correlations with plant traits. Interestingly, microbe-plant relationships were unique to specific microbiome and watering regimes, and they were found at both the whole community and taxon scales. Positive correlations between the community composition and plant traits suggest that plant-microbe interactions could contribute to variability in plant physiology; however, such interactions depended on the biotic and abiotic contexts of the soil. At the taxon scale, Pearson's correlations between plant measurements and the abundances of prevalent taxa at different resolutions (bacterial ASVs, genera, or families) identified a variety of organisms that were associated with the measured plant traits. Similar to the compositional level, all of the significant correlations that were identified were specific to unique microbiomes and watering regimes.

The soil bacterial community composition and the abundances of particular taxa may provide a partial explanation of the observed variability in plant traits within each treatment. In this experiment, biogeochemical differences between the original source soils were largely removed due to the inoculation approach used, leaving the community structure and the watering regime as the primary factors to account for the condition-specific relationships that were identified. This implies that microbial functions and interactions with a plant can change when the community structure and environment vary (53). For example, the family Devosiaceae and genus *Devosia* were positively correlated with WUEi in the agricultural microbiome, but *Devosia ginsengisoli/humi/insulae* (ASV57) was positively correlated with root biomass in the context of the forest community. Although differences between strains or across taxonomic levels may explain distinctions in specific microbe-plant interactions, closely related taxa are more likely to have common functions (64, 65). Conversely, different taxa were correlated with the same plant traits between microbiomes. These taxa may be functionally similar in the context of the plant trait, or there may be multiple mechanisms through which these bacteria are able to influence the plant trait. Functional redundancy across phylogenetically dissimilar taxa has been characterized previously (66, 67). Similarly, a variety of microbial mechanisms have been shown to influence plant root growth and other plant traits (40, 48, 68, 69). While we cannot confirm which functional driver best explains our findings without more specific microbial functional data, our results demonstrate the importance of community composition in determining plant performance outcomes.

TABLE 3 Summary of taxa potentially linked to plant function and performance[a]

Organism	ID method	Relationship with plant	Treatment (soil/water)
ASV24 (Rhizobiaceae)	Correlation	(−) Root mass	Forest / Limited
ASV57 *Devosia ginsengisoli/humi/insulae* (Devosiaceae)	Correlation	(+) Root mass	Forest / Limited
Duganella sp. (Oxalobacteraceae)	Correlation	(+) Root mass	Forest /Well-watered
Brevundimonas sp. (Caulobacteraceae)	Correlation	(+) LWC	Forest / Limited
Dyadobacter sp. (Spirosomaceae)	Correlation	(+) WUEi	Agricultural / Limited
Chthoniobacter sp. (Chthoniobacteraceae)	Correlation	(+) WUEi	Agricultural / Limited
Saccharimonadales LWQ8	Correlation	(−) WUEi	Forest / Limited
Oxalobacteraceae	Correlation	(−) LMA	Agricultural / Limited
Spirosomaceae	Correlation	(+) WUEi	Agricultural / Limited
Devosiaceae	Correlation	(+) WUEi	Agricultural / Limited
Pirellulaceae	Correlation	(+) WUEi	Agricultural / Limited
Rickettsiaceae	Abundance	(+) Forest	Forest / Either
Abditibacteriaceae	Abundance	(+) Forest	Forest / Either
Myxococcaceae	Abundance	(+) Forest	Forest / Either
Fibrobacteraceae	Abundance	(+) Forest	Forest / Either
Cytophagaceae	Abundance	(+) Forest	Forest / Either
Sphingobacteriales KD3-93	Abundance	(+) Forest	Forest / Either
Mycobacteriaceae	Abundance	(+) Forest	Forest / Either
Ilumatobacteraceae	Abundance	(+) Forest	Forest / Either
Solirubrobacterales 67-14	Abundance	(+) Forest	Forest / Either
Chthoniobacteraceae	Abundance	(+) Forest	Forest / Either
Methylacidiphilaceae	Abundance	(+) Forest	Forest / Either
Isosphaeraceae	Abundance	(+) Forest	Forest / Either
Tepidsphaerales WD2101 soil group	Abundance	(+) Forest	Forest / Either
Saccharimonadales LWQ8	Abundance	(+) Forest	Forest / Either
Acidobacteriaceae (Subgroup 1)	Abundance	(+) Forest	Forest / Either
Gallionellaceae	Abundance	(+) Forest	Forest / Either
Moraxellaceae	Abundance	(+) Forest	Forest / Either
Cellvibrionaceae	Abundance	(+) Forest	Forest / Either
Bryobacteraceae	Abundance	(+) Forest	Forest / Well-watered
Myxococcales 27F-1492R	Abundance	(+) Forest	Forest / Limited
Polyangiales Blrii41	Abundance	(+) Forest	Forest / Limited
Unknown Rhizobiales	Abundance	(+) Agricultural	Agricultural / Either
Ferrovibrionaceae	Abundance	(+) Agricultural	Agricultural / Either
Nitrospiraceae	Abundance	(+) Agricultural	Agricultural / Either
Microscillaceae	Abundance	(+) Agricultural	Agricultural / Either
Anaerolineae A4b	Abundance	(+) Agricultural	Agricultural / Either
Rubinisphaeraceae	Abundance	(+) Agricultural	Agricultural / Either
Polyangiales Blrii41	Abundance	(+) Agricultural	Agricultural / Limited

[a]Taxa were identified through the noted analyses at the ASV, genus, or family level. Inferred roles were assigned based on the particular analysis performed.

A variety of bacterial taxa that potentially contribute to increased growth and physiological performance in maize were identified and are summarized in Table 3. Many studies examining drought amelioration via microorganisms have been restricted to inoculations with single taxa or small consortia, often of cultured isolates (38, 50, 55, 60, 70–72). These studies have identified important plant-microbe interactions that have the potential to improve drought tolerance in plants, but the impact is limited, given either inoculation into sterile environments or a failure to compare efficacy in different microbial community contexts. In many cases, the applications of a single strain of cultured bacteria into the field fail to replicate the positive results identified under laboratory or greenhouse conditions (73, 74). Such outcomes are consistent with our findings that show that plant-microbe interactions that are relevant to plant performance are strongly dependent on the greater community composition. Thus, specific community structures and environmental conditions may be required for an inoculum to successfully modify plant growth or function. Inoculations of taxa into established soil microbial communities often do not persist in the long term (75), which further limits their potential utility. While working with complex microbial assemblages presents its own challenges, whole microbial communities can potentially be used to modify plant functions in the face of stress. Understanding that specific plant-microbe interactions are community dependent

provides a step forward to manipulating whole communities, such as through directed evolution (76–78), to optimize plant performance for specific conditions, species, or genotypes, and perhaps even for individual fields.

Conclusion. Using two complex soil microbial communities from distinct semiarid environments, we found that bacterial relationships with plant growth and leaf traits were dependent upon environmental context, specifically, water availability and community composition. In particular, these factors both influenced maize growth, although water limitation had a larger impact. Neither factor induced differences in leaf functional traits across treatments. Nevertheless, we identified microbial features that correlated with variability in structural and leaf functional traits within individual treatments. Our approach comparing the effects of complex bacterial communities and water availability on plant performance identified new plant-bacteria interactions. These findings provide a foundation for future studies to continue exploring the potential for plant-microbe associations from the single taxon to complex community scales to improve plant performance during drought.

MATERIALS AND METHODS

Experimental setup. A balanced 2 × 2 factorial designed experiment was used to investigate the individual and combined effects of soil microbial community composition and water limitation on the performance of maize in a controlled greenhouse setting. Maize inbred line B73 plants were grown from seed in 10 L pots containing 6 L artificial fritted clay soil (Profile Ceramic Greens Grade, Buffalo Grove, IL, USA) in a temperature-controlled greenhouse in Los Alamos, NM, USA, between February and May of 2020. The average (±standard deviation) greenhouse temperature during the experiment was 22.0 ± 2.3°C, the maximum temperature was 31.85°C, and the minimum temperature was 15.66°C. In addition to natural solar irradiance, a supplemental artificial light source was provided to maintain a 14-hour daylight period. This light source provided artificial light at a minimum irradiance of 800 μmol photons m^{-2} s^{-1} while the sun was down.

56 maize plants were grown in pots of fritted clay soil that were inoculated with one of two natural soil microbial communities that originated in a ponderosa pine forest near Los Alamos, NM, USA (35.888° N, 106.327° W), or a minimally irrigated agricultural field in Akron, CO, USA (40.154° N, 103.141° W). These two soils from a nonmanaged forest ecosystem and heavily altered agricultural field were chosen to obtain complex communities that were distinct in composition, which we expected would differentially impact plant performance. The microbial communities used in this experiment originated in environments with similar mean annual precipitation: a ponderosa pine forest (Los Alamos, NM, USA), receiving 47.73 cm y^{-1} (Western Regional Climate Center); and a minimally irrigated agricultural field (Akron, CO, USA), receiving 41.91 cm y^{-1} (51). Plants were watered with unsterilized, filtered water amended with fertilizer, namely, FloraGro (200 μL L^{-1}), FloraMicro (200 μL L^{-1}), and FloraBloom (100 μL L^{-1}) (General Hydroponics, Santa Rosa, CA, USA), under two watering regimes. Plants under the well-watered treatment received enough fertilizer-amended water to maintain soil hydration near the water holding capacity at 65% volumetric water content (VWC) three times a week, whereas water-limited soils were maintained at 45% VWC (approximately 5% greater than the wilting point) to reduce water availability without creating soil-suction conditions that were too severe for maize growth. To determine the amount of water that each pot needed, the VWC of each pot was measured using Meter TEROS 10 soil moisture sensors (Meter Environment, Pullman, WA, USA) that were installed 10 cm below the soil surface in each pot, prior to watering. The water deficit was calculated using calibration curves that were created, prior to the experiment, to be specific to our setup. This experimental design resulted in four unique microbiome/watering treatments with 14 replicate plants each. Although this approach ultimately delivered different amounts of fertilizer to the well-watered and water-limited treatments, nutrients were supplied in excess to prevent growth limitation. Porewater nutrient concentrations were measured at the end of the experiment and were expectedly high (Table S2). Porewater was collected from Rhizon samplers (Rhizosphere Research Products, Wageningen, Netherlands) that were installed in the pots. Ammonium was measured on a UV-VIS using the indophenol blue method. Nitrate and phosphate were measured via ion chromatography (IC) on a Thermo Fisher Dionex ICS-2100. Potassium was measured via inductively coupled plasma-optical emission spectrometry (ICP-OES) on a Perkin Elmer Optima 2100 DV.

Inoculation, germination, and watering procedure. Soil inoculum from the forest and agricultural sources were each created by mixing 800.0 g soil with 16.0 L sterile water in sterile carboys. Biomass approximations with colony enumeration on R2A plates and DNA extraction yield measurements that were performed on archived inoculum soil confirmed that the inoculum soils were similar in biomass, with less than a threefold difference being observed. Minor differences in the initial biomass were not expected to significantly impact plant growth, as the diluted inocula would grow over the course of the experiment and would likely achieve similar maximum biomasses in the pots. Particulates in the soil slurries were allowed to settle for 1 h before the supernatant was passed through a sterile 0.5 mm screen to remove large particles. Maize seeds were soaked in a 10% bleach solution for 10 min to sanitize the seed surface, and they were then rinsed three times via further soaking in sterile water for 10 min each. After rinsing, the seeds were soaked in 0.5 L of the designated inoculum slurry. 2 seeds were planted, 2 to 3 cm deep and 4 to 5 cm apart, in each pot. 500 mL of inoculum slurry was applied, and the pots were covered with plastic film to prevent drying during germination. After germination occurred, the plastic

film, second seedlings, and ungerminated seeds were removed. For the first 14 days after planting, all plants received sufficient water to maintain 65% VWC. After this initial growth period, the well-watered or water-limited watering regime was initiated and maintained for the remainder of the experiment.

Plant measurements and analysis. The WUEi, LMA, and LWC measurements were conducted when most of the plants within a treatment had reached the state of the tenth leaf emerging (approximately 7 weeks post germination for the well-watered plants and 9 weeks post germination for the water-limited plants). However, many of the water-limited plants had begun to develop their eleventh or twelfth leaf, prior to the collection of these measurements. The plant height and stem diameter were measured for all plants on the same day, at approximately 7 weeks post germination. The plant height was measured from the soil surface to the tip of the tallest leaf by using a ruler. The stem diameter at the soil surface was measured in two orthogonal directions using calipers. These measurements were averaged to give the reported stem diameter for each plant. The intrinsic water use efficiency (WUEi) of each plant was calculated as the ratio of the maximum photosynthesis rate to the stomatal conductance associated with this photosynthesis rate. Photosynthesis and stomatal conductance were measured simultaneously from the newest fully extended leaf (leaf number 8 or 9) using portable photosynthesis systems (LI6400; Licor Inc. Lincoln, NE, USA). Two systems were calibrated and tested prior to use and were used simultaneously. The measurement settings were 2,000 μmol $m^{-2}s^{-1}$ of photosynthetically active radiation (PAR) (above saturation), ambient atmospheric CO_2 concentration of 400 ppm, flow rate at 400 μmol s^{-1}, block temperature controlled at 20°C, and relative humidity at 30% to ensure maximal stomatal opening and photosynthesis rates under ambient conditions. Following these measurements, the tip of the same leaf was collected to measure the leaf mass per area (LMA; g m^{-2}) and the percent leaf water content (LWC; %). Leaf tips were weighed for fresh mass and scanned for leaf area (Canon Image Runner C5560i), and they were then oven dried at 60°C for 48 h before the collection of the dry mass. LMA was calculated as the area of the fresh leaf tip divided by the dry mass of the leaf tip. LWC was calculated as the dry mass divided by the fresh mass, multiplied by 100.

After measuring the plant gas exchange, above-ground growth, and leaf traits, the plants in all treatments were subjected to water starvation (full withdrawal of water) either for an additional 2 weeks or until stomatal closure was observed using portable photosynthesis systems. This period of water starvation required the harvest of the root tissue of all plants to be delayed, but it was included to compare methods of stomatal closure point measurement as part of another study that was using the same experiment. During this time, no additional growth was observed, and, after full stomatal closure, the roots were harvested, dried in an oven at 60°C for 48 h, and weighed for dry root biomass. Roots were harvested by cutting the stem approximately 2 cm above the soil and placing the root bulb and soil into a tray. The main root bulb was lifted out of the soil, shaken free of soil, and washed with water. Additionally, fine roots that were detached from the main root system were removed from the soil for approximately 2 min (to maintain consistency in the additional root mass that was collected) and were included in the total root biomass measurements.

Microbiome sample collection and analysis. Soil microbial communities were sampled immediately before taking above-ground plant samples and measurements. A 5 cm soil core was taken, using a sterile 15 mL conical tube, approximately 4 cm from the plant stem, and it was stored at −80°C. DNA was extracted from approximately 0.8 g of the homogenized soil core using a Qiagen DNeasy PowerSoil Kit with the following modifications to improve its extraction efficiency from clay soil. Lysis buffer preliquoted to the bead tubes was removed and replaced with 750 μL of sterile 1.0 M sodium phosphate ($NaH_2PO_4 \bullet 1H_2O$) in 15% molecular-grade ethanol and 60 μL solution C1 to block DNA from binding to the fritted clay. Soils were lysed using a vortex adapter at maximum speed for 10 min, and they were then incubated at 80°C for 40 min (79). The standard PowerSoil protocol was used for the remainder of the extraction, except 30 μL of buffer C6 was used for the final elution.

Soil bacterial and archaeal communities were profiled using 16S rRNA amplicon sequencing. Amplicon libraries were prepared by amplifying the V4 region of the 16S rRNA gene, using 515F-806R primers, as described previously (32). Amplicon libraries were sequenced using an Illumina MiSeq to generate 300 bp paired end reads at the Los Alamos National Laboratory sequencing facility.

Raw sequencing reads were preprocessed and demultiplexed using USEARCH (80). Dada2 was used with the default settings, unless otherwise noted, to perform quality filtering, primer removal, and denoising of the sequencing reads (81). Specifically, quality filtering was performed with filterAndTrim, using the following settings to remove primers and low quality sequences: truncLen = c(240, 200), truncQ = 2, trimLeft = c(25, 26), and maxEE = c(2, 4). Paired reads with a minimum overlap of 100 bp were merged, and only sequences with 250 to 260 bp were kept for downstream analyses. Bacterial and archaeal taxonomy were assigned to the species level, using the Silva 16S rRNA taxonomic database v.138.1 at the 80% confidence level (82, 83). Sequences classified as mitochondrion or chloroplast were removed prior to analysis.

Subsequent microbiome analyses were performed in R (R Core Team, 2020) using the "phyloseq" v. 1.32.0 (84) and "vegan" v.2.5-6 (85) packages. Samples with greater than 10,000 reads were kept for analysis and were then rarefied to an even depth of 10,000 reads per sample. The rarefied abundance data were used to calculate alpha-diversity metrics (observed taxa, Shannon diversity, evenness), beta-diversity (Bray-Curtis dissimilarity), Mantel tests, and Pearson's correlations between taxa abundances and plant measurements. Metacoder (86) was used with the rarefied count data to generate heat trees to compare taxa abundances (Fig. 4) across treatments.

The plant measurements and the alpha diversity measurements were quantitatively compared using a two-way interaction ANOVA with Tukey's *post hoc* tests. The ANOVA output was used to calculate the variance components for the plant measurements (87). The beta diversity (Bray-Curtis dissimilarity) differences were compared via a two-way interaction PERMANOVA test, using the adonis function in vegan

(85). Pairwise differences in beta diversity between each unique combination of microbiome and watering treatment were compared using the pairwiseAdonis package (88), and *P*-values were corrected for multiple tests with the Bonferroni procedure. Pearson's correlations between taxon abundance and plant metrics were calculated for taxa that were present in greater than 80% of the samples within a given treatment, using the abundances at the ASV, genus, and family taxonomic levels. Multiple test corrections were performed by calculating the false discovery rate. Community composition-plant metric correlations were assessed via Mantel tests. Euclidian and Bray-Curtis sample-pairwise distance matrices were constructed for the plant and compositional data, respectively, using the "dist" function (vegan v.2.5-7). Then, the "mantel" function (ecodist v.2.0.7) was used with 999 permutations to compute the correlations between the plant trait and compositional dissimilarity for the samples within each treatment group. The differential abundances of taxa were statistically tested using Wilcoxon rank-sum tests and the false-discovery rate multiple test correction via the "compare_groups" command that is described in the Metacoder documentation (86). For all analyses, significance thresholds were defined at the $\alpha = 0.05$ level.

Data availability. All plant data and processed microbiome (ASV and taxonomy table) data are provided as supplemental files. The unprocessed microbiome sequence data are available at NCBI's Sequence Read Archive under the accession number PRJNA780613.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, CSV file, 0 MB.

SUPPLEMENTAL FILE 2, CSV file, 1.5 MB.

SUPPLEMENTAL FILE 3, PDF file, 2.7 MB.

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