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RESEARCH ARTICLE

Drought conditioning of rhizosphere microbiome influences maize water use traits

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Abstract

Background and Aims Beneficial plant–microbe interactions can improve plant performance under drought; however, we know less about how drought-induced shifts in microbial communities affect plant traits.

Methods We cultivated *Zea mays* in fritted clay with soil microbiomes originating from contrasting environments (agriculture or forest) under two irrigation treatments (well-watered or water limited). Using this design, we investigated whether water conditioning was carried forward through the microbiome to affect a subsequent plant cohort that was subjected to either a well-watered or water limited treatment.

Results Regardless of the microbiome-origin, plants inoculated with a microbiome from a water limited legacy had traits that allowed them to avoid stress but conserve water. They produced longer roots to explore soil, generated greater soil dissolved organic carbon, potentially stimulating the microbiome, and slower soil water content loss during drought. A well-watered legacy resulted in plants that delayed permanent stomatal closure and higher photosynthetic nitrogen use efficiency. In plants with a forest-originated microbiome, a well-watered legacy and water treatment also resulted in higher rates of photosynthesis and stomatal conductance.

Conclusion These results demonstrate that soil microbiomes can be developed to influence plant

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drought performance, impacting crop resilience, using short-term microbial conditioning.

Keywords Plant–microbe interaction · Drought · *Zea mays* · Root traits · Plant function

Introduction

The frequency and duration of drought events is increasing across agricultural land area (Masson-Delmotte et al. 2021), driving growing concerns about global food supplies and bioenergy security. The effects of climate change are compounding and increasing the negative impacts of drought on worldwide crop yield (Lesk et al. 2016). These declines in crop yield not only threaten food security but cascade into severe economic costs (Boyer et al. 2013). Drought often leads to lower crop productivity by decreasing stomatal conductance, eventually limiting CO_2 uptake and photosynthesis (Farquhar and Sharkey 1982). Lower CO_2 uptake, or productivity, leads to declines in plant growth (Xu et al. 2009; Hussain et al. 2019), resulting in lowered crop yield (Ciais et al. 2005). Maintaining plant productivity and growth under limited water availability is key to maintaining crop yields as world population and food demand continues to rise (Ciais et al. 2005; Passioura 2007; Boyer et al. 2013).

There is growing interest in utilizing microbes to improve plant and ecosystem functions for specific functions, such as improved productivity (Backer et al. 2018; Cavicchioli et al. 2019; Compant et al. 2019; de Vries et al. 2020). Soil and phyto- microbiomes create close associations with their host plant and can play large roles in ecosystem functioning and plant productivity (van der Heijden et al. 2008), providing a promising solution to help alleviate plant stress. Microbial associations can increase a plant's ability to deal with drought by, for example, directly altering root growth (Fan et al. 2011; Marasco et al. 2013; Henkes et al. 2018) and water and nutrient scavenging through the extension of the root system (Orfanoudakis et al. 2010). These responses improve the plant's ability to maintain higher rates of stomatal conductance and continued CO_2 uptake during moderate drought (Ulrich et al. 2019). Microbes can also directly influence the rhizosphere soil water available to the plant by excreting exopolysaccharides that create a gel-like substance that increases soil adhesion to roots and maintains soil structure (Sandhya et al. 2009). Beyond drought tolerance, many studies have shown improved plant structure and form through microbial inoculations (Timmusk et al. 2014; Dhawi et al. 2015). Trees and crops inoculated with beneficial microbes can have higher growth (Timmusk et al. 2014; Rolli et al. 2015) and produce higher yields (Chen et al. 2021). Most of these applications focus on individual plant growth promoting bacteria or mycorrhizal fungi; however, interactions between microbes can alter their effects on plants (Lau and Lennon 2011), and developing complex microbial communities may provide a more robust method of improving plant traits (Busby et al. 2017; Compant et al. 2019). Inoculations of microbial communities can provide advantages over a single microbial strain as they may have a competitive advantage and longer persistence in natural systems.

Plants can alter the soil chemistry and microbial communities creating plant-soil feedbacks, where the feedbacks have legacies that affect the growth, function, and interactions of future plant communities (Bever 2003; Van der Putten et al. 2013). Legacies of plant community-induced shifts in microbiomes can influence future generations of microbial-plant interactions (Heinen et al. 2020), and abiotic factors, such as extreme weather, warming, and nitrogen enrichment can shift the microbial community in a way that alters future plant-plant interactions (Meisner et al. 2013). These microbial legacies can be beneficial for plant drought avoidance and tolerance, and understanding these interactions could help identify robust microbiomes that can support plant growth under limited water availability. Some plant soil-feedback studies have investigated drought effects on plant growth and competition, where drought legacies on microbiomes can give some plant species a competitive advantage over others (Kaisermann et al. 2017). Further, bacteria isolated from a droughted environment can enhance plant drought tolerance traits (Marulanda et al. 2009), and natural microbial consortia sourced from water stressed conditions can allow maize to avoid drought stress by increasing root length compared to microbiomes from non-stressed plants (Zhang et al. 2022). Additionally, one study found that a microbiome intentionally developed under drought can improve the fitness of future plant generations experiencing drought conditions (Lau and Lennon 2012), suggesting a beneficial legacy. While

these studies provide evidence that drought-induced rapid shifts in microbial communities can influence plant competition and fitness, we are still lacking information on how these stress-induced shifts in microbial communities can affect plant growth and function.

In this study we investigated microbiome legacy effects on *Zea mays* (maize) growth and function using two different source microbiomes, one originated from a forest (M_{Forest}) and one from an agricultural field (M_{Ag}), under well-watered (W_W) and limited water (W_L) conditions. We used a plant-soil feedback approach to condition the microbiomes under an initial microbial selection generation (G_0) of maize growth with these two watering conditions. We then propagated these microbiomes into a second, experimental, generation (G_1) of plants and tested for a legacy effect under the same and altered watering treatments (Fig. 1). This design allowed us to assess how a short-term drought inflicted on microbiomes originating from diverse sources affects plant growth and function. We hypothesized that the watering treatment would have a strong effect on plant growth; however, we also expected a growth-promoting legacy from microbiomes that received a W_L treatment during the microbial conditioning phase. The legacy would manifest so that microbiomes that received a W_L conditioning treatment would promote plant growth and function (photosynthesis and stomatal conductance) compared to microbiomes that received a W_W conditioning treatment. We further hypothesized that the legacy of the W_L conditioning would be stronger in plants inoculated with the microbiome originating from agricultural soil, as this microbiome was adapted to maize under low irrigation conditions.

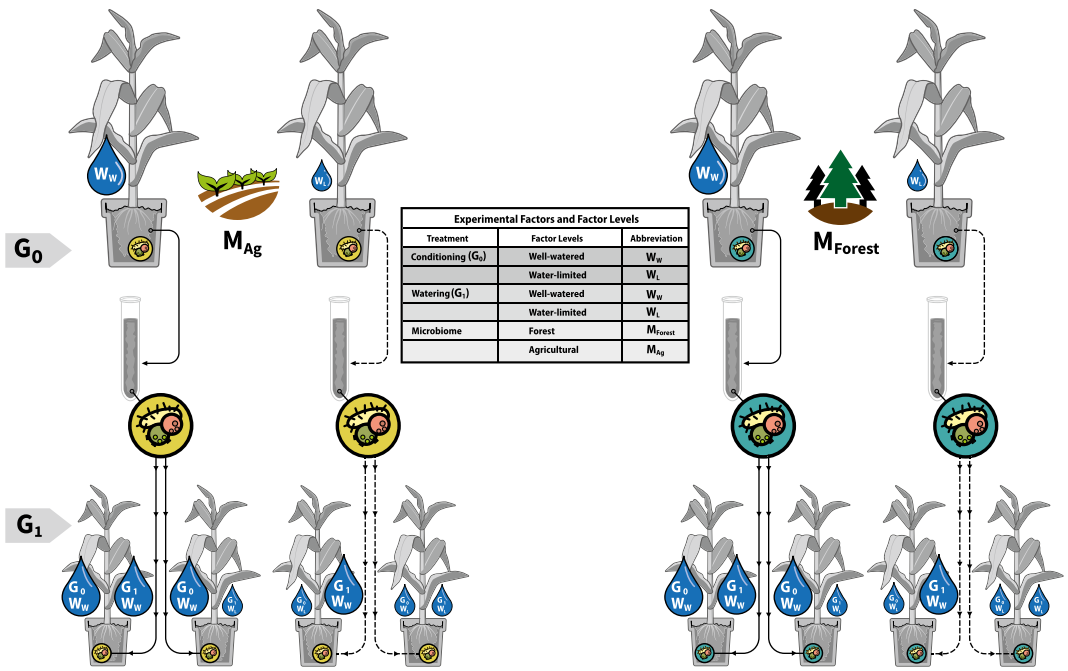

Fig. 1 Representation of experimental design. Generation 0 (G_0) describes the water conditioning phase where the microbiomes were either well-watered (W_w; large water drop) or water limited (W_L; small water drop). The parent microbiomes in the conditioning phase originated from either agricultural (M_{Ag}; yellow) or forest (M_{Forest}; green) soil. Soil was collected from each conditioning phase pot and the microbiome was transferred to a new pot and soil for Generation 1 (G_1). Half of the pots containing W_W-conditioned microbiome were selected at random to receive W_W watering treatment again at G_1, while the remaining pots containing W_W-conditioned microbiome were instead given W_L watering treatment at G_1. Likewise, half of the pots containing W_L-conditioned microbiome received W_W watering treatment and half received W_L watering treatment at G_1. This obtains all eight combinations of microbial conditioning treatment, watering treatment, and microbiome parent source (shown in the bottom row of the figures) specified by the experimental design. Image courtesy of LANL. Don Montoya

Methods

Experimental design

We investigated the effects of microbial conditioning, microbiome parent, and watering treatments on plant growth and function. We were interested in studying two levels of microbial conditioning treatment (W_L and W_W); two levels of watering treatment (W_L and W_W); and two microbiome parent sources (M_{Ag} and M_{Forest}). The experiment was performed as a completely randomized $2 \times 2 \times 2$ full factorial design, consisting of all possible combinations of these levels, giving eight combinations in total. The experiment was carried out in seven replicates per condition for a total of 56 plants.

Experimental set-up

The experiment was conducted in a greenhouse with air temperature averaging 21–22 °C during both day and nighttime hours. Fifty-six *Zea mays* plants (B73 line: USDA National Plant Germplasm System) were grown from seed under 14 h/10 h day/night cycles. To equalize the daylight hours over the season, natural light was supplemented with growth lights (ROI-E720, Grower's Choice, Ontario, CA) set to 50% of maximum natural light intensity (PPFD 2000 µmol m^{-2} s^{-1}) to produce PPFD 1000 µmol m^{-2} s^{-1} both in the morning and evening. All plants were grown in 9.8L pots in 6L 20–30 mesh size artificial, fritted clay (GreensGradeTM, PROFILE(R) Products LLC, Buffalo Grove, IL, USA) to control soil chemistry and sterility prior to inoculation. We did not autoclave the clay due to leaching issues; however, the fritted clay manufacturing process produced an extremely low microbial load. Fritted clay was rinsed multiple times prior to planting to reduce the potential for solute toxicity, especially manganese (Adams et al. 2014).

Following the experimental design, half of the 56 plants were selected at random to be inoculated with microbiome source M_{Ag} and half were inoculated with source M_{Forest}. The agricultural microbiome (M_{Ag}) was collected from a maize agricultural field from Akron, CO (40.1605° N, 103.2144° W) that has historically not been irrigated. The forest microbiome (M_{Forest}) was collected from a mid-elevation mixed conifer forest in Los Alamos, NM (35.8800° N, 106.3031° W). The two source microbiomes started with different community compositions that remained different throughout the water conditioning phase of the experiment (Moore et al., 2023). Plant inoculation consisted of inoculating both the fritted clay and the seed. The fritted clay was inoculated using a 1:20 soil–water slurry. *Z. mays* seeds were soaked in a 10% bleach solution for 10 min, rinsed three times with sterile water, and soaked in the natural soil–water slurry for 20 min. Two seeds were then planted per pot and 500 mL of the water slurry was used to water each pot.

Generation 0 (G_0) was used as the conditioning phase. Half of the plants inoculated with M_{Ag} and half of the plants inoculated with the M_{Forest} were chosen at random to be grown under well-watered (W_W) conditions at G_0; all remaining plants were grown under water limited (W_L) conditions at G_0. Plants were maintained at 64% and 45% volumetric water content, respectively. To allow for sufficient germination, all plants were maintained at 64% volumetric water content for two weeks after planting. Moisture contents were selected based on fritted clay measured saturation and the moisture characteristic curve (Steinberg et al. 2005). The 45% moisture condition was selected because it was high enough to allow plant growth, but low enough to impose a water limited condition. Soil moisture in each pot was monitored using a soil moisture probe (TEROS 10, METER Group, Inc, WA, USA), and each pot was watered to the target volumetric water content three times per week using filtered tap water enhanced with FloraGro, FloraMicro, and FloraBloom (General Hydroponics, Santa Rosa, CA, USA) fertilizers with doses of 200, 200, and 100 µL L^{-1} of water, respectively. When both seeds germinated, the smaller seedling was pruned from the pot. When neither planted seed germinated, both seeds were carefully dug up with a sterilized spatula and removed, and the pot re-planted. Plants were grown until the emergence of the 11^{th} leaf. Because the G_0 watering treatment affected plant growth rate, W_W plants were harvested at 8 weeks and W_L plants at 10 weeks. 10 cm soil cores were then collected to sample the soil and rhizosphere microbiome of each pot and stored to inoculate a new seed. Microbial communities remained distinct after Gen_0 and differences between Gen_0 and Gen_1 show that microbial communities continued to shift throughout the experimental Gen_1 (Fig. S1a).

After the completion of G_0, we used serial propagation of the microbiome (i.e., the microbiome from each pot in G_0 was used to inoculate one pot in G_1) to transfer the conditioned microbiome to a microbial selection generation with a fresh seed from the same seed batch as used in G_0 (G_1). G_1 seeds were inoculated with G_0 microbiome using the same method described above. The G_0 soil cores were collected surrounding the intact plants. Therefore, soil cores included fine roots, which were also incorporated in the inoculum slurry. All seeds were planted in fresh, rinsed fritted clay so that the conditioned microbiome, and not differences in soil chemistry, provided the legacy factor. While, we do acknowledge that our soil slurry likely had nutrient carry-over from G_0, this would be very minimal due to high dilution at planting. Seedlings were allowed to germinate and grow under W_W treatment for two weeks, after which half were given the W_W watering treatment and half were given the W_L watering treatment (Fig. 1), as specified by the experimental design. After emergence of the 11th leaf, plant height, stem diameter, and maximum photosynthesis rates were measured as described below. After measurement, the plants were subjected to a terminal drought phase where irrigation was discontinued. During this phase, stomatal conductance (g_s) and soil volumetric water content (VWC) were measured daily during the hours of 08:00–10:00 using a porometer (SC-1; Decagon Devices Inc., WA, USA). Once the newest fully-expanded leaf reached $g_s = 0$, we proceeded with the post terminal drought measurements described below.

Plant and soil traits pre-terminal drought

We measured plant growth response characteristics non-destructively starting one day before initiating the terminal drought. Plant height (cm) was measured from the base of the stem to the tip of the longest leaf using a ruler. Stem diameter (mm) was measured in two orthogonal directions approximately 2 cm from the base of the plant using calipers. Stem diameter was calculated as the averaged of two orthogonal measurements per plant.

We evaluated plant function from light saturated photosynthesis (A_{sat}) and g_s measured on the second fully expanded leaf using an open-system infrared gas exchange system (LI6400XT, LI-COR Inc, Lincoln, NE, USA). Light was controlled at saturating light (measured from light response curves; data not shown) set to 2,000 µmol m^{-2} s^{-1}. Reference CO_2 concentrations and flow rate were controlled at 400 ppm and 400 µmol m^{-2} s^{-1}, respectively. Relative humidity inside the chamber was controlled at 20%, similar to the ambient humidity inside the greenhouse. Intrinsic water use efficiency (WUE_i) was calculated as A_{sat}/g_s.

Leaf traits, such as leaf carbon and nitrogen content, are often used as proxies to describe plant function and were measured on the same leaf as plant gas exchange, from 2–3 cm excised from the apex of the leaf. Leaf area (cm^2) was measured on the apex cutting of the leaf using a scanner (Canon Image Runner C5560i) and analyzed using ImageJ v1.53 image analysis software. The leaf was placed in a 60 °C drying oven for 48 h for dry mass (g) and then ground using a ball mill (12621–1664, VWR International LLC, PA, USA) to analyze for % nitrogen (N) and % carbon using an elemental analyzer (Eurovector, Pavia, Italy). Nitrogen per unit area (N_{area}; g m^{-2}) was calculated as nitrogen per unit mass (N_{mass}; mg g^{-1}) multiplied by leaf mass/ leaf area. Photosynthetic nitrogen use efficiency (PNUE) was calculated as A_{sat} divided by N_{area}.

Plants can directly encourage microbial growth during drought by altering their root exudation chemistry (Karst et al. 2017; Preece et al. 2018; de Vries et al. 2019); therefore, we also investigated soil pore water chemistry. We analyzed the concentration of dissolved organic carbon (DOC; mg L^{-1}) and nitrate (NO_3^-; mg L^{-1}) in the pore water, which was collected using Rhizon filter pore water collectors (Rhizosphere Research Products, Wageningen, Netherlands). We placed two collectors per pot, one at the surface (1-11 cm) and one in the deeper profile (6-17 cm). To get a representative sample covering the entire pot depth, surface and deep profile samples were combined to collect at least 20 mL pore water. Samples were stored at -20 °C until thawed and analyzed for DOC using OI Analytical Aurora 1030 TOC analyzer. NO_3^- and NH_4^+ were analyzed using a Dionex ICS-2100 (Thermo Fisher Scientific Inc., USA) and Varian Cary 100Bio UV–VIS Spectrometer, respectively. 10 cm soil cores and biocrust samples were then collected for microbiome analysis (Supplemental Methods S1).

Post terminal drought traits

During the terminal drought, VWC declined. The rate of decline was obtained by fitting a linear decay function:

$$\mathrm{VWC} = a(1-b)^t, \quad (1)$$

where t indicates time passed since beginning the terminal drought in days, a is the starting VWC before beginning the terminal drought and b is the decay factor. Parameters a and b were estimated for each pot separately using non-linear least squares to determine the best fit. The W_W water treatment plants had higher starting VWC; therefore, we normalized each VWC and terminal drought day count measurement to a proportion of the highest VWC and total drought days for each plant. VWC at permanent stomatal closure was measured as the VWC on the morning when the plant reached zero g_s.

After the first fully expanded leaf reached $g_s = 0$, plants were harvested and measured for root biomass. To harvest roots, stems were cut 2 cm from the base of the plant. Root systems were carefully pulled from the pot and gently shaken to release soil. Root bulbs were washed in 500 mL sterilized filtered water. Remaining soil in the pot was hand searched for remaining fine roots. Roots were immediately frozen in -20 °C for root morphology analysis (total root length (cm); root length of < 1 mm diameter, 1–2 mm diameter, and > 2 mm diameter (cm), and root projected area (cm^2)). For root morphology analysis, roots were placed in a refrigerator to thaw for no more than 72 h. Root systems were imaged (4800 Perfection, Epson America Inc., Epson, CA, USA.) at 600 dpi and analyzed using WinRhizo pro software (Regent Instruments Inc., QC, Canada). Samples were dried at 60 °C for at least 48 h. Specific root length (SRL, m g^{-1}) of the entire root system was calculated as root length per dry root mass.

Statistical analyses

We employed the analysis of variance (ANOVA) to investigate the simultaneous effects of microbiome source, conditioning treatment, and watering treatment on each above- and belowground trait of interest, using a significance threshold of $\alpha = 0.05$. We carried out separate statistical analyses for each trait. All analyses were conducted using R statistical software (R Core Team 2020). When no statistically significant interaction effects were identified, we estimated main effects. For statistically significant main effects, we estimated and reported the difference in factor level means (W_W—W_L (G_0); W_W—W_L (G_1); M_{Forest}—M_{Ag}) and constructed a 95% confidence interval for the true difference. When statistically significant interaction effects were identified, we carried out multiple comparisons of all treatment means using the Tukey procedure to examine factor effects jointly, as it is not appropriate to combine factor levels to investigate main effects when interaction effects are present. When assumptions of a linear model were not met, we performed a Box-Cox transformation of the response Y, given by

$$Y^T = (Y^\lambda - 1)/\lambda \quad (2)$$

where λ is a parameter that is determined from the data (Box and Cox 1964; Sakia 1992).

Differences in microbial community composition were statistically compared with a PERMANOVA analysis using the *adonis* function in the R package *vegan* (Oksanen et al. 2022; see Supplemental Methods S1).

Results

Conditioning microbial legacy effect

A single generation of water-limited microbial conditioning enhanced root and stem growth in the subsequent generation regardless of the microbiome parent and water treatment. Microbiome conditioning did not affect plant height (Table 1; Fig. 2a), but plants grown under a microbiome with W_L conditioning had thicker stems than plants with a W_W conditioned microbiome (11.95 ± 0.40 mm *vs* 10.95 ± 0.45 mm, respectively, Fig. 2b). Interestingly, dry root mass was unaffected (Fig. 2c); however, there was a microbiome conditioning effect on root surface area and length of mid and coarse diameter roots. Plants showed marginal evidence for longer fine roots ($p = 0.069$), and had longer mid (440.93 ± 31.86 cm *vs* 330.25 ± 27.08 cm) and coarse (1341.88 ± 90.10 cm *vs* 1045.32 ± 101.12 cm) roots when grown with microbes having a W_L than W_W conditioning (Table 1; Fig. 2d–f). W_L conditioned

Table 1 Plant morphology ANOVA p-value results for the modeling including microbiome origin (Mbiome), watering treatment (Tmt), microbiome water conditioning (Conditioning), and all possible interactions between these factors

	Height $^{l=2*}$	Leaf count $^{l=2*}$	Stem diameter $^{l=0.6*}$	Root dry mass	Root Surface Area $^{l=0.6*}$	SRL $^{l=0.8*}$	Total Root Length $^{l=0.7*}$	Root Length $^{l=0.7*}$ (diameter < 1 mm)	Root Length $^{l=0.4*}$ (diameter 1-2 mm)	Root Length $^{l=0.8*}$ (diameter > 2 mm)
Mbiome	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Tmt	**< 0.001**	0.457	**< 0.001**	**< 0.001**	0.206	**0.025**	0.609	0.696	**0.002**	0.203
Condition	0.668	0.989	**0.011**	0.815	**0.023**	0.051	0.063	0.069	**0.003**	**0.015**
Mbiome x Tmt	0.981	0.989	0.619	0.634	0.626	0.451	0.603	0.594	0.900	0.704
Mbiome x Condition	0.297	0.152	0.387	0.282	0.341	0.623	0.351	0.346	0.522	0.518
Tmt x Condition	0.372	0.757	0.893	0.766	0.915	0.907	0.864	0.839	0.307	0.637
Mbiome x Tmt x Condition	0.822	0.735	0.472	0.366	0.163	0.169	0.141	0.142	0.139	0.259

In all cases, numerator and denominator degrees of freedom were 1,48 respectively

Bold values indicate significant response of $\alpha < 0.05$

* Variables that were transformed are noted by superscripts indicating the degree of Box-Cox transformation used, based on lambda (*l*): Transformed Variable = $(x^{\wedge}l - 1)/l$

microbes produced plants with greater root surface area than W_W conditioned microbiomes (1407.88 ± 96.17 cm^2 *vs* 1132.45 ± 102.54 cm^2; Fig. 2g). Plants grown with W_L conditioned microbes had marginally longer SRL (47.47 ± 2.62 m g^{-1} *vs* 40.74 ± 2.88 m g^{-1}) than those with W_W conditioned microbes (Table 1; Fig. 2h).

For A_{sat}, the conditioning legacy effect depended on both the watering treatment and the parent microbiome, characterized by a statistically significant three-factor interaction involving conditioning treatment, watering treatment, and microbiome treatment ($p = 0.006$, Table 2). Tukey multiple comparisons showed that M_{Forest} plants treated with W_w at G_1 obtained statistically significantly higher mean A_{sat} (17.71 ± 0.85 µmol m^{-2} s^{-1}; Fig. 3a) when given the W_w conditioning treatment than when conditioned with the W_L treatment (12.06 ± 0.67 µmol m^{-2} s^{-1}; $p = 0.011$). Additionally, M_{Forest} plants treated with W_w at G_1 also obtained statistically significantly higher mean A_{sat} than W_L M_{Ag} plants treated with the W_L conditioning treatment (11.36 ± 1.28 µmol m^{-2} s^{-1}; mean ± se; $p = 0.003$).

There was a similar three-factor interaction for g_s ($p = 0.031$). Under the W_L conditioning, varying the levels of microbiome or watering treatment had no discernable effect on mean g_s (Fig. 3b; left panel). Under the W_W conditioning, increasing the watering treatment from W_L to W_W resulted in an increase in mean g_s for M_{Forest} but not for M_{Ag}. Tukey multiple comparisons showed that M_{Forest} plants treated with W_W at G_1 obtained statistically significantly higher mean g_s (0.17 ± 0.03 mol m^{-2} s^{-1}; Fig. 3b) when given the W_w conditioning treatment than plants given three of the four treatment combination involving W_L conditioning: (1) W_L conditioning with M_{Ag} and W_L watering treatment (0.09 ± 0.03 mol m^{-2} s^{-1}; $p = 0.001$); (2) W_L conditioning with M_{Forest} and W_W watering treatment (0.11 ± 0.03 mol m^{-2} s^{-1}; $p = 0.029$); (3) W_L conditioning with M_{Forest} and W_L watering treatment (0.11 ± 0.03 mol m^{-2} s^{-1}; $p = 0.025$). Additionally, W_w-conditioned M_{Forest} plants treated with W_W at G_1 obtained statistically significantly higher mean g_s than W_w-conditioned M_{Forest} plants treated with W_L at G_1 (0.11 ± 0.03 mol m^{-2} s^{-1}; $p = 0.025$).

There were no significant pairwise differences among any other treatment combinations for A_{sat} or g_s responses. There was no conditioning effect on WUEi (Fig. 4a; data shown as differences between

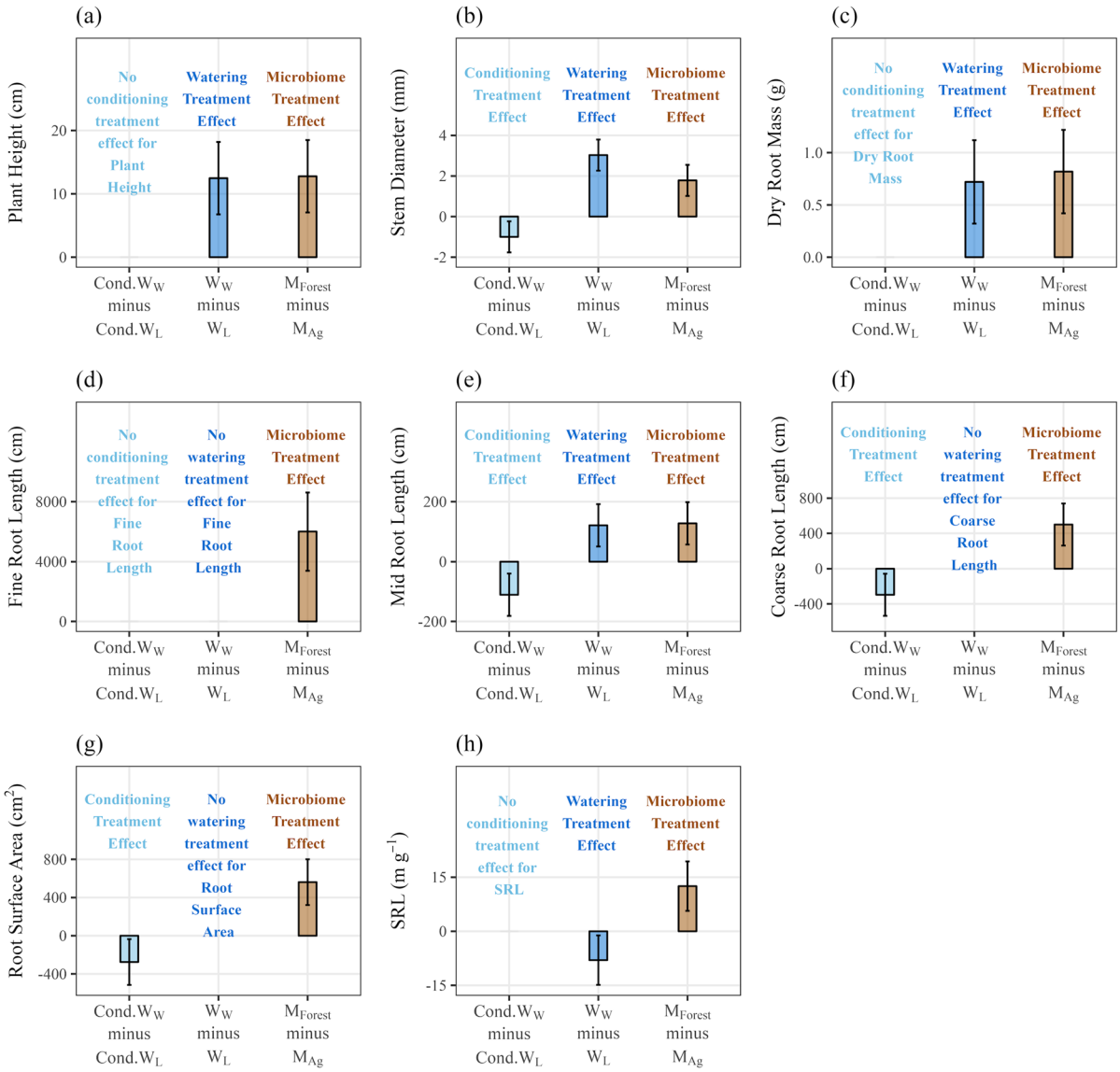

Fig. 2 Plant growth trait response differences between the two factor level means for each of the three experimental factors: microbiome conditioning (G_0), watering treatment (G_1), and microbiome parent. Effects on plant height (**a**), stem diameter (**b**), dry root mass (**c**), fine root length (**d**), mid-diameter root length (**e**), coarse root length (**f**), root surface area (**g**), and specific root length (**h**). Bars in figures show the differences in mean response between plants treated with well-watered (W_W) and water-limited (W_L) conditioning (light blue); W_W and W_L watering treatment (dark blue); and microbiomes originating from forest (M_{Forest}) and agricultural (M_{Ag}) soil (brown). Factor level mean differences are not shown for characteristics with hypothesis test results for main effects with $p > 0.05$ (Table 2)

treatment means) or leaf chemistry (Table S1; Fig. S2), but W_W conditioning increased PNUE (31.14 ± 14.21 µmol CO_2 $g^{-1}N$ s^{-1}) over W_L conditioning (25.31 ± 10.06 µmol CO_2 $g^{-1}N$ s^{-1}; Table 2; Fig. 4b).

W_W conditioned microbiomes also allowed plants to delay permanent stomatal closure longer during the terminal drought, even when the rate of VWC decline was elevated for W_W conditioning. Plants grown with W_L conditioned microbiomes reached

Table 2 Plant function ANOVA p-value results for the modeling including microbiome origin (Mbiome), watering treatment (Tmt), microbiome water conditioning (Conditioning), and all possible interactions between these factors

	A_{sat}	g_s $^{l=0.5*}$	WUE_i $^{l=0.4*}$	PNUE $^{l=0.2*}$	Days to closure $^{l=0.7*}$	VWC decline $^{l=2*}$	VWC at closure $^{l=0.9*}$	DOC $^{l=-0.3*}$	NO_3^- $^{l=2*}$	NH_4^+
Mbiome	0.487	0.354	0.555	0.489	0.236	0.337	0.982	0.357	**0.004**	0.299
Tmt	**0.047**	**0.003**	**0.005**	**<0.001**	**0.008**	**0.009**	**0.005**	**<0.001**	**<0.001**	0.447
Conditioning	**0.007**	**0.006**	0.266	**0.026**	**0.048**	**0.024**	0.050	**0.030**	0.480	0.177
Mbiome×Tmt	0.471	0.567	0.208	0.513	0.056	0.925	0.681	0.649	0.248	0.552
Mbiome×Condition	0.903	0.468	0.410	0.193	0.239	0.728	0.171	0.478	0.089	0.360
Tmt×Conditioning	0.097	0.167	0.764	0.116	0.058	0.746	0.771	0.495	0.161	0.271
Mbiome×Tmt×Conditioning	**0.006**	**0.031**	0.930	0.237	0.721	0.445	0.707	0.381	0.720	0.314

Numerator and denominator degrees of freedom for light saturated photosynthesis (A_{sat}), stomatal conductance (g_s), intrinsic water use efficiency (WUE_i), photosynthetic nitrogen use efficiency (PNUE), number of days it took for plants to close their stomata during the terminal drought (days to closure), rate of volumetric water content decline during the terminal drought (VWC decline), VWC at the stomatal closure, and NO_3 were 1,48 respectively. Dissolved organic carbon (DOC) degrees of freedom were 1,43 respectively

Bold values indicate significant response of $\alpha < 0.05$

*Variables that were transformed are noted by superscripts indicating the degree of Box-Cox transformation used, based on lambda (*l*): Transformed Variable = (x^*l*—1)/*l*

permanent stomatal closure almost two days earlier than plants grown with W_W conditioned microbiome (13.29 ± 0.66 days *vs* 15.07 ± 0.71 days, respectively; Table 2, Fig. 4c). This occurred despite an increase in the decay factor describing VWC decline over time in plants with W_W conditioned microbiomes (0.60 ± 0.02% *vs* 0.54 ± 0.02%; Table 2; Fig. 5) relative to plants with W_L conditioned microbiomes. VWC at stomatal closure did not respond to microbial water conditioning. Microbial W_L conditioning increased pore DOC over W_W conditioning (11.11 ± 0.145 *vs* 8.25 ± 0.98 mg L^{-1}; Table 1; Fig. 4d). However, pore water NO_3^- and NH_4^+ did not respond to microbial water conditioning (Table 2; Fig. 4e). Soil microbial community composition varied and showed distinct clustering by conditioning treatment (Fig. S1b; $p = 0.001$).

Watering treatment effect

The G_1 watering treatment affected some plant traits without interacting with the G_0 conditioning treatment, showing that our watering treatment imposed stress while still allowing plants to continue functioning. Aboveground growth reduced and root length increased under W_L watering treatment, even when root biomass decreased. Plants grown under the W_W treatment were taller (115.89 ± 2.32 *vs* 103.42 ± 2.28 cm; Table 1, Fig. 2a) with increased stem diameter (12.97 ± 0.35 *vs* 9.94 ± 0.29 mm; Fig. 2b) compared to those grown under W_L. Plants from W_W had greater root biomass than W_L treatment plants (3.66 ± 0.19 *vs* 2.94 ± 0.12 g; Table 1; Fig. 2c). However, when separated into root diameter classes, there was only an overall watering treatment effect for mid diameter root length with W_W treatment producing plants with longer mid root length than W_L treatment (445.99 ± 33.85 vs 325.19 ± 23.62 cm; Fig. 2e). SRL of W_W plants was shorter than for W_L plants (40.11 ± 2.48 vs 48.11 ± 2.94 m g^{-1}; Fig. 2h).

The only treatment that affected WUE_i was the watering treatment, where plants grown under the W_W treatment had lower WUE_i (0.12 ± 0.02 µmol CO_2 mmol H_2O^{-1} *vs* 0.13 ± 0.02 µmol CO_2 mmol H_2O^{-1}) than under W_L treatment (Table 2; Fig. 4a). In contrast, W_W had higher PNUE (36.73 ± 11.35 µmol CO_2 $g^{-1}N$ s^{-1} *vs* 19.71 ± 6.44 µmol CO_2 $g^{-1}N$ s^{-1}; Fig. 4b) than W_L watering treatment. This, combined with the microbiome conditioning results,

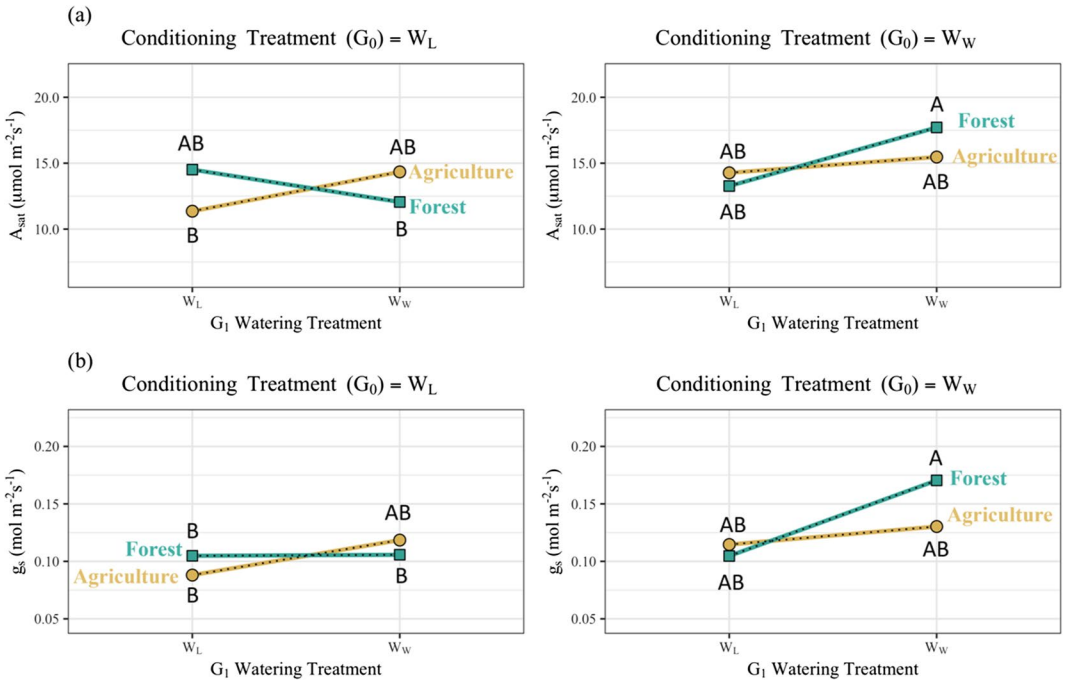

Fig. 3 Three-factor interaction plots for response of interest (**a**) saturated photosynthesis (A_{sat}) and (**b**) stomatal conductance (g_s) (**b**). The left panels contain watering treatment-by-microbiome two-factor interaction plots when the third factor, conditioning, is W_L. The right panels contain watering treatment-by-microbiome two-factor interaction plots when the third factor, conditioning, is W_W. The x-axis indicates the two water treatment levels, W_L or W_W. Letters indicate significant Tukey posthoc analysis differences between means. Interaction plots were based on the significant three-way interaction between microbiome, watering treatment, and conditioning shown in Table 2 (A_{sat} $p = 0.006$, g_s $p = 0.031$)

strongly suggest that W_W conditions are beneficial for PNUE. Leaf carbon to nitrogen ratio (C:N) was higher in W_W (33.05 ± 5.13 unitless vs 26.75 ± 0.94 unitless; $p < 0.001$; Table S1; Fig. S2a) compared to W_L. N_{mass} was lower in W_W (12.53 ± 2.02 mg g^{-1} *vs* 15.95 ± 2.85 mg g^{-1}; $p < 0.001$; Table S1; Fig. S2b) than W_L. Similarly, N_{area} was lower in the W_W (0.43 ± 0.09 g m^{-2} *vs* 0.71 ± 0.14 g m^{-2}) than W_L watering treatment ($p < 0.001$; Table S1; Fig. S2c).

Plants grown under W_L watering treatment experienced stomatal closure ~ 2.5 days more quickly (12.93 ± 0.54 days *vs* 15.43 ± 0.77 days; Fig. 4c) than plants grown under a W_W treatment. Similarly, the VWC decay factor was higher in plants given the W_W treatment (0.60 ± 0.01% *vs* 0.54 ± 0.02%VWC day^{-1}) than in plants given W_L. VWC at stomatal closure was lower for the W_L watering treatment (21.62 ± 0.35%VWC) than W_W (23.06 ± 0.34%VWC; Table 2). W_L watering treatment pots also had higher DOC (14.98 ± 1.28 mg L^{-1} *vs* 5.38 ± 0.26 mg L^{-1}; Fig. 4d) than W_W treatment pots. Pore water NO_3^- was almost three times higher in W_L compared to W_W pots (33.21 ± 3.64 mg L^{-1} *vs* 11.36 ± 2.21 mg L^{-1}; Fig. 4e). NH_4^+ did not respond to water treatment (Table 1). Soil microbial community composition varied and showed distinct clustering by watering treatment (Fig. S1b; $p = 0.001$).

Parent microbiome effect

Plant growth was strongly influenced by the parent microbiome, where above- and belowground growth were all higher in M_{Forest} compared to M_{Ag}. Plants with a microbiome parent originating from M_{Forest} were taller (116.04 ± 1.88 cm *vs* 103.27 ± 2.34 cm; Table 1, Fig. 2a) than M_{Ag}. Plants with M_{Forest} parent had thicker stems (12.34 ± 0.40 mm *vs* 10.56 ± 0.41 mm; Fig. 2b) than M_{Ag}. Plants with a microbiome originating from M_{Forest} had higher root biomass (3.71 ± 0.67 g *vs*

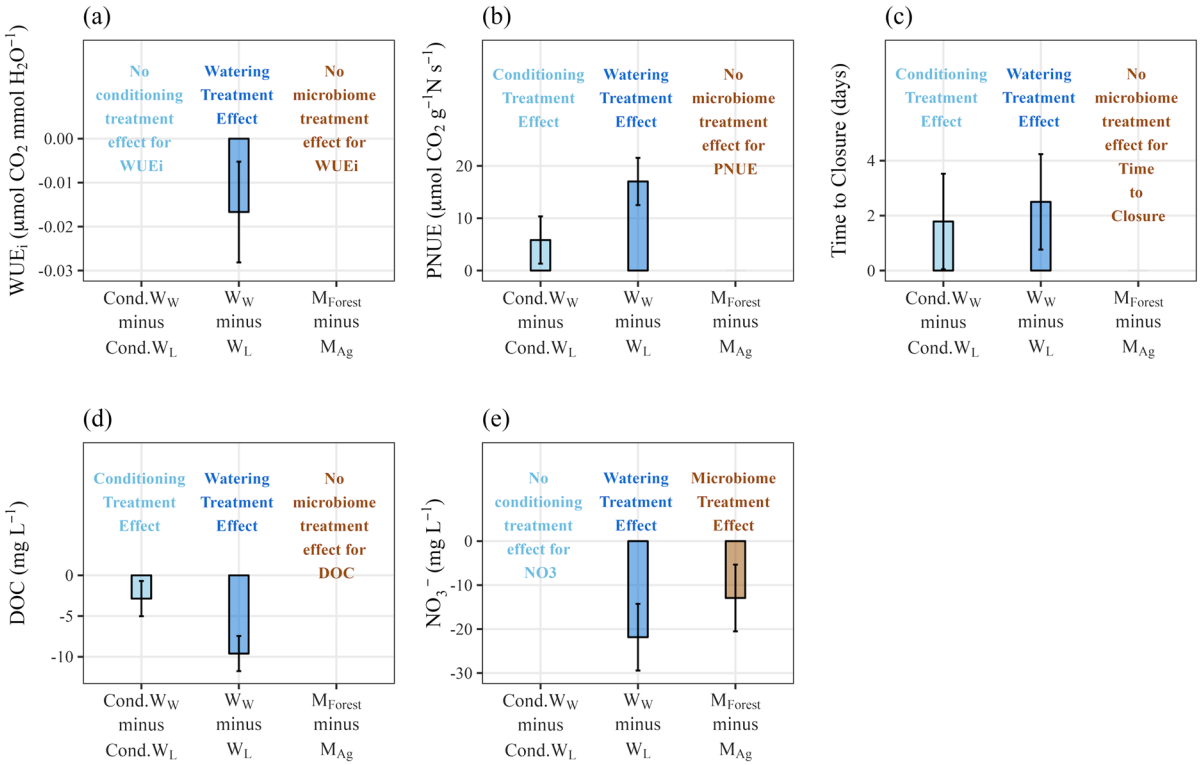

Fig. 4 Plant function response differences between the two-factor level means for each of the three experimental factors: microbiome conditioning (G_0), watering treatment (G_1), and microbiome parent. Effects on intrinsic water use efficiency (WUE_i; **a**), photosynthetic nitrogen use efficiency (PNUE; **b**), time to stomatal closure (**c**), pore water dissolved organic carbon (DOC; **d**), and nitrate (NO_3^-; **e**). Bars in figures show the differences in mean response between plants treated with well-watered (W_W) and water-limited (W_L) conditioning (light blue); W_W and W_L watering treatment (dark blue); and microbiomes originating from forest (M_{Forest}) and agricultural (M_{Ag}) soil (brown). Factor level mean differences are not shown for characteristics with hypothesis test results for main effects with $p > 0.05$ (Table 1)

$2.89 \pm 0.0.18$ g) than plants with M_{Ag}. Plants that had a parent microbiome originating from M_{Forest} had longer fine ($14{,}879.50 \pm 653.78$ cm; Table 1; Fig. 2d) mid (449.31 ± 23.63 cm; Fig. 2e), and coarse (1443.38 ± 67.60 cm; Fig. 2f) roots than M_{Ag} fine (8879.64 ± 1132.76 cm), mid (321.87 ± 33.40 cm), and coarse (943.82 ± 103.83 cm) roots. M_{Forest} produced higher root surface area (1550.81 ± 59.88 cm^2; Fig. 2g) than M_{Ag} (989.52 ± 108.39 cm^2). M_{Forest} microbiome plants had a higher SRL (50.37 ± 2.10 m g^{-1}; Fig. 2h) than M_{Ag} plants (37.84 ± 2.94 m g^{-1}).

The parent microbiome did not affect WUEi, PNUE, leaf chemistry traits, pore water DOC, or traits describing time to stomatal closure or soil VWC at wilting (Table 2). NO_3^- (28.75 ± 4.01 mg L^{-1}) was almost double in M_{Ag} compared to M_{Forest} (15.82 ± 2.79 mg L^{-1}; Fig. 4e). NH_4^+ did not respond to parent microbiome (Table 1).

M_{Forest} demonstrated a higher abundance of nitrogen-fixing bacteria during the conditioning phase (G_0). This trend continued and was more pronounced in G_1, where M_{Forest} showed a higher abundance (hits) of nitrogen-fixing bacteria than M_{Ag} (Fig. S2).

Discussion

Our experiment showed that just a single generation of water treatment conditioning generated a microbial community that could carry forward its water treatment legacy to influence the structure and function of a new maize plant. Root and belowground traits were strongly influenced by a W_L legacy with root

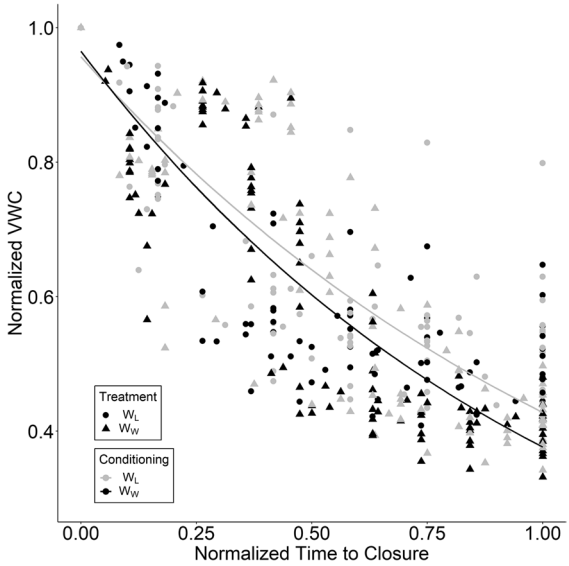

Fig. 5 Volumetric water content (VWC) decline during the terminal drought. Normalized to highest content per pot, the rate that VWC declined until the plant reached the wilting point where stomatal conductance = 0 for plants that received the well-water conditioning (W_W; black) or water limited conditioning (W_L; gray) microbiomes. Symbols represent the current watering treatment: W_L (circle) or W_W (triangle)

surface area, specific root length, lengths of mid- to coarse-roots, and soil pore water dissolved organic carbon increasing in plants inoculated with a W_L conditioned microbiome. A W_W conditioned microbiome also positively affected some plant functional traits. Plants with a W_W conditioning legacy kept stomata open longer before reaching their point of permanent stomatal closure, had higher photosynthetic nitrogen use efficiency, and had higher rates of photosynthesis and stomatal conductance under M_{Forest} and W_W treatment. These results provide important evidence of how quickly microbiomes can shift to affect the function of future plant growth. Our findings provide a promising experimental design approach toward the development of microbiomes to secure agriculture under future climate stressors.

Water conditioning legacy on plant structure

Many studies have shown that microbiomes influence plant belowground growth, where plant growth promoting bacteria increases root dry or fresh biomass (Marulanda et al. 2009; Marasco et al. 2013; Schwartz et al. 2013) by, e.g., altering plant signal cascades to increase lateral root growth or promoting production of root hairs that increase plant surface area for water uptake (Creus et al. 2005; Molina-Favero et al. 2008). Our watering conditioning induced a legacy effect, such that depending on the trait, the W_L conditioning either benefitted or disadvantaged plant structure in relation to drought independent from the original microbiome source. Plants with a microbiome originating from W_L conditioning had greater root length due to shift in the morphology of their root system such that they produced longer roots per biomass investment (e.g., longer SRL) than plants growing with a microbiome that originated from W_W conditioning. Greater root length (Fig. 2, S5; Table 1), theoretically assists plants growing under limited water availability in foraging for water (Comas et al. 2013; Marasco et al. 2013). Similarly, plants that originated from the W_L conditioning had thicker stems, even though height did not increase. Plant-associated microbiomes, particularly plant growth promoting bacteria, can either increase (Bashan et al. 2012; Molina-Romero et al. 2017) or decrease stem diameter (Molina-Romero et al. 2017). Greater stem thickness could lead to higher rates of water transport or, overall, higher biomass. The longer root length resulting from W_L conditioning could have increased plant water uptake; therefore, thicker stems, if associated with larger conduits and stem cross sections, could have developed to better facilitate water movement through the plant (Abd El-Rahim et al. 1998). Although we did not specifically measure hydraulic conductance in this study, greater water transport through the stem often leads to greater primary productivity (Brodribb and Feild 2000; Mencuccini 2003; Liu et al. 2020). However, during times of water scarcity, greater water transport can cause more rapid water uptake from the soil and eventually lead to more rapid water deficits (Leakey et al. 2006). In our experiment, W_L conditioning still had a soil water retention advantage over W_W conditioning in that W_L-conditioned plants lost VWC more slowly during the terminal drought (Table 2; Fig. 5); therefore, this may not apply to our system. Overall, these results suggest that microbiomes quickly respond to drought in a way that affects plant growth traits toward water uptake and that shift is conserved in the next plant cohort.

Water conditioning legacy on plant functioning

While W_L legacy resulted in beneficial plant structural shifts, whether W_W or W_L legacy was beneficial differed between plant functional traits. The W_L conditioning resulted in greater DOC (Fig. 2d), showing the important control that the microbial legacy had on DOC. Pre-experimental growth substrate (fritted clay + fertilized water) pore water DOC was quite low (~ 5 mg l^{-1}); however, it is likely that the fertilized water caused some accumulation of DOC over time in our pots. Even so, we saw a conditioning treatment effect on DOC regardless of the water treatment; therefore, the conditioning treatment effects are due to alterations of the microbiome during the conditioning phase. DOC treatment differences could be an indicator of either increased microbial metabolism of soil organic carbon, cells sloughing off during growth, or direct exudation by roots (Calvo et al. 2019). Root exudates play a direct role in signaling and providing a carbon source for the microbial community that can help alleviate drought stress (Kiers et al. 2011). Although severe droughts may limit root exudation (Preece and Peñuelas 2016; Karst et al. 2017), greater carbon exudation or DOC is commonly found in systems exposed to moderate drought compared to a control treatment (Sanaullah et al. 2012; Preece et al. 2018). The amount of DOC has been shown to be controlled by microbial taxa (Sanaullah et al. 2012; Albright et al. 2020). ^{14}C labeling studies have shown that drought-induced increases in DOC do not necessarily lead to increases in recently exuded C in microbial biomass (Sanaullah et al. 2012), suggesting DOC is directly coming from the plant instead of increased microbial biomass. The legacy of W_L conditioning promoted higher DOC in the soil, suggesting that a drought adapted microbiome stimulates C exudation or root sloughing from the plant that, in principle, could promote microbiome activity that may also benefit the plant under drought stress.

A W_W conditioning treatment benefitted M_{Forest}, which produced higher rates of photosynthesis and stomatal conductance under well-watered conditions. However, due to higher rates of photosynthesis and stomatal conductance in the forest soil when microbiomes experienced W_W during both the conditioning and water treatment phase, W_L during the conditioning or water treatment phases seemed to prohibited gas exchange rate increases (Fig. 3). It is possible that the M_{Ag} treatment did not experience an observable W_W conditioning treatment legacy because this microbiome was already adapted to maize drought conditions. Less responsive stomata are more commonly found in more drought tolerant species (Skelton et al. 2015; Henry et al. 2019) and, while often associated with lower stomatal conductance, lower stomatal sensitivity and less efficient xylem can help plants maintain hydraulic safety (Gleason et al. 2017). Further, we hypothesize that plants grown under M_{Forest} combined with W_W water treatment and W_W conditioning may have experienced upregulation of plant gas exchange compared to the W_L treatment because M_{Forest} was enriched with potential nitrogen fixing bacteria (Figs. S3, S4). N fixation could positively influence photosynthetic capacity and CO_2 uptake via impacts of increased N on photochemistry (Henneron et al. 2020). We note that further experiments are needed to definitely attribute function to these microbial taxa. These are taxa that could explain treatment differences, and nitrogen fixation is one potential mechanism, but we acknowledge that these taxa and other taxa in our systems could be driving different functions and, thus, the different plant responses observed between our treatments. Even so, rhizospheric nitrogen fixing bacteria play a key role in plant nutrient availability as they are able to directly reduce atmospheric nitrogen, creating higher nitrogen availability to plants while also producing phytohormones and solubilizing phosphorus (Swarnalakshmi et al. 2020). This could lead to higher nitrogen availability for the photosynthetic apparatus (Cleveland et al. 1999; Reed et al. 2011) and, therefore, increased rates of photosynthesis.

Photosynthetic nitrogen use efficiency (PNUE) also experienced a watering treatment legacy effect; however, this was not dependent on parent microbiome (Fig. 4b). W_W conditioning led to plants with higher PNUE than plants with W_L legacy, independent of the parent microbiome. This is aligned with the Least-cost Hypothesis that states that plants optimize their traits to efficiently use and acquire the more limiting resource (either water or nitrogen) (Field et al. 1983; Wright et al. 2003). In our study, W_L microbiome conditioning tended to produce lower rates of photosynthesis, (significant interaction effect, Table 2); however, N_{area} did not differ between plants with different microbiomes (Table 2). This suggests that the plants experiencing the W_W treatments were

better able to utilize similar concentrations of leaf nitrogen. Water and nitrogen are the factors that most limit global crop growth (Mueller et al. 2012), and importantly, these results show that the microbiome plays a role in this relationship, where W_W conditioning of the microbiome allowed the plant to maximize plant nitrogen utilization per the rate of photosynthesis.

Time to stomatal closure strongly controlled by microbial community

Contrary to our hypothesis, corn plants that were inoculated with a W_W conditioned microbiome were able to last longer during the terminal drought before reaching the point of permanent stomatal closure (Fig. 4c). This suggests the microbiomes retained a feature from W_W conditioning that allowed them to prolong plant activity even under drought. The microbial community can have direct and indirect effects on how long plants keep stomata open during drought and have specifically been shown to delay wilting in rice (Redman et al. 2011). Microbes can exude exopolysaccharides that directly retain water and nutrients within the rhizosphere (De Caire et al. 1997; Alami et al. 2000; Mehboob et al. 2009). In addition, microbes can control plant hormonal signals and can reduce abscisic acid induced stomatal closure (Perrig et al. 2007; Kang et al. 2014). In our system, we also found that pots with W_W conditioning lost soil water more quickly than pots with a W_L legacy, yet these plants also took longer to close their stomata (Fig. 5). This extended time to stomatal closure under faster soil drying might be accomplished by altering plant hormonal signals or by allowing the plants to better access the more limited water available in these pots.

Implications and conclusions

We found that just a single generation of plant growth under different watering conditions induced rapid shifts in microbial function to influence a subsequent cohort of plant structure and function under varying watering treatments. These results present novel microbiome selection methods to improve plant performance relative to other studies that have used mycorrhizal fungi (Bowles et al. 2016), plant growth promoting bacteria (Rolli et al. 2015), and arid region microbiomes (Marulanda et al. 2009) in that they show that, regardless of the microbiome origin, we can very quickly modify the entire microbiome to produce plant traits of interest using droughting techniques. Similarly, we know from previous studies that drought alters the legacy of microbial communities (de Vries et al. 2019); however, our work further shows that shifting the microbial community alone can influence plant structure and function.

We hypothesized a priori that, of our two microbiomes, the microbiome originating from non-irrigated agricultural soil would show a stronger microbial legacy response to drought than the forest originated microbiome. Surprisingly, our results do not support this hypothesis. Except for A_{sat} and g_s grown under M_{Forest} and W_W treatment (Fig. 3), there were no clear differences between the conditioning legacies of the two microbiomes even though had different starting compositions (Tables 1 and 2; Moore et al. 2023). What was clear, however, was that the M_{Forest} promoted greater plant growth than M_{Ag} (Figs. 2e–g, 5), a trend that occurred regardless of watering treatment and further supports results discussed in Moore et al. (2023). The shorter plants and lower root mass seen in M_{Ag} plants might suggests that, similar to our W_L legacy effects, plants exposed to M_{Ag} would use less water and might be able to withstand drought for longer periods of time independently of the microbiome conditioning treatment. However, the decrease in aboveground growth was not accompanied by increased root length or area that would improve the plant's ability to access sources of water (Fig. 2; Comas et al. 2013). There is a trade-off associated with drought tolerance and plant size where a smaller plant might have a greater ability to survive but the smaller, drought adaptive traits often lead to lower productivity. Our results suggest that M_{Ag}, when compared to M_{Forest}, might actually be limited in its ability to shift in response to drought in a way that is beneficial for the crops growing under water limitation. Further, depressed growth in M_{Ag} plants could have been influenced by the yield drag phenomenon, where years of agricultural plant growth in the same field decreases yield through an altered microbiome (Johnson et al. 1992; Benitez et al. 2021). While the difference in growth suggests important functional differences between plants grown in the presence of different microbiomes, it is important to note that

height alone might not indicate improved plant yield, especially under drought where light interception is key (Comas et al. 2019). The impacts of microbiomes from a historically droughted environment on plant drought tolerance traits are currently under investigated. However, the differences we found between the two original parent microbiomes are opposite to a recent study by Zhang et al. (2022) who showed that a microbiome sourced from a droughted grass conferred higher maize drought tolerance than a microbiome sourced from irrigated agricultural conditions. Our results, however, agreed with Zhang et al. (2022) in that the microbiome able to best improve plant traits came from a natural soil rather than controlled, agricultural conditions. These results have important implications for agricultural applications trying to improve plant growth or yield through modification of the microbiome, where it is important to perform rigorous investigations of the community being amended. Further, sourcing that a microbiome from agricultural soil that has already gone through generations of selection under plant species and nutrient amendments might not produce the most effective microbial community to improve plant performance.

Our study shows that it is possible to rapidly induce a shift in complex microbial communities to alter plant performance. Examples showing that microbial communities can be pressured to modify composition in a way that alters plant function, rather than plant growth alone, are rare; however, our results are aligned with a study where a microbiome from a three-generation drought experiment improved aspects of plant fitness, such as fruiting and flowering time (Lau and Lennon 2012). In addition, Panke-Buisse et al., (2015) also adjusted plant flowering time through microbiome directed evolution. These results suggest that, while directly trying to evolve plants in a timely manner in response to global change may be an unlikely or impractical solution for improving plant structure and function in future environments (Lau et al. 2007; Lau and Terhorst 2020), achieving improved plant performance by imposing short-term treatments on microbial communities may be a promising avenue to ameliorate plant drought stress.

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Data availability Data are available as supplemental dataset.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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