Soil microbes increase switchgrass germination but not seedling growth under drought

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Abstract

Purpose Soil microbial communities can mitigate the negative effects of drought on early-stage plant growth. However, the magnitude of this benefit may depend on both the microbial community's previous host associations and the plant's developmental stage.

Methods We conducted a greenhouse experiment to investigate how microbial presence (autoclaved bulk vs. live bulk soils) and the microbial community's association history (bulk soil vs. rhizosphere soil) affect germination and seedling growth during drought, as well as how drought and life-stage alter the assembly of the inoculated communities. Our focal plant was switchgrass (*Panicum virgatum*), a target bioenergy crop frequently used in native prairie restorations.

Results We found that drought reduced growth by 59% and germination by 41% compared to ambient conditions, and that microbial presence altered drought responses. Seeds with microbes (live bulk soil) had 83% greater germination and 72% higher survival than seeds in autoclaved soils, and these effects were similar under both precipitation regimes. In contrast to microbial presence, the inoculated communities' association history did not affect plant responses. We did find that plant growth-stage altered bacterial community assembly; bulk and rhizosphere bacterial communities were initially similar, and responded similarly to drought, but they diverged at the end of the experiment only with a germinating seed.

Conclusion We show that soil microbes can increase germination and mitigate early-stage drought stress but that microbial association history may not strongly affect plant drought responses. Furthermore, interactions between soil community history and germination may be a critical, yet understudied, driver of microbiome assembly.

Introduction

Increasing frequency and intensity of droughts is a major threat to plant productivity worldwide (Hoegh-Guldberg et al., 2018; Leng & Hall, 2019). Soil microbes have the potential to mediate plant drought stress by altering resource availability and plant traits (de Vries, Griffiths, Knight, Nicolitch, & Williams, 2020; Naylor & Coleman-Derr, 2018). For instance, bacteria can produce phytohormones that stimulate root growth and rhizosheath development which helps plants access water during drought (Jochum, McWilliams, Pierson, & Jo, 2019; Zhang et al., 2020). These plant-growth-promoting bacteria can benefit plants under many conditions, but their impact is often greater under drought stress (Rubin, van Groenigen, & Hungate, 2017). Most of our understanding of microbially-mediated drought tolerance comes from studies that focus on single microbial strains (Jochum et al., 2019; Ngumbi & Kloepper, 2016; Rubin, van Groenigen, & Hungate, 2017) yet plants interact with diverse microbial communities in the soil. This discrepancy makes it unlikely that studies of single microbial strains will help us predict plant drought responses to soil communities in field conditions, as plant drought responses may be dependent on the characteristics of these diverse microbial communities. For instance, drought-tolerant taxa become enriched under drought stress, which can increase plant drought tolerance (reviewed by Naylor & Coleman-Derr, 2018). Studies across the globe report similar shifts in plant-associated soil communities under drought (Ochoa-Hueso et al., 2018; Xu & Coleman-Derr, 2019), yet it is unknown if certain community characteristics have greater potential to mitigate plant drought stress.

One of the most important soil community characteristics that affects plant-microbial interactions is the communities' previous associations with plant hosts (Bever et al., 2010; Van der Putten et al., 2013). Plants impose strong selective forces on soil communities near their roots by secreting carbon-rich exudates that recruit microbes from the surrounding bulk soil (Sasse, Martinoia, & Northen, 2018). This zone of selection is called the rhizosphere and is often characterized by a less-diverse, but more host-associated subset of microbes (Philippot, Raaijmakers, Lemanceau, & Van Der Putten, 2013). Studies using microbial isolates suggest that these rhizosphere microbes have stronger effects (both positive and negative) on their plant hosts compared to host-neutral microbes isolated from bulk soil (Luo et al., 2019; Marasco et al., 2012; Timmusk et al., 2014). Yet it is unknown if these responses to single strains are predictive of plants' interactions with whole rhizosphere and bulk soil communities. In many cases, soils with a history of host interactions have more negative effects on host growth than soils conditioned by different host species (i.e. negative plant-soil feedbacks; reviewed by Kulmatiski, Beard, Stevens, & Cobbold, 2008). Similarly, studies find that soils near conspecifics have a more negative effect on seedling establishment than more distant soils (Bagchi et al., 2010; Bell, Freckleton, & Lewis, 2006; Packer & Clay, 2000). These negative effects are often driven by species-specific pathogens (Bagchi et al., 2010; Luo et al., 2019), but their abundance and activity is predicted to change under drought (Pugnaire et al., 2019; van der Putten, Bradford, Pernilla Brinkman, van de Voorde, & Veen, 2016), making it even more important to study how host-associated microbes affect plants under drought.

Plants are more responsive to host-associated microbes and drought stress during seedling establishment (James, Svejcar, & Rinella, 2011; Leck, Parker, Simpson, & Simpson, 2008; Miller, Perron, & Collins, 2019). Despite this, most studies on plant-microbial interactions and drought focus on later developmental stages (Fry et al., 2018; Kaisermann, de Vries, Griffiths, & Bardgett, 2017; Zolla, Badri, Bakker, Manter, & Vivanco, 2013; but see Ulrich et al., 2019). Plants are known to associate with distinct microbial communities throughout development (Chaparro et al., 2013; Na et al., 2019), so it is likely that plants' interactions with soil microbes during drought also differ by life-stage. Still, because most drought studies focus on plant growth after germination, little is known about how early-stage microbial interactions affect plant drought tolerance. Studies show that germinating seeds and developing roots secrete exudates that begin the initial stages of microbial assembly (Nelson, 2018; Shade, Jacques, & Barret, 2017; Torres-Cortés et al., 2018), so it is likely that these early -stages of microbial assembly could play an important, yet understudied, role in plant drought tolerance.

Switchgrass (*Panicum virgatum*), a perennial C4 grass native to North America, is one example of a plant whose initial-establishment and yields are limited by drought-sensitive early life-stages (Barney et al., 2009; Seepaul, Macoon, & Reddy, 2012). Studies on single strain inoculations of fungal (Ghimire, Charlton, & Craven, 2009; Ghimire & Craven, 2011) and bacterial (Wang, Seiler, & Mei, 2016) isolates suggest that early-stage switchgrass growth and drought tolerance could be mediated by beneficial microbial interactions. Studies on whole soil communities, on the other hand, report that switchgrass growth is negatively affected by host-associated soil communities (Bauer, Mack, & Bever, 2015; Hawkes, Kivlin, Du, & Eviner, 2013; McKenna, Darby, & Yurkonis, 2018). Therefore, we expect that switchgrass seedling establishment and drought tolerance will be more negatively affected by growth in switchgrass rhizosphere soil than by growth with host-neutral bulk soil communities. Understanding how soil microbes affect switchgrass establishment and drought tolerance has the potential to inform both conservation and agricultural practices, as switchgrass is frequently used in prairie restorations and is also considered a leading candidate for lignocellulosic bioenergy production (Casler, 2012).

We used switchgrass as the focal species in a greenhouse experiment to determine how soil communities affect its germination and seedling establishment under drought stress. We asked three questions: 1) Does microbial presence (autoclaved vs. live bulk soils) affect plant germination, seedling growth, and survival under drought? 2) Does a microbial community's association history (rhizosphere vs. bulk soils) alter its effect on plant drought responses?, and 3) Do the inoculated soil bacterial communities change in response to drought and plant life-stage? We hypothesized that soil microbial communities will have an overall positive effect on germination and growth and this benefit would be more pronounced under drought stress. Second, we hypothesized that under drought plants inoculated with rhizosphere soil would have lower growth and germination than in bulk soil due to the greater abundance of host-associated soil pathogens exacerbating drought stress. Finally, we hypothesized that drought would alter the soil bacterial communities associated with germinating seeds and seedlings and that these shifts would be driven by an enrichment of drought-adapted taxa.

Methods

Experimental overview

Switchgrass (var. Cave-in-Rock) seeds and seedlings were exposed to ambient and drought conditions in the presence of three microbial treatments: autoclaved bulk soil, and live bulk and rhizosphere soil (Figure 1a). Each of these factors (2 life-stage x 2 precipitation x 3 microbial treatments) contained 15 replicates (180 total pots) which were split into five randomly-positioned blocks separately for seeds and seedlings to control for environmental gradients across the greenhouse (Figure 1b). The greenhouse was sterilized twice with MicroBLOC greenhouse disinfectant (FloraLife, South Carolina, USA) prior to use and temperatures were maintained between a maximum of 26.7°C during the day and minimum of 15.6°C at night with 14 hours of artificial lighting.

Precipitation treatments

Watering treatments were based on meteorological data from W.K. Kellogg Biological Station Long-Term Ecological Research Site (https://lter.kbs.msu.edu/datatables/448). The ambient precipitation treatment was 3.03 mm per day based on average daily rainfall in May and June from 1997 to 2017 (4 mL day^{-1} pot^{-1}), while the drought treatment was based on the rainfall conditions during a severe drought in 2012 with daily rainfall averaging 0.86 mm in May and June. Droughts in the Midwest are predicted to occur through decreases in the frequency of rain events, as opposed to the size of events (Hoegh-Guldberg et al., 2018); therefore, pots in our drought treatment were watered 4 mL every three days to mirror the daily average during the drought.

Microbial treatments (presence and association history)

We used three soil inoculum treatments – rhizosphere, bulk, and autoclaved bulk soils – to manipulate microbial presence and the microbial association history (Figure 1a). We collected inoculum soils on June 4th, 2018 (May 2018 was characterized by 2.3 times more rainfall compared to the May in 1997-2017 average) from established switchgrass monocultures (var. Cave-in-Rock; replicate 6; established in 2008) at the Great Lakes Bioenergy Research Center Intensive site at the W.K. Kellogg Biological Station in Hickory Corners, Michigan. Bulk and rhizosphere soils were collected from five randomly chosen switchgrass individuals. The paired sampling of rhizosphere and bulk soils were maintained as blocking factors in the greenhouse and statistical analyses to capture plot-level soil heterogeneity. We excavated entire switchgrass plants to approximately 15 cm in depth and vigorously shook plants to collect all loosely-adhering soil (hereafter rhizosphere soil). Bulk soil was collected by digging pits (approximately 15 cm in depth) at least 1 m from any switchgrass plant after removing groundcover vegetation. The soils were sieved (2 mm) and half of the bulk soil was autoclaved three times to be used as the autoclaved treatment. We filled bleach sterilized pots (164 mL, Ray Leach "Cone-tainer" SC10 pots, Stuewe and Sons, Oregon USA) with 90% autoclave sterilized sand and vermiculite (50:50) and approximately 16 mL of soil (microbial treatment descriptions below). Subsamples of bulk and rhizosphere soils were frozen at -20°C for microbiome and nutrient analyses.

Two plant life-stages: germination and seedling growth

Plant establishment is dependent on successful germination and initial seedling growth (Leck et al., 2008), so we prioritized these two life-stages for our evaluation of microbially-mediated drought responses. We examined the effect of soil microbes and drought on germination and seedling growth by varying when the plants were exposed to the microbial treatments (Figure 1b). For the germination-stage treatment (hereafter germinants), seeds were sterilized for 10 minutes in 10% bleach then stratified on sterilized filter paper saturated with nanopure water for 16 days at 4°C before being sowed into pots at a rate of 10 seeds per pot. For the seedling-stage treatment (hereafter seedlings), we sterilized and stratified seeds (10 days), we then germinated seeds in flats with sterilized sand and vermiculite (50:50) for six days before the start of the experiment. Due to the potential for mechanical damage during transplant, any seedlings that died within 7 days were replaced. The two life-stages were established in the microbial soil treatments and topped with autoclave sterilized sand to reduce cross-contamination during watering.

Greenhouse measurements

For the germinants, germination was recorded daily for one week before pots were thinned to one germinant. For the seedlings, we measured the initial height prior to transplanting into the microbial treatments. Seedling survival was recorded daily and height and leaf number weekly throughout the experiment. At the end of the experiment (34 days), the final height, leaf number, and survivorship for all germinants and seedlings was recorded. We evaluated seedling root traits and rhizosheath (details below) on five replicates per treatment (one replicate each from 5 greenhouse blocks). Germinants were not assessed for root traits or rhizosheath, as their roots were too small for the collection of rhizosheath soils. The remaining seedling and germinant pots were harvested for plant tissues (roots and shoots) and soils. Plant tissues were dried at 60°C for >72 hours and weighed. Homogenized soils from each pot were collected for DNA extractions (stored at -20°C), gravimetric soil moisture content (5 g dried at 60°C), and soil nutrients (12 g processed immediately, described in *Microbial biomass and soil nutrient assays*).

Root trait and rhizosheath measurements

We assessed the seedlings' rhizosheath, defined as soils that physically adhere to plant roots after gentle shaking (Pang, Ryan, Siddique, & Simpson, 2017), using five replicates per treatment. Prior to harvesting the seedlings, we saturated the pots with nanopure water to control for variation in rhizosheath due to soil moisture. After carefully excavating the seedlings, they were vigorously shaken for 10 seconds to remove loosely-adhering soils. Seedlings were then placed in a 50 mL centrifuge tube with 25 mL water and shaken at 3500 rpm for 5 minutes on an orbital shaker table. After dislodging the rhizosheath, the roots were removed and stored at 4°C and the remaining rhizosheath was weighed. The rhizosheath was calculated by subtracting the weight of the 50 mL tube and water from the weight of the tube with water and dislodged rhizosheath; this weight was normalized by dry root weight and any negative values were converted to 0 for all analyses.

After rhizosheath collection, the roots were scanned by placing roots with 200 mL water in a glass scanning bed (Epson Perfection v600 desktop scanner). A control scan with a ruler was used to convert pixel numbers to root length (cm). The roots were scanned at 1200 dpi and we calculated total root length with GiA Roots software (Galkovskyi et al., 2012). Following scanning, roots were dried at 60°C for 72 hours and weighed, and specific root length (total root length/ total dry root mass) was calculated.

Microbial biomass and soil nutrient assays

Chloroform-fumigated and unfumigated potassium sulfate (0.5 M) extracts were used to assess biomass carbon (MBC), dissolved organic carbon (DOC), as well as soil nitrate (NO_3^-) and ammonium (NH_4^+) concentrations. Fumigated and unfumigated extracts were analyzed on a TOC analyzer (Shimadzu TOC-VCPH) and MBC was calculated by subtracting total C of the unfumigated samples from the fumigated sample and dividing the difference by previously calibrated extraction efficiency (K_{EC}: 0.45) (Joergensen, 1996; Vance, Brookes, & Jenkinson, 1987). Unfumigated extracts were used to determine soil inorganic NH_4^+ and NO_3^- with colorimetric 96-well plate assays. Ammonium and nitrate concentrations were determined using previously described 96-well plate assays and absorption values were read on a Synergy HTX plate reader (BioTek, Winooski, Vermont, USA)(nitrate - Patton & Kryskalla, 2011; ammonium - Sinsabaugh et al., 2000). Soil N assays were completed on a subset of the seedling ($n \geq 3$ per treatment) and germinant soils ($n \geq 1$ per treatment because of low germination and survival), as well as the initial soil inocula (n = 5 per treatment) which were initially frozen, thawed and extracted in the same way as the experiment soils. We focus on soil N results from the seedling soils due to its higher replication. Microbial biomass and DOC were only assessed on the seedling ambient-exposed soils (n = 5 per microbial treatment).

DNA extraction and sequencing

DNA was extracted from 0.25 g of soil from the initial soil inocula (n = 4 per treatment) and harvested seedling- and germination-stage soils (n = 4 per treatment) using the PowerSoil DNA kit (Qiagen) standard protocol on a KingFisher Flex (Thermo Fisher, Massachusetts, USA). DNA libraries were prepped and sequenced by the Michigan State University RTSF Genomics Core (East Lansing, Michigan, USA). The V4 hypervariable region of the 16S rRNA gene was amplified using Illumina compatible, dual indexed primers 515f/806 (Kozich, Westcott, Baxter, Highlander, & Schloss, 2013). Sequencing was carried out in a 2x250bp paired end format using a MiSeq v2 500 cycle reagent cartridge.

Bioinformatics

Sequences were merged, quality filtered, clustered into OTUs, and classified using USEARCHv11 pipeline (http://drive5.com/usearch/) (Edgar, 2010). Prior to merging the forward and reverse reads, 80 bp were trimmed from the trailing end of the reverse reads to increase merging success. Cutadapt (Martin, 2011) was used to remove any residual base adapter and primer base pairs, and then reads were quality filtered (EE <1 and reads < 250bp)(Edgar & Flyvbjerg, 2015). Reads were then dereplicated and clustered into OTUs at 97% similarity using UPARSE (Edgar, 2013) which were classified against SILVA v123 rRNA database (Yilmaz et al., 2014) using SINTAX (Edgar, 2016). A phylogenetic tree of representative sequences was built using PASTA with default settings (Mirarab et al., 2015). OTUs that classified as Chloroplast, Mitochondria, or non-bacterial/archaea at 80% confidence were filtered from the community. Additionally, any OTU with less than three reads and samples with less than 250 reads (one sample) were removed from the community. The final community had 8,864 OTUs and 972,894 reads. The community matrix was then rarefied to 10,000 reads per sample for all future statistical analyses using the vegan R package (Oksanen et al., 2017).

Analysis

Seedling and germinant responses were analyzed in both a full model and separately (described below) using mixed effects models and type 3 sum of squares with the lme4 and lmertest package in R v4 (Bates, Maechler, Bolker, & Walker, 2015; Kuznetsova, Brockhoff, & RHB, 2017; R Core Team, 2018). We first used a full model with precipitation, microbial treatments, life-stages, and their interactions to determine the relative importance of the treatments for each life-stage. We then used split models with the same design to differentiate the effects of microbial presence (Q1: autoclaved bulk versus live bulk) and soil history (Q2: bulk versus rhizosphere) for life-stages separately. We included a random blocking factor to control for soil sampling location and pot position in the greenhouse. For the germination rates, a negative bionomial generalized linear mixed effect model was used. Post-hoc pairwise comparisons were conducted using emmeans package with a Tukey adjustment (Lenth, 2019). Seedling initial height before transplanting did not differ among any of the microbial (p = 0.41) or precipitation (p = 0.95) treatments, so was not included as a covariate in analyses. Germination rate was calculated by the number of seeds that germinated out of 10 seeds for every pot.

Multivariate analyses of community composition were performed on Weighted-Unifrac distance matrices from the rarefied community. Differences in microbial community composition were analyzed using PRIMER v6 with the same models described above (Clarke & Gorley, 2006). We identified taxa that drove community dissimilarity among treatments using a simper analysis in the vegan package (Oksanen et al., 2017). Changes in the soil communities pre- and-post experiment were analyzed by plotting pairwise Weighted-Unifrac dissimilarity from starting soil community, paired by block factor. All plant response and microbial community data and analysis scripts are publicly available (data and code:

https://github.com/Idereske/Ulbrich_etal_2021_Switchgrass_seedlings/Switchgrass; 16S FASTA files: NCBI Sequence Read Archive, accession number PRJNA720265).

Results

Effect of drought on germinants and seedlings

Drought effectively reduced soil moisture by an average of 42% across all microbial treatments ($p = 0.008$, Table S1, Figure S1), compared to ambient conditions. Precipitation also affected soil nutrients, with 29% higher nitrate ($p < 0.001$) and 3.45 times higher soil ammonium under drought compared to ambient ($p = 0.031$, Table S6, Figure S2).

Drought negatively affected switchgrass germination and seedling growth. Germination rate was 41% lower under drought than ambient conditions ($p = 0.038$, Table S2, Figure 2a). Drought also tended to slow the rate of germination, with seeds in drought conditions germinating on average 1.3 days slower than seeds in ambient conditions ($p = 0.066$, Table S2, Figure 2b). Drought did not significantly reduce germinant survival across all microbial treatments ($p = 0.803$, Table S2, Figure 2c), but did affect seedling survival and biomass. Seedlings in drought conditions had 22% lower survival and 59% lower total biomass relative to those in ambient conditions ($p < 0.001$, Table S3, Figure 3b). Drought also altered the seedlings' root morphology, with seedlings in drought conditions having 29% greater specific root length (SRL) than those in ambient conditions ($p = 0.030$, Table S3, Figure 2c). Seedling rhizosheaths were not influenced by precipitation or microbial treatments ($p > 0.05$, Table S3, Figure S3).

Effect of microbial presence on plant drought responses

Live and autoclaved soils had significantly different nutrient content and soil bacterial communities, and both likely contributed to the effect of the microbial presence treatment on plant responses. Consistent with other studies, we found that the autoclaved soils were not completely sterile by the end of the experiment but they had lower microbial diversity (Gebhardt, Fehmi, Rasmussen, & Gallery, 2017; Lau & Lennon, 2011). By the end of the experiment (34 days), microbial biomass did not differ among the treatments ($p = 0.434$, Table S6, Figure S5), but autoclaving did alter community composition ($R^2 = 0.40$, $p = 0.007$, Figure S6 & S7) and reduce bacterial richness by 3.4 times in the final communities ($p < 0.001$, Table S5, Figure 4). Soil nutrients also differed between the autoclaved and live soil: the autoclaved soil had seven times higher ammonium than the live soil at the start of the experiment ($p < 0.001$) and 15 times higher ammonium at the end of the experiment ($p < 0.001$, Figure S2). Soil nitrate did not differ across microbial treatments at the beginning of the experiment ($p = 0.477$), but the live soil had 45% higher nitrate concentration than autoclaved soils under drought by the end of the experiment ($p = 0.008$, Table S6, Figure S2).

Microbial presence significantly affected both life-stages but had a greater effect on germinant responses. Though there were few interactions between microbial presence and precipitation (Table 1), seeds in live soil receiving ambient precipitation had 90% greater germination ($p = 0.009$) and germinated an average of 4.5 days faster than seeds in autoclaved soil ($p = 0.019$, Table 1, Figure 2a & 2b). These microbially-mediated effects were also observed under drought, but the pairwise differences were not significant ($p > 0.35$). Microbial presence also increased germinant survival under both precipitation regimes ($p = 0.021$, Table 1, Figure 2c). Under ambient conditions, germinants in live soil had 33% greater survival while under drought germinants in live soil had 170% greater survival than those in autoclaved soil. Only two germinants survived in autoclaved soils under drought, but those that survived had significantly greater biomass and root:shoot ratios than the germinants in live soil ($p = 0.034$, Table 1, Figure S4).

Microbial presence did not affect seedling survival under drought ($p > 0.30$, Table 2, Figure 3a), but there were strong interactions between precipitation and microbial presence on seedling biomass and specific root length (SRL). Under ambient conditions seedlings had 59% less total biomass in live than autoclaved soil ($p < 0.001$), but there were no differences under drought ($p = 0.867$, Table 2, Figure 3b). Seedling SRL, on the other hand was affected by microbial presence only under drought conditions: the seedlings in live soil had 40% lower SRL than those in autoclaved soil under drought ($p = 0.018$) and there were no differences in ambient conditions ($p = 0.190$, Table 2, Figure 3c). Seedling root:shoot ratio did not significantly differ by microbial treatment ($p > 0.10$, Table 2, Figure S3).

Effect of host-microbial association history on plant drought responses

The initial bulk and rhizosphere microbial treatments did not differ in bacterial community composition ($p > 0.55$; Figure 5), diversity ($p > 0.7$, Figure 4a & 4d), or soil nitrogen content (nitrate $p = 0.251$, ammonium $p = 0.851$, Figure S2a & S2d). Additionally, the inocula

type had no significant effect on any germinant or seedling responses (Table 1 & 2, Figure 2 & 3) or affect plant responses through interactions with precipitation regime (Table 1 & 2) or life-stage (Table S4).

Effect of drought and plant life-stage on inoculated bacterial communities

As predicted, drought altered bacterial community composition in both life-stages but had surprisingly little effect on bacterial diversity. Drought had no effect on bacterial diversity in germinant soils ($p > 0.5$, Table 3, Figure 4c & 4f), but marginally reduced diversity in the seedling soils (bacterial richness $p = 0.058$, Table 3, Figure 4b & 4e). The inoculated rhizosphere and bulk soil communities had similar responses to drought for both life-stages (precip.×mic.inoc.×life-stage $p > 0.06$, Table S4), with precipitation explaining 12% and 15% of the variation in the final germinant and seedling bacterial communities, respectively (Table 3, Figure 5a & 5b). Differences in the communities were driven by a drought-induced enrichment of taxa belonging to diverse bacterial phyla, including Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Gemmatimonadetes and Proteobacteria (Table S7 & S8, Figure S7). Approximately half of the OTUs enriched under drought for both plant life-stages were Actinobacteria, a phylum that composed ~1.65% of the total reads for either life-stage's bacterial community.

We also investigated shifts in the trajectory of the final rhizosphere and bulk soil communities by comparing their composition to the initial soil inocula. Both microbial treatments diverged from the initial soil inocula by the end of the experiment (Figure 4c & 4d), but the drivers of these changes differed by life-stage (mic.inoc.× life-stage $p = 0.006$, Table S4). The microbial association history explained 29% of the variation in the final germinant-associated bacterial communities but had no effect on the structure of the final seedling communities (Table 3, Figure 4a & 4b). Changes in the seedling-associated bacterial communities were driven more by precipitation than inocula type, such that the two inocula communities remained similar under ambient conditions but diverged under drought (Table 3, Figure 5c). In contrast, the germinant-associated inocula communities diverged in both precipitation regimes (Table 3, Figure 5d). Under both precipitation treatments, the bulk soil bacterial community was more different from its initial community than the distance between the final and initial rhizosphere community. The larger shift in the final bulk than rhizosphere communities was likely driven by a greater loss of bacterial taxa in the bulk soil: the final bulk bacterial community had 50% fewer taxa at the end of the experiment, while the rhizosphere community only lost 33% of the original taxa (Figure 4a & 4c).

Discussion

Microbial presence affects germination, but not seedling growth

We first hypothesized that microbial presence would improve switchgrass germination, growth, and survival under drought. We found partial support for this since microbial presence increased germination rate and survival but not seedling growth under both precipitation regimes. Other studies also report increased switchgrass germination with microbial presence, but these studies used fungal isolates (Debebe, 2005; Ghimire et al., 2009; Ghimire & Craven, 2011). Though the mechanism for increased switchgrass germination in live soils is unknown, it could be driven by microbes that produce germination-stimulating hormones or suppress seed-borne pathogens (reviewed by Schiltz, Gaillard, Thiombiano, Mesnard, & Gontier, 2015). The germinants in live soil also had greater survival under drought, perhaps through associations with microbes that produce extracellular polysaccharides (EPSs) that can maintain soil moisture near the seed (Roberson & Firestone, 1992; Sandhya, Z., Grover, Reddy, & Venkateswarlu, 2009). It is also possible that these germinant responses were influenced by greater soil nitrogen in the autoclaved soils, but studies show that elevated nitrogen levels are more likely to increase – not decrease – germination and survival (Fawcett & Slife, 1978; Mullen, Kassel, Bailey, & Knapp, 1985; Sexsmith & Pittman, 1963; Monaco et al., 2016).

We observed few interactions between precipitation regime and microbial presence on plant responses, yet microbial benefits to germination trended towards being stronger in ambient conditions. This result is contrary to our initial hypotheses that microbial benefits would be greater under drought, but may be influenced by the field-collected inoculated microbial communities being pre-conditioned to ambient, not drought conditions. Previous work suggests that microbial communities from water-stressed environments provide more benefits to plants under drought (Giauque & Hawkes, 2013; Lennon, Aanderud, Lehmkuhl, & Schoolmaster, 2012; Timmusk et al., 2011). Because the microbial inocula in this study were collected from annually fertilized fields experiencing above-average rainfall during the sampling month, it is plausible that they had reduced capacity to increase germination and growth under drought.

We found little evidence that microbes increased growth of switchgrass seedlings. Surprisingly, seedlings in autoclaved soil were six times larger than those in live soil, but autoclaved soils also increased soil nutrients (Figure S2b). It is possible that stimulated plant growth could have been driven by methodological artifacts of increased soil nutrients, not microbial absence. We also saw higher nitrogen in autoclaved drought soils, but water stress may have prevented stimulated growth. Seedlings under drought did have a higher specific root length (SRL) in autoclaved soils. Investing in long, thin roots (high SRL) is a strategy plants use to increase root surface area and, thus, their ability to access limted resources (Comas, Becker, Cruz, Byrne, & Dierig, 2013). Though previous studies have found increased SRL under autoclaved driven nutrient flushes (Petipas et al., 2020), we predict that the drought-exposed seedlings invested in longer roots to compensate for the lack of beneficial microbes (e.g. EPS-producing bacteria or mycorrhizal fungi; Augé, 2001; Sandhya et al., 2009) because SRL only responded to microbial presence under drought. In contrast, rhizosheath formation was not influenced by drought or microbial inocula, perhaps because it is only important under more severe droughts (Liu et al. 2019). Future studies should investigate how microbial interactions influence plant root development under drought, as the impact of mycorrhizae on root traits is far from resolved (Brejda, Moser, & Vogel, 1998; Ghimire et al., 2009; Hetrick, Wilson, & Leslie, 1991).

In contrast to microbial presence, we found microbial association history did not affect any germinant or seedling responses. We initially hypothesized rhizosphere soils would exacerbate plant drought stress because host-specific pathogens in the rhizosphere inocula would limit switchgrass growth more than the bulk soil inocula, but instead both inocula types limited switchgrass growth. The seedlings in both live microbial treatments were smaller than those in the autoclaved soil, perhaps due to less soil nitrogen as discussed previously, or perhaps due to greater pathogen pressure. Previous work on switchgrass suggests that host-specific pathogens are common and have large effects on early switchgrass growth (Bauer et al., 2015; Hawkes et al., 2013; McKenna et al., 2018). Our study expands upon previous work by suggesting that the host-associated characteristics of rhizosphere communities may extend beyond the rhizosphere in perennial grasslands. This overlapping nature of rhizosphere and bulk soils is likely unique to mature perennial stands (Liang et al., 2016; Vieira et al., 2019), as studies report greater divergence in rhizosphere and bulk soil taxonomy and function in annual cropping systems (Fan et al., 2017; Marasco et al., 2012; Shi et al., 2016).

Drought and seed germination alter bacterial composition, and inoculant community assembly

As we hypothesized, drought altered the structure of the inoculated bacterial communities for both life-stages. These drought-induced shifts in the soil communities were driven by an enrichment of monoderm bacterial phyla (Actinobacteria, Firmicutes, Chloroflexi) groups which have been previously observed to increase under drought across diverse plant hosts (Xu & Coleman-Derr, 2019). Actinobacteria had the greatest enrichment under drought, and this group is suggested to increase drought tolerance in C4 grasses like switchgrass (Fitzpatrick, Copeland, Wang, Guttman, & Kotanen, 2018; Naylor, Degraaf, Purdom, & Coleman-Derr, 2017). Our study highlights that it is still unclear how these drought-tolerant taxa may increase plant drought tolerance; despite their enrichment, we found only one plant response – germinant survival – to increase in live soils under drought. To better understand how these drought-enriched microbial groups influence plant drought tolerance, we suggest future studies assess their microbial traits that can affect plants, such as EPS production, and, further, identify if plant interactions with these groups change throughout development, as our study suggests.

In addition to drought-induced changes in the inocula communities, an unexpected finding was how their assembly differed by life-stage. Initially, the bulk and rhizosphere communities did not differ in composition or diversity. By the end of the experiment, these two inocula communities diverged in germinant soils with changes in bulk soil treatments driving the divergence. It is possible this shift in community composition mirrors the transition from bulk to rhizosphere through root exudates selecting for less diverse and more copiotrophic communities (Philippot et al., 2013). Such selective secretions are released during both germination (Barret et al., 2015) and root growth (Sasse, Martinoia, & Northen, 2018a; Zhalnina et al., 2018), but few studies focus on germination-stage processes (but see Torres-Cortés et al., 2018). Differences in the chemical composition of seed and root exudates could contribute to differences in the germinant- and seedling-associated communities (Vančura & Stotzky, 1976). In fact, some bacterial isolates grow more in response to seed than root exudates (Yaryura et al., 2008; Zheng & Sinclair, 1996), further suggesting that seed exudates are an understudied yet critical driver of microbial assembly.

The lack of divergence in the inoculated bacterial communities during seedling growth may have also been influenced by their initial six days in sterile conditions. Germinating in sterile conditions is common practice for many greenhouse studies (Fry et al., 2018; Naylor et al., 2017; Zolla et al., 2013), yet because root exudates are influenced by microbial crosstalk and differ in sterile conditions

(Oburger & Jones, 2018; Sasse et al., 2018), it is likely that an initial period of growth in sterile conditions could alter root exudates and, in turn, the seedlings' effect on microbial interactions after transplanting. No studies to our knowledge have evaluated how germinating in sterile conditions affects later plant-microbial interactions, but this potentially confounding effect could contribute to issues with scaling results from greenhouse (sterile germination) to field studies (germination in live soil)(Beals et al., 2020; Forero, Grenzer, Heinze, Schittko, & Kulmatiski, 2019).

Implications and Conclusions

We show that soil communities may help overcome challenges with seedling establishment that plague many native plant restorations (James et al., 2011; Lauenroth & Adler, 2008) and agricultural landscapes (Ellis, 1992; Farooq, Barsa, & Wahid, 2006). We found that plants are most sensitive to microbial communities during germination and that these microbial interactions may alleviate early-stage drought stress. Though microbial communities may not have strong effects on initial plant growth, they alter longer-term drought tolerance through effects on plant traits such as SRL. We also found evidence that the interaction between microbial history and germination is a critical driver of microbiome assembly, since the bacterial communities unexpectedly diverged in the soil with germinating seeds. These potentially stronger plant-microbial interactions during germination could be harnessed to promote early-stage microbial assembly and drought tolerance, thus furthering restoration success. Furthermore, germination effects on microbial communities may corroborate studies that find seed-based microbial inoculants, which interact with germinating seeds, to outperform inoculants applied to soils of established seedlings (Rubin et al., 2017). In sum, germinating seeds begin the selection of soil microbial communities and the feedbacks that may have lasting effects on plant population establishment and productivity in the face of drought.

Declarations

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Author contributions: TCU and LB conceived of the project, with input from SE; all authors contributed to experimental design; TCU, LB, HE, and SH conducted the experiment and performed all lab assays; LB analyzed the data; TCU and LB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data Availability: All plant and soil data are available at https://github.com/ldereske/Ulbrich_etal_2021_Switchgrass_seedlings/Switchgrass; DNA Fasta files are available at NCBI Sequence Read Archive, accession number PRJNA720265.

Code Availability: https://github.com/ldereske/Ulbrich_etal_2021_Switchgrass_seedlings/Switchgrass

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Tables

Table 1. Germinant responses to precipitation, microbial presence (autoclaved vs. bulk), and microbial inocula association history (bulk vs. rhizosphere). Chi-square, F-statistic and p-values provided; significant p-values ($p < 0.05$) are bolded.

	Factor	Germination rate (Number of seeds that germinated/pot)	Survival (given germination)	Days to first germination	Germinant total biomass (given germination)	Germinant root:shoot
		χ^2, *p*	χ^2, *p*	*F*, *p*	*F*, *p*	*F*, *p*
Microbial Presence	Precipitation	0.803, 0.370	0.13, 0.722	**$4.28_{1,29}$, 0.047**	**$147.58_{1,22}$, <0.001**	**$9.70_{1,22}$, 0.005**
	Microbial Presence	**4.15, 0.042**	**5.31, 0.021**	**$8.82_{1,29}$, 0.006**	**$152.17_{1,22}$, <0.001**	$2.13_{1,22}$, 0.159
	Precip.* Mic.Presence	0.019, 0.889	1.07, 0.300	$1.85_{1,30}$, 0.183	**$187.19_{1,22}$, <0.001**	**$8.87_{1,22}$, 0.007**
Microbial Association History	Precipitation	**5.68, 0.02**	0.40 0.529	$1.07_{1,40}$, 0.307	**$8.71_{1,34}$, 0.006**	$1.00_{1,33}$, 0.324
	Microbial Inocula	0.015, 0.90	0.04 0.843	$40.26_{1,40}$, 0.867	$0.31_{1,34}$, 0.581	$0.85_{1,32,}$ 0.363
	Precip.* Mic.Inocula.	1.42, 0.23	0.001 0.994	$0.007_{1,39}$, 0.934	$0.29_{1,34}$, 0.589	$0.48_{1,31}$, 0.492

Table 2. Seedling responses to precipitation, microbial presence (autoclaved vs. bulk), and microbial inocula association history (bulk vs. rhizosphere). Chi-square, F-statistic and p-values provided; significant p-values ($p < 0.05$) are bolded. 'NA' indicates factors not included in the model due to the models being a perfect fit (i.e. 100% survival under ambient precipitation).

	Factor	Total biomass (g)	Seedling survival	Specific root length (cm g dry root^{-})	Seedling root:shoot	Seedling rhizosheath soil mass (g soil g dry root^{-1})
		F, *p*	χ^2, *p*	*F*, *p*	*F*, *p*	*F*, *p*
Microbial Presence	Precipitation	**$13.25_{1,56}$, <0.001**	NA	$4.84_{1,10}$, 0.052	$1.11_{1,50}$, 0.297	$1.49_{1,10}$, 0.253
	Microbial Presence	**$10.34_{1,56}$, 0.002**	0.80, 0.370	$1.61_{1,10}$, 0.233	$0.06_{1,50}$, 0.810	$2.49_{1,10}$, 0.065
	Precip.* Mic.Pres.	**$8.86_{1,56}$, 0.004**	NA	**$9.53_{1,10}$, 0.011**	$1.58_{1,50}$, 0.214	$0.09_{1,10}$, 0.772
Microbial Association History	Precipitation	$1.01_{1,52}$, 0.319	NA	$0.17_{1,12}$, 0.686	$2.49_{1,50}$, 0.121	$0.02_{1,15}$, 0.886
	Microbial Inocula	$0.53_{1,52}$, 0.469	0.87, 0.351	$0.002_{1,12}$, 0.962	$0.28_{1,50}$, 0.600	$1.56_{1,15}$, 0.230
	Precip.* Mic.Inoc.	$0.05_{1,52}$, 0.820	NA	$1.46_{1,12}$, 0.250	$0.06_{1,50}$, 0.815	$0.13_{1,15}$, 0.724

Table 3. Final germinant- and seedling-associated bacterial community details from pots inoculated with live soil microbial treatments (bulk and rhizosphere). F-statistic and p-values provided; significant p-values ($p < 0.05$) are bolded.

	Factor	Bacterial richness	Bacterial Inverse Simpson	Bacterial community composition (Weighted Unifrac)		Distance from initial community (Weighted Unifrac)
		F, p	*F, p*	R^2	*p*	*F, p*
Germinant	Precipitation	$0.05_{1,12}$, 0.828	$0.06_{1,12}$, 0.811	**0.12**	**0.010**	$0.01_{1,7}$, 0.914
	Microbial Inocula	**$13.81_{1,12}$, 0.003**	**$12.52_{1,12}$, 0.004**	**0.29**	**0.020**	**$10.21_{1,6}$,0.018**
	Precip.* Mic.Inocula.	$0.03_{1,12}$, 0.877	$0.01_{1,12}$, 0.906	0.05	0.147	$0.001_{1,7}$, 0.976
Seedling	Precipitation	$4.39_{1,12}$, 0.058	$1.05_{1,12}$, 0.325	0.15	0.089	$1.63_{1,12}$, 0.225
	Microbial Inocula	$0.04_{1,12}$, 0.838	$0.24_{1,12}$, 0.634	0.04	0.663	$0.21_{1,12}$, 0.655
	Precip.* Mic.Inocula.	$2.14_{1,12}$, 0.169	$3.66_{1,12}$, 0.080	0.12	0.157	**$5.12_{1,12}$, 0.043**

Figures

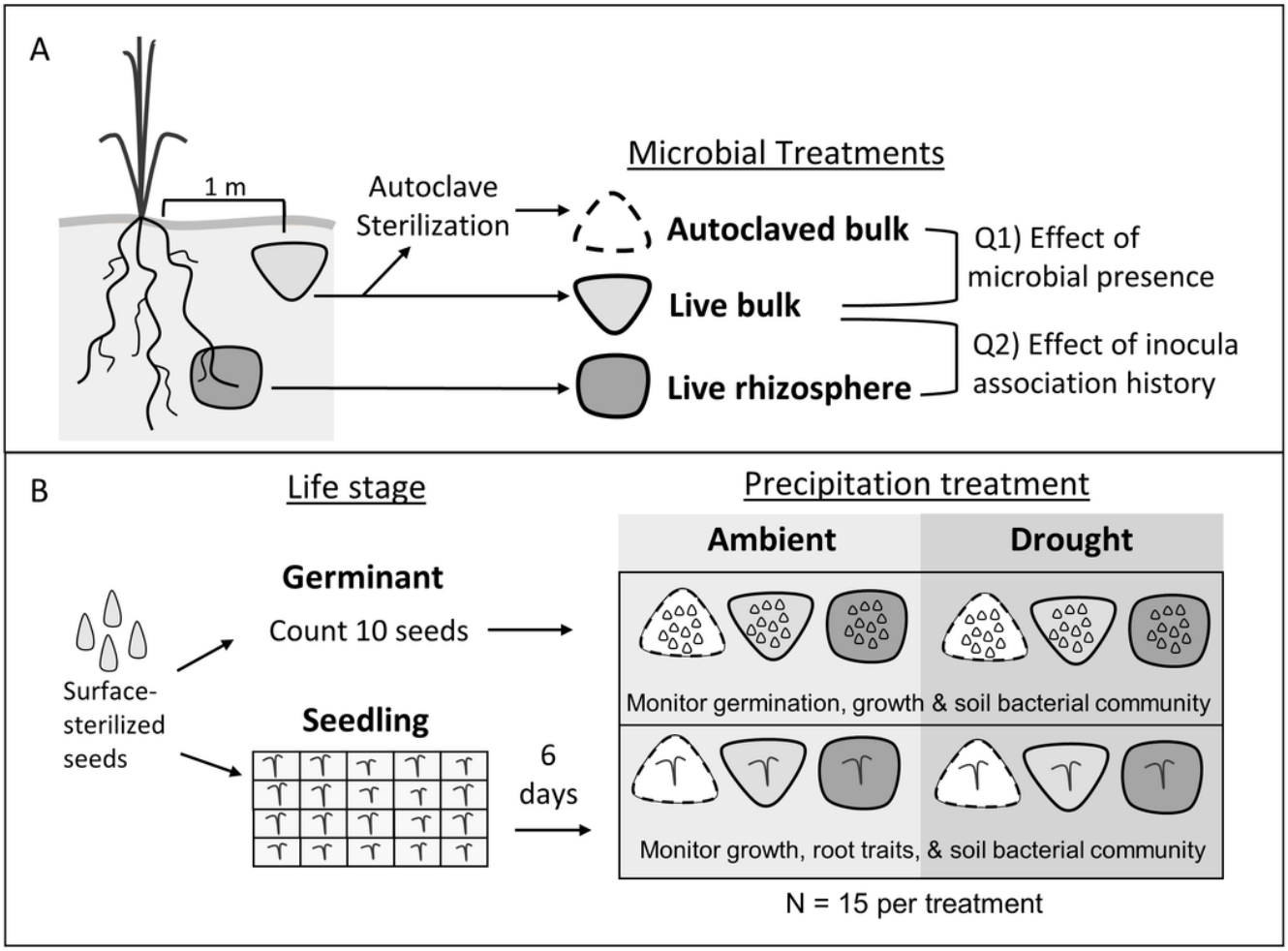

Figure 1

Experimental design showing A) three microbial treatments that were used to evaluate the effect of (Q1) microbial community presence and (Q2) inocula association history on B) switchgrass germination and growth and changes in soil bacterial communities.

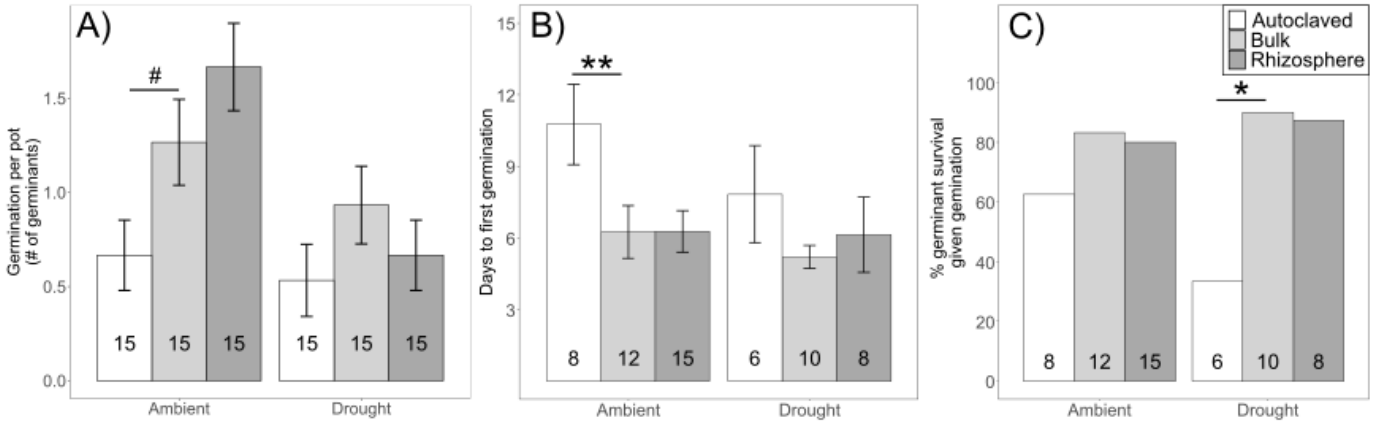

Figure 2

Effect of precipitation and microbial treatments on switchgrass germinant responses: A) proportion of pots with at least one germinant, B) number of days until first germination, and C) percent seed survival given germination. Error bars represent ±1 standard

error from the mean and numbers indicate replicates analyzed for each treatment. Horizontal bars and asterisks represent significant pairwise differences, * p < 0.05, ** p < 0.01, *** p < 0.001.

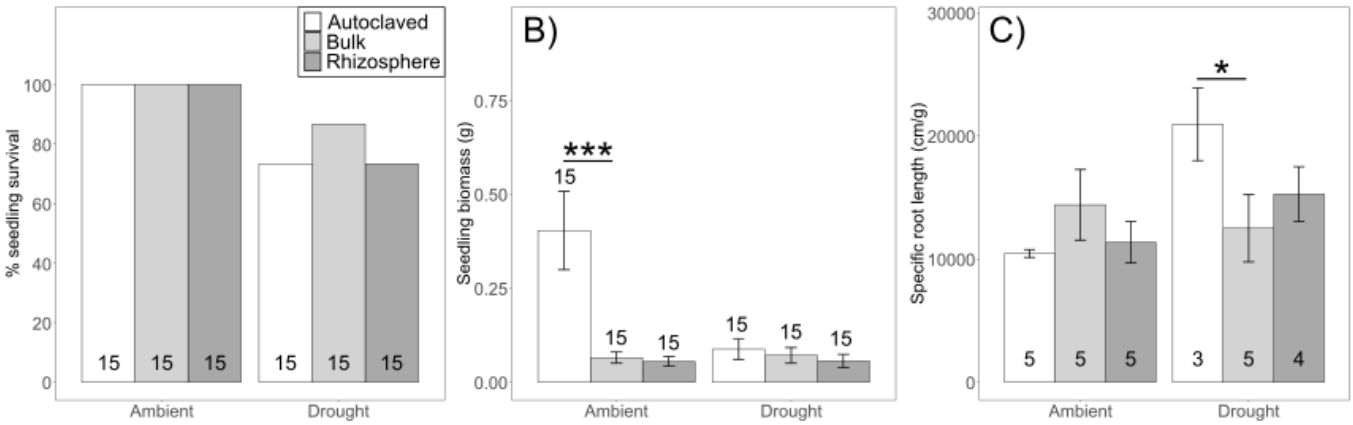

Figure 3

Effect of precipitation and microbial treatments on switchgrass seedling responses: A) percent seedling survival, B) seedling biomass, and C) specific root length. Error bars represent ±1 standard error from the mean and numbers indicate number of replicates analyzed for each treatment. Horizontal bars and asterisks represent significant pairwise differences, when present * p < 0.05, ** p < 0.01, *** p < 0.001.

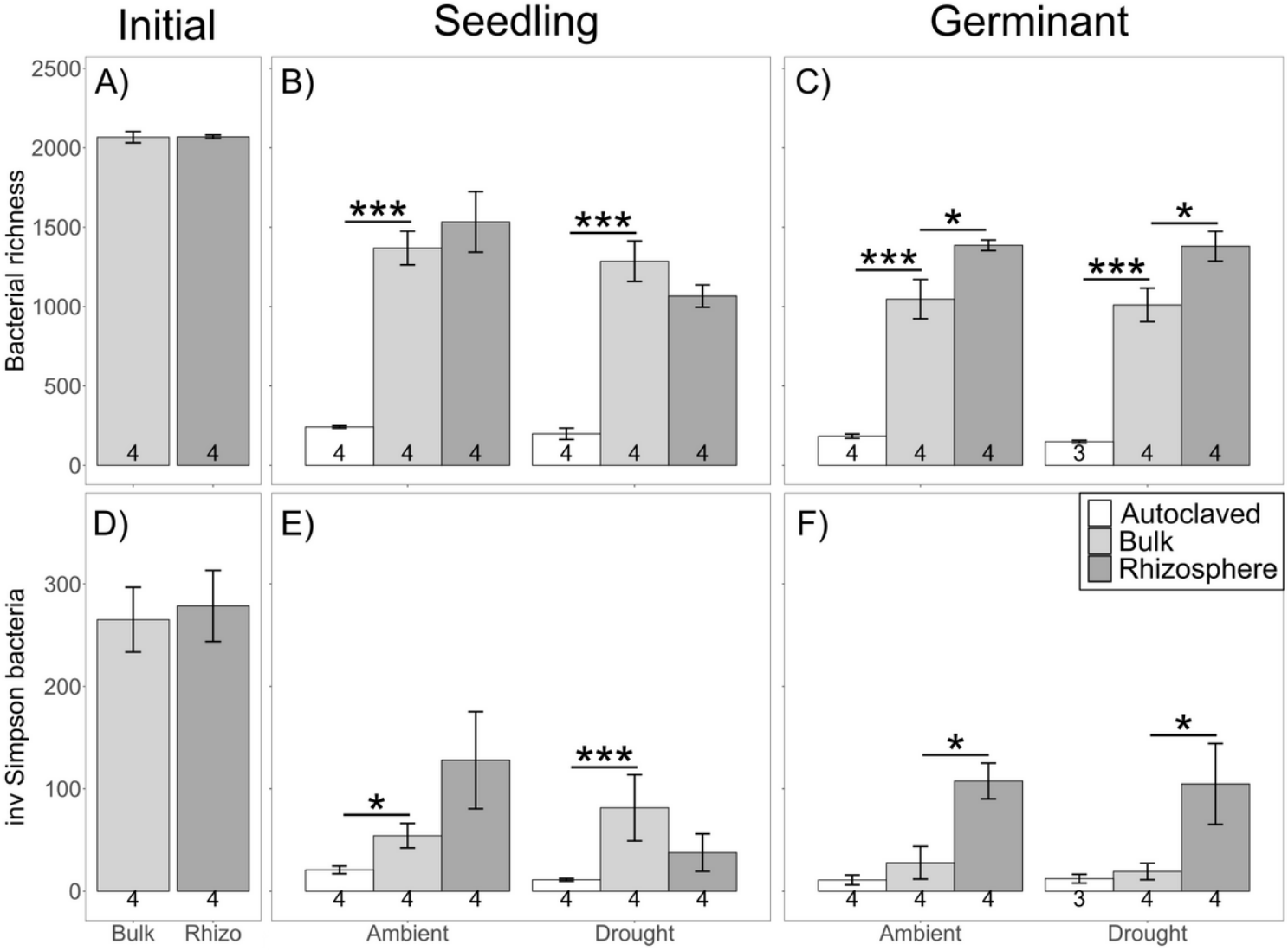

Figure 4

Differences in bacterial richness and Inverse Simpson diversity of initial live soil inocula (A,D) and the effect of precipitation and microbial treatment on final seedling- or germinant-associated bacterial richness (B,C) and Inverse Simpson diversity (E,F). Error bars represent ±1 standard error from the mean and numbers indicate replicates analyzed for each treatment. Horizontal bars and asterisks represent significant pairwise differences, * p < 0.05, ** p < 0.01, *** p < 0.001.

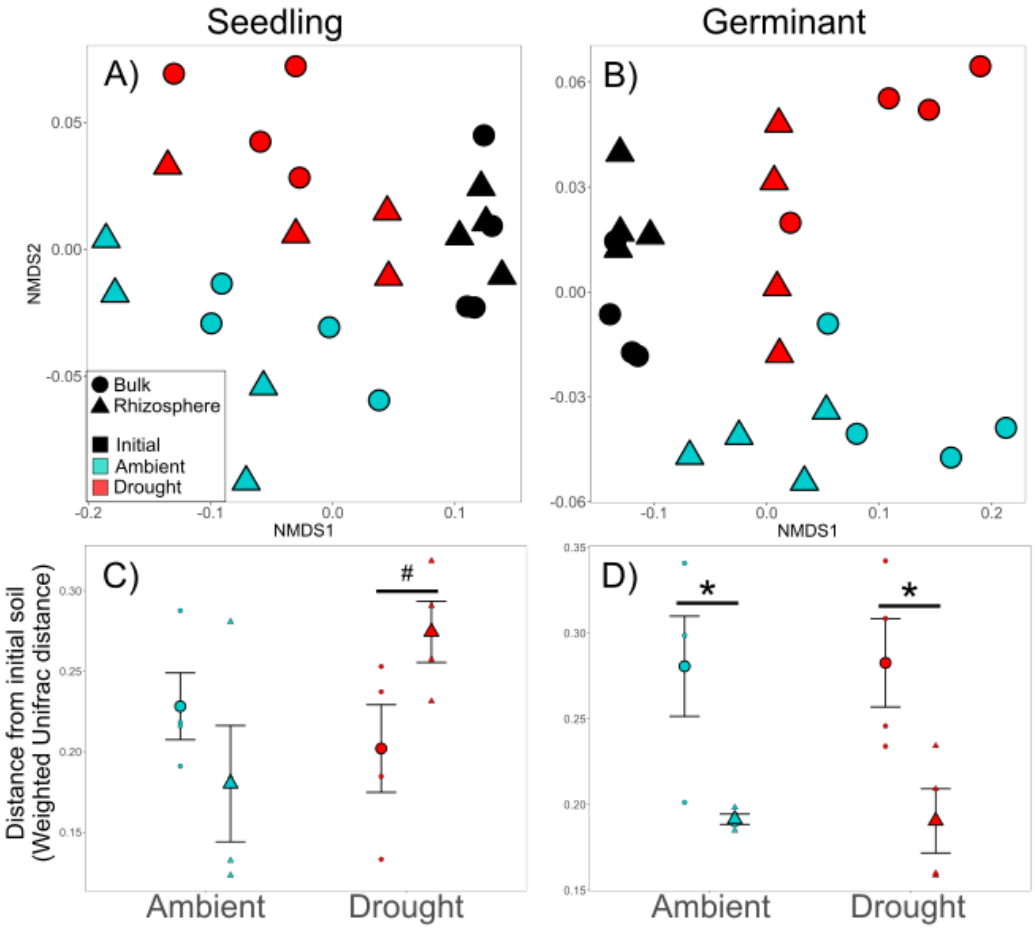

Figure 5

Weighted Unifrac NMDS represents differences between initial soil inocula (black) and final bacterial community composition driven by precipitation (drought – red or ambient – blue) and microbial treatment (bulk soil – circle, rhizosphere soil – triangle) for switchgrass A) seedling- and B) germinant-associated bacterial communities. Pairwise Weighted Unifrac distances between final seedling- (C) and germinant-associated (D) bacterial communities compared to the initial bulk (circle) and rhizosphere (square) inocula after exposure to ambient (blue) and drought (red) precipitation treatments. Each point represents the dissimilarity from each rhizosphere or bulk soil sample (n = 1 sample) to the initial soil from the same block at the start of the experiment. Bars represent ±1 standard error from the mean of each treatment. # p < 0.10, * p < 0.05, ** p < 0.01, *** p < 0.001. Autoclaved soils were excluded from these figures but are included in supplemental figures S6.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ULBRICHetal2021PlantSoilSupplemental.pdf