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RESEARCH

Effects of Drought Stress on the *Panicum hallii* Microbiome over a Growth Cycle

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Abstract

Forecasted increases in drought events resulting from climate change pose a serious threat to bioenergy crop yields, and improving the resilience of crops against water stress is essential to bioenergy development. Many plants exhibit a conserved drought response that is partially mediated by their associated microbiomes. *Panicum hallii*, a close relative of the promising bioenergy crop switchgrass, is a model organism for this grass due to its genetic tractability and short generation time. Nevertheless, little is known regarding the effects of drought on its associated microbiome. A comprehensive analysis of bacterial and archaeal microbiomes of drought-exposed *P. hallii* combining 16S rRNA amplicon and shotgun metagenomic data supports a drought response similar to that observed in other crops and offers new insights. Over the course of one growth cycle, exposure of *P. hallii* to drought led to a decrease in bacterial diversity in the rhizosphere-associated microbiome, corresponding with a decrease in Proteobacteria (Pseudomonadota) and Bacteroidota and an increase in Actinobacteriota (Actinomycetota). Genome-resolved metagenomic analysis further revealed an enrichment in Patescibacteria and specific Bacteroidota under drought. Functional analysis linked the changes in community structure with increased capacities for complex carbohydrate metabolism, molecular salvaging, chemotaxis, and antioxidant accumulation. By confirming that drought stress effects similar changes to the *P. hallii* rhizosphere microbiome as observed in switchgrass and other plants, we reinforce the strength of *P. hallii* as a model organism for crop improvement studies and emphasize the value of a dual amplicon and shotgun-based analysis for a comprehensive interpretation of community composition.

Keywords: Actinobacteria, Actinobacteriota, Actinomycetota, bioenergy, drought, *Panicum*, plant microbiome

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Data availability: Sequencing data for individual metagenome samples was deposited in the NCBI Short Read Archive (SRA) under the BioProject accession numbers PRJNA710742 to PRJNA710965. Specific BioProject, BioSample, and SRA accession numbers are provided in Supplementary Table S9.

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e-Xtra: Supplementary material is available online.

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As climate change worsens due to increasing carbon dioxide in the atmosphere, biofuels represent a key strategy for carbon-neutral energy production. At the same time, more frequent drought events caused by climate change are likely to diminish crop yields (IPCC 2023), making it imperative that biofuel crops selected for development be resilient to drought and capable of cultivation on marginal lands as extreme heat and arid conditions become increasingly common. One crop at the forefront of biofuel production is switchgrass (*Panicum virgatum*), prized for its drought tolerance and high biomass yield (Beschoren da Costa et al. 2022; Lovell et al. 2018; Schmer et al. 2008; Somerville et al. 2010). *Panicum hallii*, a close relative of switchgrass, serves as a genetic model for C4 perennial grasses, as it is more genetically tractable, has a shorter generation time, and is smaller in stature, making it more amenable to laboratory studies (Lovell et al. 2018; Lowry et al. 2013, 2015; Meyer et al. 2012). This self-fertilizing grass can be found across a geographically expansive range in the southwest United States and northern Mexico and has two naturally occurring ecotypes with

distinct morphologies that mimic those of switchgrass: the xeric variety associated with upland habitats (*P. hallii* var. *hallii*) and the mesic variety found in lowland habitats (*P. hallii* var. *filipes*) (Gould 1975; Khasanova et al. 2019; Lowry et al. 2014, 2015; Porter 1966; Singer et al. 2019; Waller 1976).

To establish *P. hallii* as a model for *P. virgatum* and generally for crop improvement studies, it is necessary to evaluate and compare the plant's response to variable environmental conditions. In particular, understanding the impact of soil water availability on biomass production will be critical to the development of resiliency strategies (Beschoren da Costa et al. 2022; Naylor and Coleman-Derr 2018). *P. hallii* var. *filipes* typically grows in mesic regions of the southern United States, which can experience anywhere between 13 and 76 cm of rainfall annually (Palecki et al. 2021). It is not native to California, and thus, exposure to drought in a region that typically does not experience more than 38 cm of rainfall annually will likely produce a stress response.

Plant fitness and microbiome composition are determined by a myriad of biotic and abiotic factors with complex interactions (Barnes and Tringe 2022; Bennett and Klironomos 2019; Compant et al. 2019; Naylor and Coleman-Derr 2018). Water scarcity directly impacts microbes and plants by reducing soil pore connectivity, increasing solute concentration, and limiting substrate diffusion, triggering adaptive responses as well as a feedback loop between host and microbe (Gebauer et al. 2022; Naylor and Coleman-Derr 2018; Schimel et al. 2007; Stark and Firestone 1995; Trivedi et al. 2020). Plants undergo physiological changes in response to prolonged conditions of water limitation that in turn affect their interactions with nearby microbes. These responses include changes in root morphology, carbon efflux to soil, and the root exudate profile (Hasibeder et al. 2015; Naylor and Coleman-Derr 2018). Enhancing root growth to access deeper parts of the soil profile with higher moisture levels will introduce new bacterial species to the rhizosphere environment, expanding the range of functional diversity from which to recruit (Delmont et al. 2011; Kawasaki et al. 2016; Naylor and Coleman-Derr 2018; Zhang et al. 2017). As the rate of translocation of newly assimilated carbon to roots decreases and the composition of exudates alters, resilient bacteria with the ability to adapt and utilize a wider range of metabolites are likely to be recruited (Badri et al. 2013; Naylor and Coleman-Derr 2018).

It is well established that the root-associated microbiome plays a significant role in improving the drought tolerance of plants by facilitating increased nutrient uptake and shoot biomass, as well as through osmolyte production (Khasanova et al. 2023; Mendes et al. 2013), and these stress responses are highly conserved among various plant hosts (Hestrin et al. 2021; Naylor and Coleman-Derr 2018; Xu and Coleman-Derr 2019). In fact, microbes have even been shown to be responsible for facilitating the local adaptations to varying precipitation levels that have led to divergence between the *P. hallii* var. *hallii* and *P. hallii* var. *filipes* ecotypes (Khasanova et al. 2023). Nevertheless, the effects of drought on the *P. hallii*-associated microbial community over a growth cycle have not been formally studied. Here, we explored the changes in diversity and structure that occur within the rhizosphere bacterial and archaeal microbiome of *P. hallii* var. *filipes* under drought stress. Although fungi are known to enhance the drought tolerance of plants (Ruiz-Lozano et al. 2016; Yue et al. 2024), we omitted them from this analysis, as it has been shown that fungal community members are less sensitive to drought compared with the bacterial community (Barnard et al. 2013; de Vries et al. 2018; Gao et al. 2022; Sarkar et al. 2022). We also refrained from analyzing other plant-associated eukaryotes, due to the fact that their impact on plant growth is less understood and that most prior studies focused their investigations on bacterial and archaeal community members (Hestrin et al. 2021; Mendes et al. 2013; Xu and Coleman-Derr 2019). A dual amplicon and shotgun-based approach was employed for robust assessment of community composition, as well as to investigate key taxa for plant growth-promoting features that may support their enrichment under drought stress.

Materials and Methods

Growth room germination

P. hallii var. *filipes* (also known as ecotype FIL2 or *P. hallii* var FIL2) seeds were vapor-phase sterilized at 20°C for 4 h according to Nguyen et al. (2016). Sterilized seeds were then germinated onto agricultural soil retrieved from the UC Davis Plant Sciences Field Station site into SC10 Cone-tainers (Stuewe and Sons), with three seeds planted per cone-tainer (see "excavation zone" in Fig. 1). The soil used in this study was classified as sandy clay loam, originating from an arid climate with no precipitation in the summer months. Germination conditions were 28°C and 12-h day/night cycles under Fluence Ray series (Fluence Bioengineering) growth lights with approximately 500 μmol/s^2 and twice daily watering. Two-week-old seedlings were transferred from cone-tainers into the field site.

Field experimental setup and sampling

The field experiment layout (38°31′57.36″N, 121°46′58.8″W) is depicted in Figure 1. The field was tilled prior to the start of the experiment to ensure homogeneity of all the soil within the designated plot area. Control and drought-treated samples were divided into subplots separated by a buffer zone of 10 ft. The decision to separate samples by treatment into two distinct subplots, as opposed to randomizing control and drought plots across the field, was justified based on the fact that it was not possible to guarantee that water from irrigated beds would not carry over into droughted beds and become accessible to drought-treated plant roots in the subsurface. Each treatment subplot was 15 × 50 ft and consisted of 10 raised beds (Fig. 1A). Each bed contained two rows of double drip irrigation spaced 20 in. apart from each other, with eight seedlings planted along each row (separated by 18-in. spacers) (Fig. 1B). Another buffer zone (10 × 110 ft.) separated the experimental zone from the excavation zone. Soil volumetric water content (VWC) sensors were installed in each bed, integrating over 5- to 12-cm soil depth. Soil tension sensors were installed at 5-, 10-, and 15-cm soil depth. A fence enclosed the entire zone to protect plants and sensor cables. A total of 320 *P. hallii* seedlings were transplanted into the beds in September 2019, evenly distributed between drought and control beds. Seedlings were given 2 weeks to acclimate in the field, with daily watering for 1 h, prior to sampling. After 2 weeks, watering was stopped for 50% of beds, whereas it was continued daily for the other 50% of beds.

Sampling of shoots and roots was conducted at eight distinct time points, with 10 replicate plants per drought/control treatment. For downstream analyses, these time points were further grouped into early- (1 to 6 days), mid- (8 to 10 days), and late-stage (14 to 29 days) time frames (Table 1). Plants were randomly sampled at each time point to minimize spatial bias between control and drought plots. Plant height and aboveground biomass were collected for each plant. Plant height was measured with a ruler in the field with the plants still in the ground. After plant height measurements, plants were destructively sampled for all other analyses. Aboveground biomass was measured by cutting the entire aboveground portion of the plant off, drying it for a week at room temperature, and then weighing the dry biomass. Soil samples were taken by excavating 5 × 5 × 5-cm^3 (depth A) and 5 × 5 × 10-cm^3 (depth B) soil quadrats per plant. Quadrats containing soil and root fragments were packed in aluminum foil and set on ice for transportation to the laboratory,

where they were kept at 4°C until further processing the next day. Roots with rhizosphere soil were separated from surrounding soil with tweezers, flash frozen in liquid N_2, and stored in 50-ml tubes at −80°C until further processing. Rhizosphere soil was defined as being attached to or immediately surrounding roots following shaking. Rhizosphere soil for DNA extraction was washed off the roots with a buffer (0.1× phosphate-buffered saline buffer with 0.1% Triton X-100) on a tabletop shaker (200 rpm for 15 min). Rhizosphere samples belonging to the same plant and depth were pooled for sequencing and stored at −80°C. Bulk soil samples were retrieved away from plants but within beds of respective treatments (within three quadrats distance, or 15 cm from the center) and at the same depths (0 to 5 and 5 to 15 cm) as the rhizosphere soil and frozen at −80°C. In total, 618 field samples were collected, including 329 rhizosphere samples (171 control and 158 drought-treated) and 289 bulk soil samples (139 control and 150 drought-treated).

TABLE 1
Experiment sampling time points and time frames

Time point	Days after drought onset	Days since germination	Time frame
0	1	37	Early
1	3	39	Early
2	6	42	Early
3	8	44	Mid
4	10	46	Mid
5	14	50	Late
6	18	54	Late
7	29	65	Late

Soil chemistry analysis

Soil samples were sent to the Soil, Water, and Forage Testing Laboratory at the Texas A&M AgriLife Extension for analysis. Three drought-exposed samples and three control samples from time points 1 and 7 (12 samples total) were tested for concentrations of nitrate-nitrogen (NO_3-N), carbon (total and organic), phosphorus, potassium, calcium, magnesium, sulfur, and sodium by standard methods (Texas A&M AgriLife Extension Service 2012). Conductivity (Rhoades 1982) and pH (Schofield and Taylor 1955) were also measured for each soil sample. In brief, NO_3-N was extracted using a 1 N KCl solution. A 10:1 ratio of 1 or 2 M KCl to <2 mm

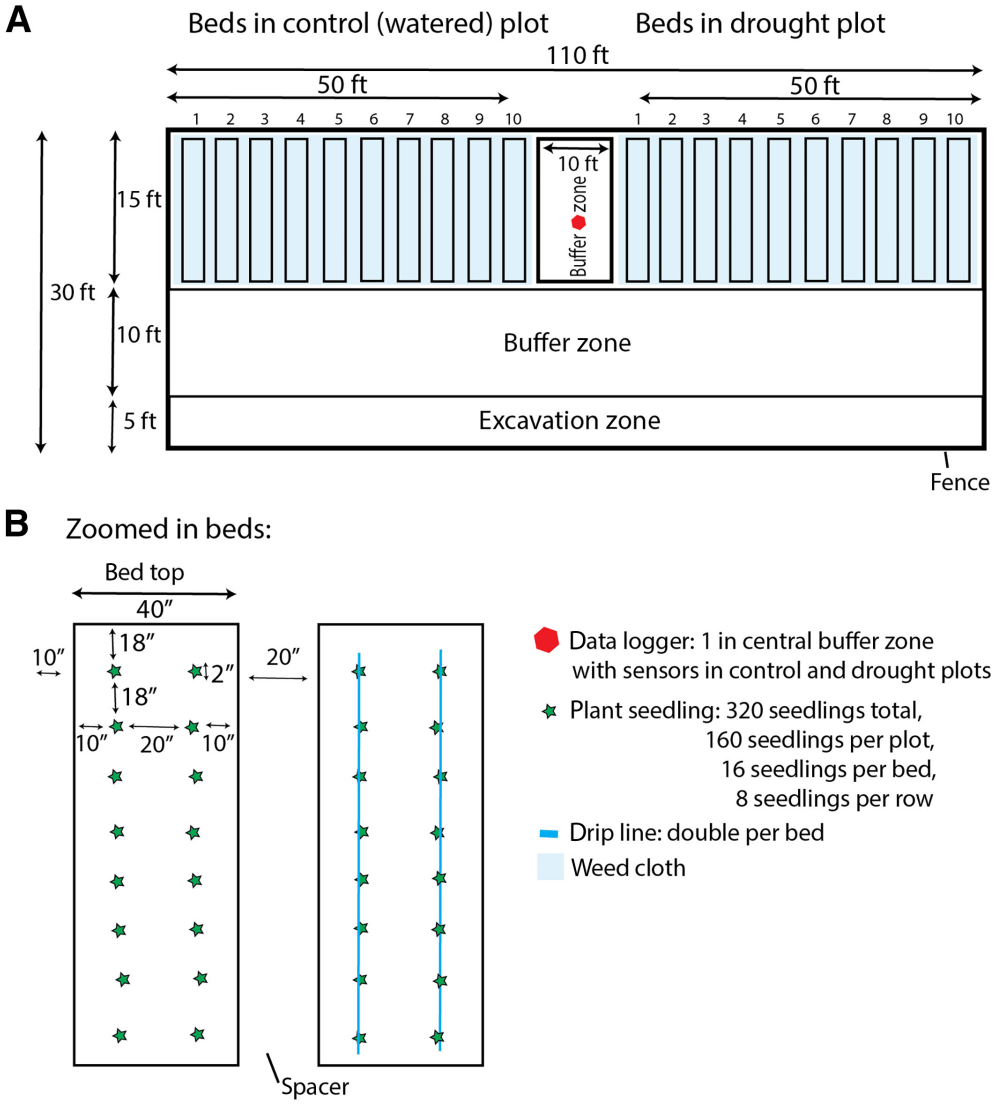

Fig. 1. Experimental design at UC Davis field site. **A,** Overview of plot setup. Seedlings were grown in the laboratory in soil retrieved from the excavation zone. **B,** Detailed layout of plant seedlings within an individual bed and spacing between beds.

pulverized soil was prepared, from which a minimum of 2 g of soil was volumetrically or gravimetrically placed in an extraction cup or flask and orbitally (1 in. orbital throw) agitated at 200 opm for 5 min, followed by filtration through a Whatman #2-equivalent filter (Kachurina et al. 2000; Keeney and Nelson 1982). Nitrate was reduced to nitrite using a cadmium column and measured by a spectrophotometer. Organic and inorganic carbon were measured by a combustion procedure after soil samples were ground to pass an 80-mesh screen (McGeehan and Naylor 1988; Schulte and Hopkins 1996; Storer 1984). Organic carbon was determined by reducing the primary sample ignition furnace to 650°C and measured directly with a total carbon analyzer (Texas A&M AgriLife Extension Service 2012). Phosphorus, potassium, calcium, magnesium, sulfur, and sodium were extracted with Mehlich III extractant, and concentrations were determined by inductively coupled plasma (Mehlich 1978, 1984). Soil electrical conductivity and pH were measured in a 1:2 soil/water extract of the soil prepared with deionized water. Following the addition of water, samples were stirred and allowed to equilibrate for at least 30 min. Conductivity was measured using a conductivity probe (umho/cm), and pH was measured using a hydrogen selective electrode. All measurements are reported on a dry soil basis only.

Amplicon and shotgun metagenomic sequencing

For rhizosphere DNA extractions, root wash solutions were centrifuged, supernatant was decanted, and 0.25 g of the resulting rhizosphere soil was used for DNA extraction using the Qiagen High-Throughput PowerSoil kit (Qiagen). For bulk soil, 0.25 g of frozen soil was extracted with the same kit. DNA concentrations were quantified using a Quant-iT dsDNA Assay Kit, and fluorescence was measured with a Tecan Infinite M1000 Pro microplate reader.

The V4 16S ribosomal RNA (rRNA) gene was amplified using a single-step PCR with custom dual-index 515F and 806R primers (Price 2022). A controlled microbiome sample was used as a positive control when establishing the primer sets and PCR conditions used for these samples, and PCR-grade water was used as a negative control on each plate. The PCR conditions were as follows: initial denaturation for 3 min at 94°C, followed by 30 cycles of denaturation for 45 s at 94°C, annealing for 10 s at 78°C and then for 1 min at 50°C, elongation for 1.5 min at 72°C, and a final elongation step for 10 min at 72°C before holding at 4°C. Resulting PCR products were quantified using a Qubit high-sensitivity DNA assay kit (Thermo Fisher Scientific), then pooled at equimolar amounts into two pools. Pools were submitted to the California Institute for Quantitative Biosciences (QB3-Berkeley) Vincent J. Coates Genomics Sequencing Lab for sequencing on an Illumina MiSeq in two 2 × 300 paired-end runs. The raw data were demultiplexed, and primers/adapters were removed using a custom Perl script, demulti.pl (Price 2022).

Shotgun metagenome library generation and sequencing was performed according to the Joint Genome Institute (JGI) standard procedures. Plate-based DNA library preparation for Illumina sequencing was performed on the PerkinElmer Sciclone NGS robotic liquid handling system. One nanogram of DNA was fragmented and adapter ligated using the Nextera XT kit (Illumina). The ligated DNA fragments were enriched with 12 cycles of PCR and purified using Coastal Genomics Ranger high-throughput agarose gel electrophoresis size selection from 450 to 600 bp. The prepared libraries were quantified using the KAPA Biosystems next-generation sequencing library qPCR kit and run on a Roche LightCycler 480 real-time PCR instrument. Sequencing of the flowcell was performed on the Illumina NovaSeq sequencer using NovaSeq XP V1 reagent kits, S4 flowcell, following a 2 × 151 indexed run recipe.

Amplicon and shotgun metagenomic data processing

For amplicon data, raw sequences from each MiSeq run were combined and processed together in QIIME2 v. 2021.11 (Bolyen et al. 2019). Raw reads were trimmed to remove primer sequences, and low-quality bases were removed from the end of both the forward and reverse reads (trimmed at first base < Q30). Remaining reads were denoised and assigned to amplicon sequence variants (ASVs) at 100% sequence similarity in DADA2 (Callahan et al. 2016). The SILVA database release 138.2 (Quast et al. 2013) was used to assign taxonomy to each ASV. It should be noted that updated phylum nomenclature for several taxa was proposed in 2018 using genome-based phylogenetic analysis, including designating Actinobacteria as Actinobacteriota, Bacteroidetes as Bacteroidota, and Proteobacteria as Pseudomonadota (Whitman et al. 2018). Actinobacteriota has since been updated to Actinomycetota in the Genome Taxonomy Database (GTDB) (Oren and Garrity 2021). However, the SILVA database used for 16S amplicon taxonomy in this study has not been fully updated to the new taxonomy system due to the lack of whole-genome data for many lineages represented only by 16S rRNA gene sequences, and therefore, the terms Actinobacteriota and Proteobacteria are referenced when discussing phylum-level classification of 16S amplicon data. In all other discussions, including those involving metagenome-assembled genomic data, which were classified using the GTDB, the phyla are referred to as Actinomycetota and Pseudomonadota, respectively.

Shotgun metagenome data were processed according to the JGI standard procedures. BBDuk v.38.94 (Bushnell 2014) was used to remove contaminants, trim reads for adapter sequences, remove trailing homopolymers containing at least 5 Gs, correct for when quality dropped to 0, and remove reads containing at least 4 N bases, reads with an average quality score <3, and reads that were not at least 51 bp in length or 33% of the full read length. Reads mapping with BBMap (Bushnell 2014) to masked human, cat, dog, and mouse references or common microbial contaminants were also removed. Read correction was performed using BBCMS v.38.90 (Bushnell 2014), discarding any reads with <2 k-mer counts, and requiring at least 60% of a read's k-mers to have a count ≥2 to pass.

Shotgun assembly and metagenome-assembled genome (MAG) recovery

Assembly of individual metagenome samples was performed using metaSPAdes v.3.15.2, with k-mer sizes of 33, 55, 77, 99, and 127 (Nurk et al. 2017). BBMap v.38.86 (Bushnell 2014) was used to map input reads to the final assembly and generate coverage information with the following options: break up FASTA reads >500 bases and map ambiguous reads with multiple top-scoring locations by selecting one top-scoring site randomly. As per the IMG Binning Pipeline (part of the DOE JGI Metagenome Workflow), genomes were binned using MetaBAT (Kang et al. 2015), with a minimum contig cutoff size of 3,000 bp and the 'superspecific' parameter for maximum specificity. The taxonomic lineage of individual contigs was determined based on IMG taxonomy, removing any contigs not affiliating with the consensus lineage. Genome completeness and contamination values were estimated using CheckM (Parks et al. 2015) and assigned quality scores (high, medium, low) as per the Genomic Standards Consortium-compliant Minimum Information on a Metagenome-Assembled Genome (MIMAG) (Bowers et al. 2017). For reference, a high-quality [HQ] MAG must be >90% complete with <5% contamination, a medium-quality [MQ] MAG must be at least 50% complete with <10% contamination, and a low-quality MAG indicates <50% completeness and <10% contamination. It should be noted that "bin" will be used to refer to any assembled collection of contigs, regardless of assembly quality, and

MAG will be used to refer to only sufficiently complete bins (MQ or HQ as determined by MIMAG standards). Bin taxonomic lineage was determined by consensus of scaffold taxonomic lineage and GTDB toolkit r220 (Chaumeil et al. 2020). Only MQ and HQ bins were loaded into IMG for analysis. For a detailed description of binning methods, please see Clum et al. (2021).

Initial assemblies of individual metagenome samples assembled with metaSPAdes recovered very few MAGs per individual metagenome sample. Only 85 out of 106 field samples generated MAGs, and most samples (60%) recovered only between one and three MAGs. The highest recovery, 10 MAGs, was achieved in only two samples. Therefore, a combined assembly of all 106 metagenome samples was performed to increase the probability of MAG recovery. According to the JGI protocol, filtered reads were co-assembled with MetaHipMer2 v.2.2.0.0-master, using k-mer sizes of 21, 33, 55, 77, and 99 (Hofmeyr et al. 2020) on 500 CPU nodes on the NERSC Perlmutter system. Contigs smaller than 500 bp were removed. Alignment information was determined by mapping reads to the assembly reference with BBTools v.38.99 (Bushnell 2014), with slightly reduced sensitivity but at a faster speed and with significantly reduced memory, and by mapping ambiguous reads with multiple top-scoring locations by selecting one top-scoring site randomly. Coverage was determined by running the pileup.sh script of the BBTools suite v.38.99, which calculates per-scaffold or per-base coverage information from an unsorted sam or bam file (Bushnell 2014).

Binning of the combined assembly was performed using the same IMG Binning Pipeline used for individual metagenome samples, as described above. In an effort to recover more MAGs for functional analysis and for additional insight, the quality of bins generated from the combined assembly was reassessed with CheckM2 (Chklovski et al. 2023). To account for slight differences in quality assessment as a result of the machine learning approach used by CheckM2, and to retain any bins that were close to 50% complete, bins were determined to be of sufficient quality for analysis if they met the MIMAG requirement for MQ when assessed by either CheckM or CheckM2. For any bins determined to meet the threshold for MQ, gene calling was performed using Prodigal v.2.6.3 (Hyatt et al. 2010), and functional annotation was performed with eggNOG-mapper v.0.5.1-2-g1032ebf using eggNOG database v.5.0.2 (Cantalapiedra et al. 2021; Huerta-Cepas et al. 2019) as part of the moducomp pipeline v.0.2-KPCT-2-g6cad6d8 (Richardson et al. 2023; Villada and Schulz 2025).

Diversity analyses

All diversity analyses were conducted in R v.4.3.3 (R Core Team 2020). Outputs from QIIME2 were merged using the *phyloseq* package (McMurdie and Holmes 2013), filtered to remove ASVs identified as eukaryota or "unassigned" at the domain level, and further filtered to remove those assigned to chloroplast or mitochondria. Several samples with low read counts (<500 reads) were removed, resulting in a dataset with an average read count of 15,520 reads per sample. Alpha diversity was calculated using the Shannon index (measure of species richness and evenness). Although the developers of *phyloseq* strongly advise against rarefying counts (McMurdie and Holmes 2013), we recognize that this is a highly debated topic, with more recent studies supporting rarefaction (Bindels et al. 2025; McMurdie and Holmes 2013; Schloss 2024; Willis 2019). To avoid bias when calculating alpha diversity metrics, each sample was rarefied to 5,000 reads, with repeated subsampling to this depth, allowing 95.4% of samples to be retained for analysis. Plots were created using *ggplot2* (Wickham 2016). Sample read counts were transformed using the center-log ratio, and community composition was compared via Euclidean distances (Aitchison et al. 2000) and visualized with principal coordinate analysis. Multivariate homogeneity of group dispersions (via betadispers) and permutational multivariate analysis of variance were performed using the *vegan* package (Oksanen et al. 2025).

For shotgun metagenome analysis, filtered metagenome reads were mapped with CoverM v.0.7.0 (Aroney et al. 2025) to a set of 1,194 dereplicated bins (including low-quality bins, from an original 1,198 bins) generated from the combined assembly of all metagenomic samples. Reads with <75% of bases aligning or with <95% identity were excluded from mapping. Resulting counts, generated from mapping reads to genomes, were used to generate an abundance matrix comparable to ASV data.

Differential abundance analyses of ASVs were performed using DESeq2 (Love et al. 2014), as well as Analysis of Compositions of Microbiomes with Bias Correction 2 (ANCOM-BC2) (Lin and Peddada 2024), with false discovery rate correction using the Benjamini–Hochberg procedure. It should be noted that despite the common practice of using DESeq2 for differential abundance analyses, it was developed for differential gene expression from RNA-seq data, and several of the assumptions made for the corresponding data and built into the model are not valid when applied to DNA sequencing data (Weiss et al. 2017). Because this differential abundance model does not account for the compositional nature of microbiome datasets, it is sensitive to the negative correlation bias and can result in a higher false discovery rate (Gloor et al. 2017; Thorsen et al. 2016; Weiss et al. 2017). The model assumes that most of the community members are not differentially abundant and that any taxa that decrease in abundance are balanced by those that increase in abundance, which does not hold true for complex and diverse environments such as soil (Weiss et al. 2017; Xia 2023). Therefore, in addition to following the standard procedure of applying DESeq2 for differential abundance analysis to facilitate accurate comparison of our results with the existing literature, we also assess differential abundance of amplicon data with the more appropriate method, ANCOM-BC2, which was developed directly for microbiome analysis. ANCOM-BC2 recognizes the inherent compositional nature of this data, controls well for false discovery rate, and has been found to produce highly consistent results across studies (Gloor et al. 2017; Lin and Peddada 2024; Nearing et al. 2022; Weiss et al. 2017). Differential abundance of MAGs was only calculated with DESeq2, as the developer of ANCOM-BC2 notes that this software was not specifically benchmarked for MAG datasets (Lin 2023). Count normalization for DESeq2 was performed using DESeq2's median of ratios method, which considers sequencing depth and RNA composition (although it was applied to microbiome data in this case: 16S rRNA composition for amplicon data and MAG composition for shotgun data). Heatmaps were generated using *pheatmap* (Kolde 2025). Additional metagenomic profiling of shotgun data was performed using sylph v.0.7.0 (Shaw and Yu 2025) and SingleM v.0.19.0 (Woodcroft et al. 2026). Quality-filtered metagenomic reads were used as input for sylph, whereas raw, unfiltered metagenomic reads were used as input for SingleM analysis, to ensure that reads were of sufficient length for analysis.

Metabolic analysis of the rhizosphere-associated bacterial and archaeal microbiome

Only MQ and HQ bins (as determined by CheckM or CheckM2) were functionally annotated and considered for analysis, for a total of 388 MAGs. The abundance of KO terms, PFAMs, and CAZymes, as annotated by eggNOG-mapper, was compared between drought-enriched and non-drought-enriched bins by a Mann–Whitney *U* test. Moducomp v.0.5.1 (Richardson et al. 2023; Villada and Schulz 2025) was used to calculate metabolic complementarity between

rhizosphere community members. Symclatron (Villada et al. 2025), a symbiont classifier software, was used to assess microbial lifestyle and detect symbionts.

Results

Impacts of drought stress on soil and plant morphology

Soil nutrient levels (Supplementary Table S1) were fairly consistent between drought-exposed and control samples and remained so over the course of the experiment. From time point 1 (3 days after drought onset) to time point 7 (29 days after drought onset), droughted samples maintained an average pH of 7 (pH of 7.2 for control samples). Conductivity increased slightly from an average of 91.7 to 120.3 μmhos/cm (increased from 100.7 to 107.7 μmhos/cm for control samples) at time point 7. Potassium levels were initially higher in droughted samples as compared with control samples, presumably due to plot heterogeneity, and decreased from 196.0 to 187.0 ppm over the 4 weeks, while slightly increasing from 178.7 to 183.0 ppm in control samples. For both experimental treatments, total organic carbon averaged 0.63%, phosphorus levels remained between 37.7 and 39.7 ppm, and magnesium levels averaged approximately 1,300 ppm throughout the experiment. Calcium levels slightly dropped from 1,441.7 to 1,371.0 ppm for droughted samples (and increased from 1,374.0 to 1,377.7 ppm for control samples) from time point 1 to 7, and sodium levels averaged between 26.3 and 27.4 ppm (slight increase from 27.5 to 33.1 ppm for control samples). Sulfur levels slightly increased from 4.2 to 7.0 ppm for droughted samples while remaining between 5.3 and 5.6 ppm for control samples. Nitrate concentrations in droughted samples ($P = 0.01$) increased from 1.3 to 6.3 ppm over the experiment while staying between 2.0 and 3.0 ppm for control samples. Statistics for soil nutrient concentrations are provided in Supplementary Table S2. Due to the small sample size per treatment group per time point ($n = 3$), normality could not be assessed, and parametric assumptions could not be met. Therefore, data are presented as individual values with means ± standard deviations, and we refrain from statistical inference given the limited replication.

Phenotypic measurements were collected for all 320 plant samples to assess the effects of the natural environment and drought on plant structure. Over the course of the 4-week experiment, plants in the drought group experienced a reduction in soil VWC to less than 25% (Fig. 2A). Given that this level approaches the permanent wilting point for sandy clay loam (approximately 20% VWC; Hanson et al. 2000; Ratliff et al. 1983) and that the VWC measurements appeared to level off several days prior to the experiment's conclusion, we can confirm a simulated drought. Plants exposed to drought ($n = 10$) initially grew slower than control plants ($n = 10$), but after 2 weeks, plants exposed to drought began growing at an even pace with watered samples (Fig. 2B). Normality was assessed using the Shapiro–Wilk test within each treatment group at each time point, confirming normal distribution in each case (data not shown). No statistically significant differences (calculated by Welch's t test) were observed between the heights of control and drought-exposed plants at any time point ($P > 0.05$ at every time point; Supplementary Table S3). Thus, restricting water access in the field indeed produced a drought, but water limitation did not negatively impact plant height. Similarly, no statistically significant differences (calculated by a Mann–Whitney U test) were observed between the biomass yields of control and drought-exposed plants at any time point ($P > 0.05$ at every time point; Supplementary Table S4). For plant biomass data, normality was also assessed using the Shapiro–Wilk test, and treatment groups at several time points showed deviations from normality (data not shown).

Influence of experimental factors on bacterial and archaeal diversity and composition

In addition to assessing the effect of drought treatment on bacterial and archaeal diversity and composition, other variables considered for their impact on the bacterial community associated with *P. hallii* include plant compartment (bulk soil versus rhizosphere; Fig. 3), depth of sampling (from 0 to 5 cm and 5 to 15 cm below the soil surface), and time point (over the course of 4 weeks) (Supplementary Fig. S1). Plant compartment ($F_{(1,513)} = 23.049$, $R^2 = 0.038$, $P = 0.001$), depth ($F_{(1,513)} = 2.962$, $R^2 = 0.005$, $P = 0.001$), treatment ($F_{(1,513)} = 1.524$, $R^2 = 0.002$, $P = 0.002$), and time point ($F_{(7,513)} = 1.431$, $R^2 = 0.016$, $P = 0.001$) all had significant effects on microbial composition, as determined by

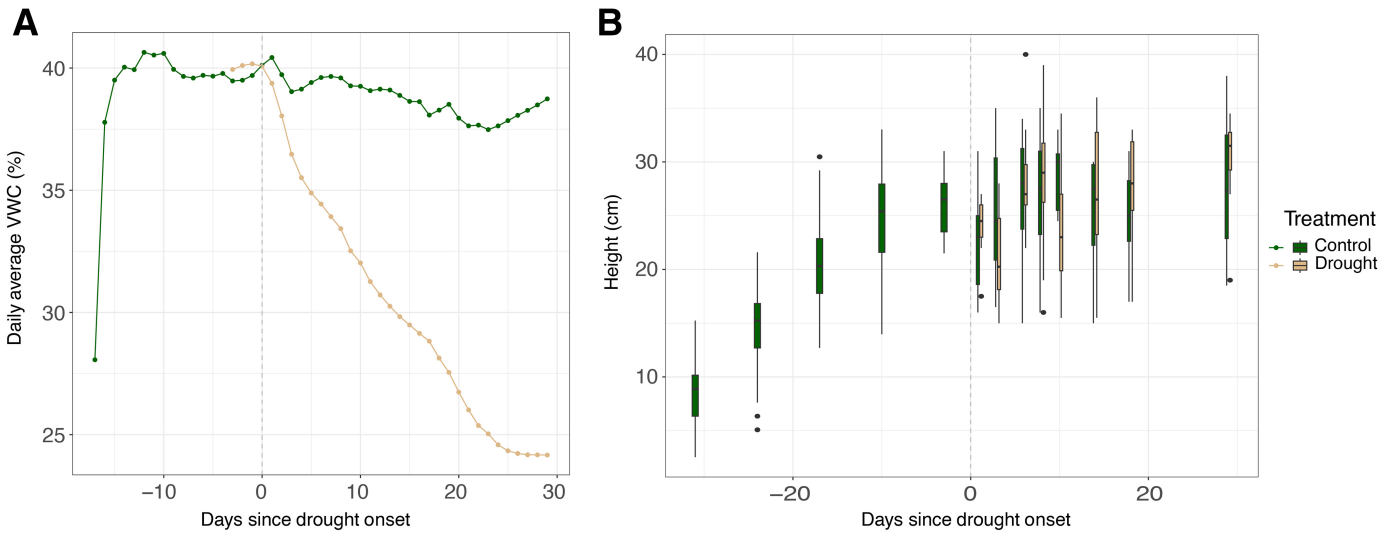

Fig. 2. Phenotypic changes observed in *Panicum hallii* control and drought-exposed samples over the duration of the experiment. **A,** Soil volumetric water content (VWC) measured daily, beginning 2 weeks prior to drought exposure, and **B,** plant heights measured beginning from 1 month prior to the start of drought exposure. Treatment groups were established 3 days prior to drought exposure.

permutational multivariate analysis of variance (Supplementary Fig. S1). Additionally, there were significant interaction effects between compartment–treatment ($F_{(1,513)} = 1.439$, $R^2 = 0.002$, $P = 0.003$), compartment–time point ($F_{(7,513)} = 1.514$, $R^2 = 0.017$, $P = 0.001$), compartment–depth ($F_{(1,513)} = 1.687$, $R^2 = 0.003$, $P = 0.001$), and treatment–time point ($F_{(1,513)} = 1.063$, $R^2 = 0.012$, $P = 0.041$) on bacterial diversity. Analysis of multivariate homogeneity of group dispersions revealed that although both control and droughted rhizosphere communities experience reduced dispersion over time, this decrease in variability was only significant under drought conditions (pairwise comparison between early- and late-stage drought permuted $P = 0.001$; data not shown). Late-stage droughted rhizosphere communities had the smallest average dispersion among all treatment × time frame groups (data not shown). However, the low variance explained by any individual variable or interaction, further evident by the sum of the resulting first two principal coordinate axes (<8%), suggests that the effect size of any of these parameters, individually or in combination, is small and diffuse over the high dimensional community structure.

Overall, a decrease in bacterial and archaeal diversity was observed in the rhizosphere over time, and this decrease was more pronounced under drought stress (Fig. 4A, right panel). In contrast, bulk soil bacterial and archaeal diversity increased over time (Fig. 4A, left panel). Regardless of experimental condition, the rhizosphere-associated microbial communities contained more Proteobacteria ($P_{adj} < 0.001$), Bacteroidota ($P_{adj} < 0.001$), Firmicutes ($P_{adj} < 0.001$), and Verrucomicrobiota ($P_{adj} < 0.001$), as well as several other less abundant phyla, whereas the bulk soil communities contained more Acidobacteriota ($P_{adj} < 0.001$) and Gemmatimonadota ($P_{adj} < 0.001$), among other less abundant phyla (Fig. 4B). Under drought, the rhizosphere community experienced an increased abundance of Actinobacteriota ($P_{adj} < 0.001$) compared with control samples, with their abundance significantly higher at later time points ($P_{adj} < 0.001$). This enrichment in Actinobacteriota under prolonged drought exposure was not seen in bulk soil and corresponded with a decrease in abundance of Proteobacteria ($P_{adj} < 0.001$), Bacteroidota ($P_{adj} = 0.02$), and Bdellovibrionota ($P_{adj} < 0.002$).

Differential abundance analysis with DESeq2 revealed that all late-drought rhizosphere communities (time points 5 to 7) had increased abundances of Actinobacteriota ($P_{adj} < 0.001$), including ASVs within *Streptomyces*, *Glycomyces*, and Micrococcaceae, whereas the identity of taxa enriched in late-stage control rhizosphere communities varied at each time point within the late time frame (Fig. 5).

Similar results were determined from differential abundance analysis with the microbiome-specific analysis framework ANCOM-BC2 (Supplementary Fig. S3). In this case, more than a dozen distinct Actinobacteriota ASVs were determined to be enriched in late-drought rhizosphere communities, of which three taxa met the pseudo-count sensitivity threshold for statistical significance. In accordance with DESeq2 results, the majority of the taxa depleted in late-stage droughted rhizosphere samples pertained to Proteobacteria (Gamma-) and Bacteroidota.

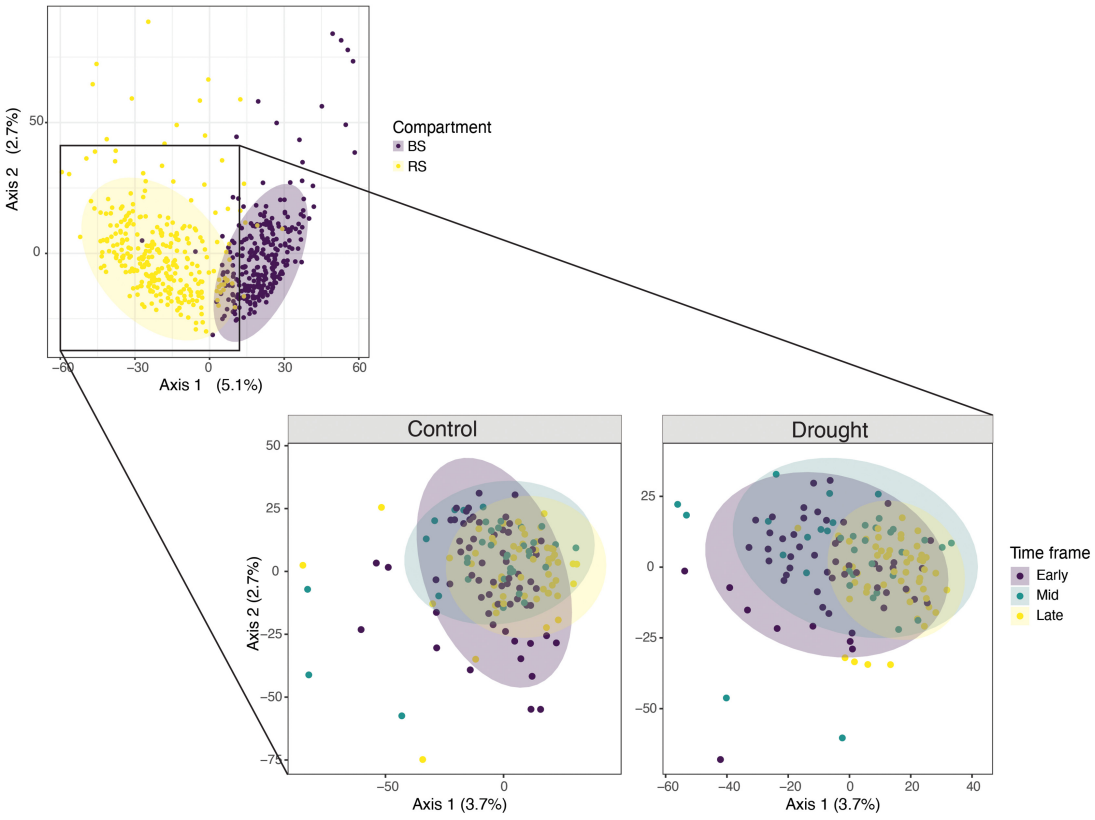

Fig. 3. Microbial composition differs between soil compartments and with treatment over time. Principal coordinate analysis (PCoA) plots performed on Aitchison distances (Euclidean distances of centered log-ratio transformed data) displaying effect of soil compartment (BS, bulk soil; RS, rhizosphere) on microbial composition of *Panicum hallii* samples. Inset PCoAs show changes in rhizosphere-associated microbial community composition over time for control versus drought-treated samples. Early time frame refers to plants sampled between 1 and 6 days after drought onset, mid time frame refers to plants sampled 8 to 10 days after drought onset, and late time frame refers to plants sampled 14 to 29 days after drought onset.

Differential abundance of MAGs

A total of 1,198 microbial bins (Supplementary Table S5) were recovered from a combined assembly of field sample metagenomes, of which 297 were originally determined to be MQ and nine HQ by CheckM. Quality assessment of the 892 "low-quality" bins with CheckM2, which more accurately assesses genome quality for novel MAGs, revealed that 82 of these bins met the MQ MAG criteria according to MIMAG standards (Bowers et al. 2017; Chklovski et al. 2023). The original dataset of 306 MQ and HQ bins was also reassessed with CheckM2, which reassigned completeness values to six MAGs below 50%. Despite the updated designation of these six MAGs as low-quality bins, they were retained for functional analysis due to the proximity of their completeness values to the MIMAG threshold and the slight variability in results between CheckM2 runs. Therefore, 388 MAGs were ultimately considered to be of sufficient quality for functional analysis. Taxonomic assignment of all 1,198 bins classified most as bacteria in known phyla (777), although 290 bins were labeled "unclassified bacteria," 21 were classified to known archaeal phyla, 8 were labeled as "unclassified archaea," and 102 could not be classified to either domain. Considering only bins that could be assigned at the phylum level, the majority pertained to Pseudomonadota (193), Bacteroidota (170), Patescibacteria (106), Acidobacteriota (80), Verrucomicrobiota (36), Gemmatimonadota (31), Myxococcota (30), Chloroflexota (24), and Actinomycetota (20), with 15 bins or fewer assigned to each remaining phylum. Of the bins determined to be MQ or HQ (388 total), the majority were classified as Pseudomonadota (99), Bacteroidota (96), Patescibacteria (73), Acidobacteriota (26), Verrucomicrobiota (17), and Chloroflexota (11), with 10 or fewer bins assigned to each remaining phylum. Notably, only five Actinomycetota bins were recovered, all MQ.

Considering the dereplicated dataset of 1,194 bins for read mapping resulted in 4.4 to 30.1% of reads from any metagenome sample mapping to bins. Differential abundance analysis with DESeq2 revealed 126 bins were enriched under late-stage drought stress, and 120 bins were depleted under this condition. Of the bins determined to be enriched under late-stage drought (including low-quality bins), 43 pertained to Bacteroidota (half of which were of the genus *Chryseosolibacter*), 15 pertained to Patescibacteria, 5 to Pseudomonadota, and 5 to Verrucomicrobiota. Forty-one drought-enriched bins could not be assigned at the phylum level, and any other phylum identified corresponded to fewer than five bins each (Supplementary Fig. S4). Only one Actinomycetota bin (*Streptomyces* sp.) was recovered in the late-stage drought-enriched dataset,

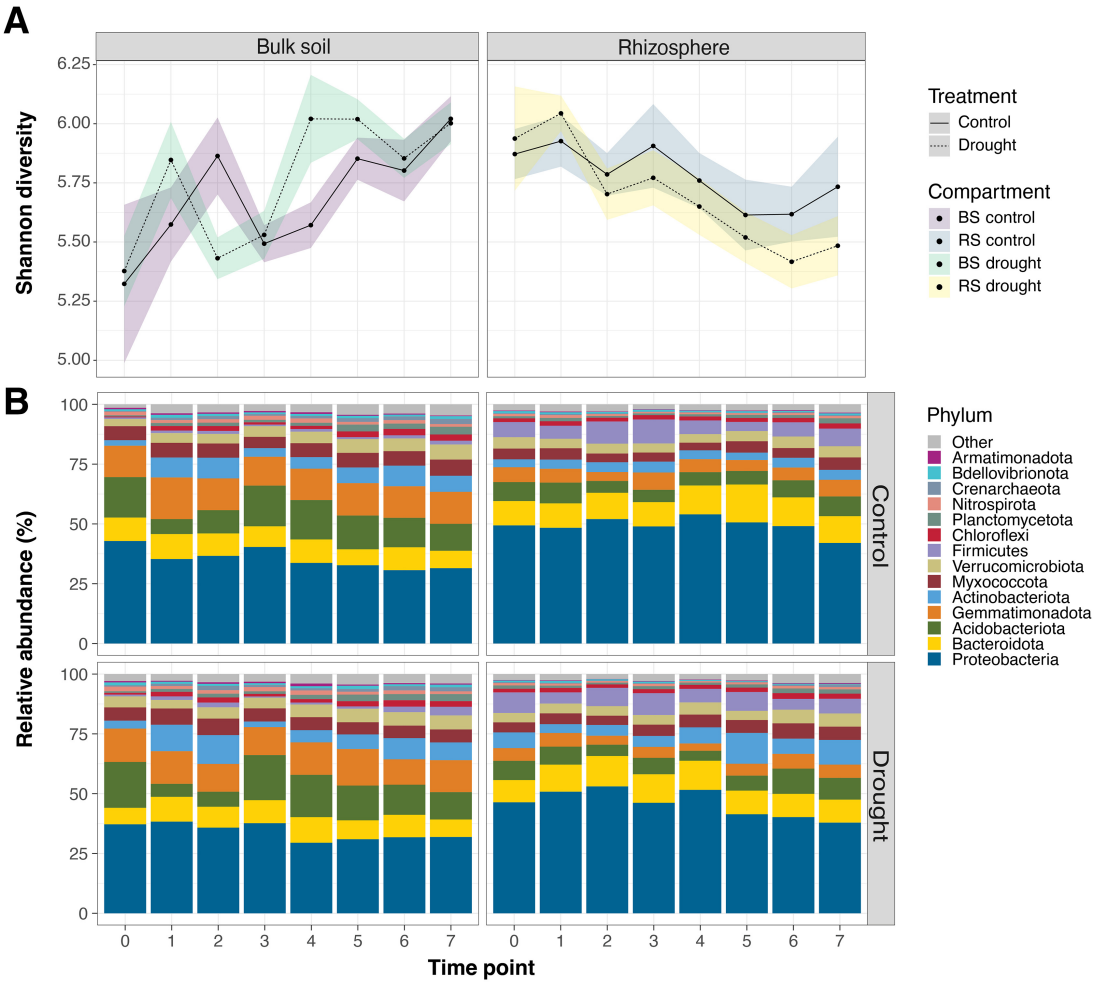

Fig. 4. Microbial diversity in the bulk soil (BS) and rhizosphere (RS) over time. **A,** Shannon diversity and **B,** relative abundance of phyla in control versus drought-treated samples by plant compartment (bulk soil and rhizosphere). The phylum category "Other" encompasses phyla that did not have a relative abundance of at least 1% in at least one sampling time point within a compartment–treatment group (Supplementary Fig. S2). Analysis of variance reveals significant interaction effects between compartment–time point ($P < 0.001$) on the Shannon index.

of low quality (<36% complete). Considering only MQ and HQ MAGs recovered, the majority of those enriched under prolonged drought were Bacteroidota (23 MAGs), followed by Patescibacteria (11 MAGs) and five or fewer MAGs each of Pseudomonadota, Verrucomicrobiota, Acidomicrobiota, Chloroflexota, Nitrospirota, and the archaeal Thermoproteota (Fig. 6; for the complete heatmaps displaying all 246 differentially abundant bins between late-stage control and drought-treated samples, please refer to Supplementary Figs. S4 [late-stage drought-enriched bins] and S5 [late-stage drought-depleted bins]). It is important to note that two of the late-stage drought-enriched Patescibacteria MAGs are of the class Saccharimonadia, which are known obligate epibionts of Actinomycetota (Actinobacteriota) (He et al. 2015). In fact, analysis with Symclatron classified all of the recovered Patescibacteria MAGs as symbiotic (from 55 out of 73 MAGs that could be classified with >72.5% confidence), four of which were further identified as obligate intracellular.

The 120 drought-depleted bins consisted mainly of Pseudomonadota members (40) (Supplementary Fig. S5), followed by Acidobacteriota (15), Bacteroidota (11), Gemmatimonadota (9), and Verrucomicrobiota (6). Thirty-two bins from this dataset could not be assigned at the phylum level, and any other phylum identified corresponded to three or fewer bins each. The presence of multiple closely related taxa was very common in this dataset, emphasizing the fact that particular bacterial lineages were responsible for the shifts in community composition. Eleven of the fifteen late-stage drought-depleted Acidobacteriota bins were of the same family (UBA2999; <85% pairwise average nucleotide identity [ANI]), six of the 11 Bacteroidota bins were of the same genus (*Flavobacterium*; <81% pairwise ANI), and six of the nine Gemmatimonadota belonged to the genus JACDDX01 (<86% pairwise ANI). Additionally, at least three closely related MAGs of multiple distinct genera of the phylum Pseudomonadota were identified, including CADEEJ01 (of the family Steroidobacteraceae; <85% pairwise ANI), *Hydrogenophaga* (<81% pairwise ANI), *Pararheinheimera* (<85% pairwise ANI), *Pseudomonas E* (<84% pairwise ANI), and *Telluria* (<81% pairwise ANI). Considering only MQ and HQ MAGs recovered, the majority of those depleted under late-stage drought stress were Pseudomonadota (18 MAGs), followed by Bacteroidota (7 MAGs), Verrucomicrobiota (6 MAGs), and four or fewer MAGs each of Acidobacteriota, Chloroflexota, Eisenbacteria, Gemmatimonadota, and Myxococcota (Fig. 6).

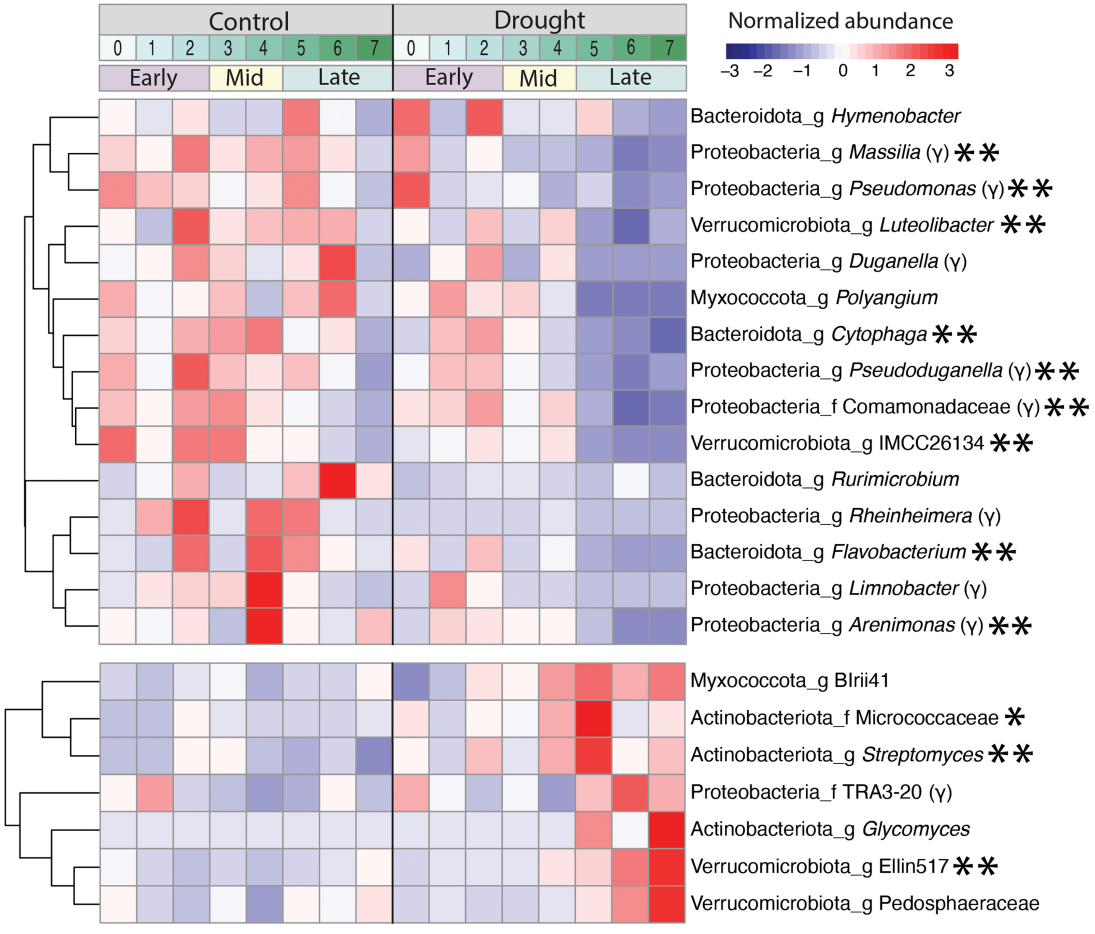

Fig. 5. Differentially abundant microbial taxa (amplicon sequence variants [ASVs]) between control and drought-treated rhizosphere communities over time as determined by DESeq2. Heatmap shows *z*-score standardized DESeq2-normalized abundances ($P_{adj} < 0.05$). ASVs confirmed by ANCOM-BC2 analysis are denoted with a single asterisk (*) or two asterisks (**) if they successfully passed the sensitivity analysis for pseudo-count addition at different thresholds. The sensitivity analysis was performed to adjust for the increased false positive rate that may occur due to necessary transformation of zero counts in the data before log transformation. Taxonomic identity depicts phylum and lowest level of identification as provided by SILVA (family [f] or genus [g]), with Proteobacteria ASVs further distinguished as class Gammaproteobacteria (γ). Note: SILVA classified the late-stage drought-enriched Verrucomicrobiota ASV with duplicate assignments at different taxonomic ranks (Pedosphaeraceae assigned to family and genus).

Despite the large relative abundance of Bacteroidota MAGs identified in both bin sets, distinct members appeared to be enriched versus depleted under drought stress. In fact, there was no overlap between the genus-level identities of Bacteroidota bins that were determined to be enriched versus depleted. Considering only the differentially abundant MQ and HQ MAGs, 52% of the Bacteroidota bins enriched under late-stage drought stress were of the same genus, *Chryseosolibacter*, and at least two closely related MAGs each of the genera *Flavisolibacter*, *Niastella*, and JAEUUG01 were among the enriched bins. On the other hand, the most commonly identified Bacteroidota bins in the late-stage drought-depleted dataset belonged to the genus *Flavobacterium* (71%).

Metabolic analysis and syntrophic interactions of drought-enriched rhizosphere-associated microbes (bacteria and archaea)

From a total of 9,006 uniquely annotated KO terms among the MQ and HQ MAGs, 17 are overrepresented in the MAGs of late-stage drought-enriched taxa ($P_{adj} < 0.05$; Supplementary Fig. S6). Of those that could be characterized (14 terms), these KO terms are mainly associated with signaling and lipid metabolism, as well as metabolism of starch and sucrose, sulfur, cofactors and vitamins, carotenoid biosynthesis, RNA degradation, and metabolism and transport of amino acids (cysteine and methionine).

From a total of 7,849 uniquely annotated PFAMs, 52 domains are overrepresented in the MAGs of late-stage drought-enriched taxa ($P_{adj} < 0.05$; Supplementary Fig. S7). Of those with known functions, an abundance of domains associated with glycosyl/glycoside hydrolases (GHs; 8 domains) indicates a propensity for complex carbohydrate metabolism among these bacteria. Differential abundance analysis of CAZymes specifically identified two overrepresented GH families, GH20 and GH29. Additionally, several overrepresented domains are associated with cell signaling, stress response, nutrient acquisition, molecular salvaging, horizontal gene transfer (HGT), chemotaxis, or cell motility, as well as metabolism of lipids, cofactors and vitamins, and amino acids. Domains linked with stress response include enzymes associated with secondary metabolites (chalcone and stilbene synthases), regulation of reactive oxygen species (squalene epoxidase, phytoene desaturase), and the plant hormones indole-3-acetic acid (IAA) and jasmonate (GH3-family enzyme). PFAM domains implicated in nutrient acquisition are tied to salvaging of various molecules, including deoxyribonucleosides (deoxynucleoside kinase), nucleotides and phosphate (S1/P1 nuclease), DNA (exonuclease), and plant biomass degradation (pectate lyase, carbohydrate esterase sialic acid-specific acetylesterase).

The overwhelming majority of these highlighted KO terms and PFAMs are overrepresented in Bacteroidota genomes. As Patescibacteria have highly reduced genomes, it is no surprise that most of these ortholog groups and domains are absent or underrepresented in these MAGs. Combined with their symbiotic nature, these features indicate that they likely rely on a host or other microbe

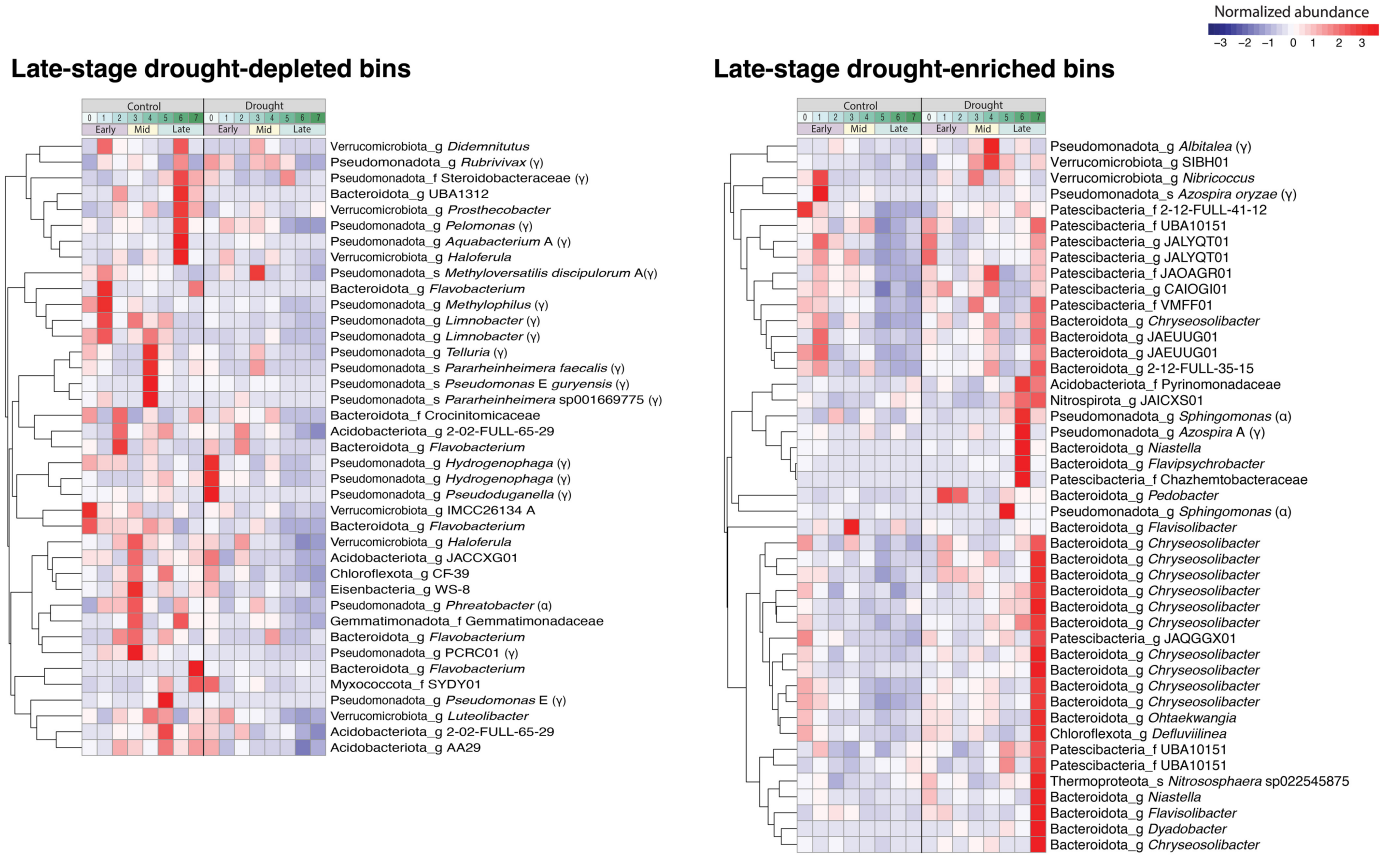

Fig. 6. Differentially abundant ($P_{adj} < 0.05$; *z*-score standardized DESeq2-normalized abundances shown) rhizosphere-associated metagenome-assembled genomes (MAGs) of medium or high quality between late-stage control and drought-treated *Panicum hallii* samples. Taxonomic identity depicts phylum and lowest level of identification as provided by GTDB-Tk (family [f], genus [g], or species [s]), with Pseudomonadota MAGs further distinguished as class Alphaproteobacteria (α) or Gammaproteobacteria (γ). Note: GTDB-Tk classified several MAGs with duplicate assignments at different taxonomic ranks (2-12-FULL-35-15 assigned to order, family, and genus; JAQGGX01 and SIBH01 assigned to family and genus).

to fulfill metabolic requirements, as supported by lifestyle analysis with Symclatron. Indeed, prior investigation of over 800 microbial communities identified metabolic cross-feeding as a major determinant of species co-occurrence and noted how these exchanges enable efficient use of limited resources (Zelezniak et al. 2015). Syntrophic analysis of MAGs identified 10 of the 11 late-stage drought-enriched Patescibacteria as possessing putative syntrophic interactions with three individual Actinomycetota (data not shown). Several of these interactions are involved in energy production (citrate cycle, glyoxylate cycle), nucleotide synthesis (de novo purine biosynthesis, nucleotide sugar biosynthesis, adenine ribonucleotide biosynthesis, guanine ribonucleotide biosynthesis, pyrimidine ribonucleotide, and deoxyribonucleotide biosynthesis), amino acid synthesis (pentose phosphate pathway, cysteine, lysine, threonine, and glutathione biosynthesis), and carbohydrate metabolism (gluconeogenesis, glycolysis), underscoring the extent to which Patescibacteria must depend on a host for essential biomolecules (Srinivas et al. 2024; Tian et al. 2020; Wang et al. 2023).

Discussion

The bioenergy crop switchgrass and its relative *P. hallii* are drought-tolerant, as compared with many crops, but still exhibit growth limitations under severe water restriction (Liu et al. 2015; Lovell et al. 2018; Morrow et al. 2014). Here, we characterized the bacterial and archaeal rhizosphere microbiome of *P. hallii*, as well as the shifts in composition that occur under moderate drought stress, to gain insight into the resiliency of this community under a shifting climate. 16S rRNA amplicon sequencing and analysis revealed a stress response of *P. hallii*-associated microbes similar to those of other crops. Additional bioinformatic analysis tailored to microbiome data, coupled with shotgun metagenome sequencing, obtained a more accurate and robust view of the bacterial and archaeal community dynamics of *P. hallii* under drought stress, highlighting associations between well-known drought-tolerant taxa and historically elusive symbionts.

Using methods similar to previous studies, we observed an enrichment of Actinobacteriota in the *P. hallii* rhizosphere under drought stress. Such an enrichment has been observed in the closely related *P. virgatum*, as well as in the rhizosphere/roots of sorghum, rice, various wheat species, barley, rye, oat, maize, potato, pearl millet, teff, tall fescue, purple plume grass, Indiangrass, Chinese silver grass, and *Brachypodium distachyon*, among other plants (Faist et al. 2023; Fitzpatrick et al. 2018; Hartmann et al. 2017; Naylor et al. 2017; Santos-Medellín et al. 2017; Wipf et al. 2021; Xu et al. 2018). Similar trends have even been observed in the cactus *Cereus jamacaru*, as well as in leguminous tree species *Mimosa tenuiflora* and *Piptadenia stipulacea* (Nessner Kavamura et al. 2013; Taketani et al. 2017). However, it is critical to note that the majority of these studies have relied solely on analysis of amplicon sequencing data with bioinformatic techniques adapted from RNA-seq (e.g., DESeq2) (Canarini et al. 2021; Faist et al. 2023; Fitzpatrick et al. 2018; Gebauer et al. 2022; Santos-Medellín et al. 2017; Xie et al. 2021; Xu et al. 2018). As previously discussed, the standard practice of using DESeq2 to assess the differential abundance of ASVs/operational taxonomic units disregards the compositional nature of microbiome data and can result in a higher false discovery rate (Gloor et al. 2017; Thorsen et al. 2016; Weiss et al. 2017). Furthermore, relying solely on amplicon sequencing data limits our ability to link specific microbial taxa with metabolic function. Thus, we also analyzed MAGs generated from shotgun metagenome samples to identify features that may explain why particular bacteria and archaea are enriched in the rhizosphere community when exposed to prolonged drought conditions.

Initial investigation of amplicon 16S rRNA gene sequences revealed that the bacterial community associated with the rhizosphere of *P. hallii* undergoes a shift in diversity and composition similar to that reported in other plants under prolonged water limitation. In many crops, drought causes a decrease in microbial diversity over time, corresponding to an enrichment in Actinobacteriota (Naylor and Coleman-Derr 2018), both of which were observed in *P. hallii* in this study. The validity of our results is strengthened by the fact that these conclusions are maintained regardless of the model applied for differential abundance analysis. The next step in our investigation was to incorporate MAG data to link enriched microbes with relevant stress tolerance and plant growth-promoting genes. However, distinct biases unique to amplicon and shotgun metagenomic sequencing limit interpretation of results. Although ASV analysis determined Actinobacteriota to be the main phylum enriched under drought stress, no Actinomycetota MAGs were identified as differentially abundant. In fact, very few Actinomycetota MAGs were recovered from the combined assembly (5 out of 388 total MQ/HQ MAGs, or 1.3%). This is in contrast to the 7.6% of total ASVs assigned as Actinobacteriota detected in late-stage drought samples, or 12.0% of unique genera observed among the ASVs (Table 2). The difference in diversity of Actinomycetota (Actinobacteriota) detected from the two datasets is likely attributable to the difficulty of resolving Actinomycetota MAGs from short-read sequencing approaches. Actinomycetota (Actinobacteriota) genomes are characterized as GC-rich and consist of highly repetitive regions, often resulting in fragmented assemblies without adequate coverage (Gomez-Escribano et al. 2016; Jørgensen et al. 2024; Seshadri et al. 2022). Examination of shotgun data through alternative approaches (ANI-based detection through k-mer containment or detection of specific amino acid stretches in single-copy marker genes) identifies a much higher diversity of Actinomycetota (19.9 to 25.6% at the species level or 9.0 to 20.1% at the genus level), proving a notable presence of members of this taxon despite the lack of representative MAGs. For detailed calculations, please refer to Supplementary Tables S6 (for Actinobacteriota/Actinomycetota) and S7 (for Patescibacteria). Various biases inherent in sequencing techniques (discussed later on) and classification algorithms yield inconsistent relative abundance calculations, complicating accurate comparison at the phylum level (Supplementary Table S8). For instance, sylph only classifies reads that can be assigned to the species level, resulting in a reduced assessment of relative abundance when compared with SingleM for taxa that are not well classified (e.g., Patescibacteria). Furthermore, SingleM requires raw metagenomic reads for analysis, whereas sylph works with quality-trimmed reads. Therefore, within-phylum diversities, as opposed to relative abundances, are presented for different shotgun data analytical methods.

The ability to link microbes with functions relevant to plant growth promotion depends on the extent to which we can recover and resolve sufficiently complete genomes. Although 16S rRNA gene amplicon analysis reveals an enrichment in Actinobacteriota (Actinomycetota) under drought conditions, the lack of resolved Actinomycetota MAGs led us to rely on reference genomes of closely related species or genera for associated stress-tolerance features that may support their enrichment. ASVs pertaining to Micrococcaceae, *Streptomyces*, and *Glycomyces* spp. displayed the most significant increases in relative abundance in the rhizosphere under drought stress in this experiment, indicating that members of these lineages could play a role in improving host tolerance to drought. There are several reasons rhizosphere communities become enriched in Actinomycetota as water availability decreases. First, as oligotrophs, bacterial members of this phylum are particularly adapted to low soil moisture levels, due to their thick cell walls and ability to sporulate (Naylor and Coleman-Derr 2018).

Second, less restrictive substrate preferences enable Actinomycetota to utilize inorganic nitrogen and recalcitrant carbon sources, both of which are abundant in low-moisture soils (Naylor and Coleman-Derr 2018). Third, they have the ability to constitutively produce and accumulate osmolytes (as opposed to activating these pathways in response to a stressor), thus providing them with a competitive advantage over other taxa (Naylor and Coleman-Derr 2018). Diverse osmolyte-accumulating bacteria, including endophytic Actinomycetota, have been shown to improve the osmotic-adjustment ability of plant hosts (Ngumbi and Kloepper 2016; Niu et al. 2022; Sandhya et al. 2010; Selim et al. 2019).

In addition to their tolerance to arid conditions, Actinomycetota are well known for their ability to produce bioactive compounds with various ecological functions, including plant growth promotion (Boukhatem et al. 2022; Omar et al. 2022; Sathya et al. 2017; Suárez-Moreno et al. 2019). These compounds facilitate a symbiotic lifestyle between Actinomycetota and nonmotile hosts and enable the host organism to obtain or compete for nutrients and other resources, as well as ward off predators and protect against disease (Benson and Silvester 1993; Bérdy 2005; Wang et al. 2021). Low moisture levels trigger a physiological response in Actinomycetota to induce biofilm formation or to begin synthesizing compounds to outcompete other taxa for limited resources (Bouskill et al. 2016; Chenu 1993; Naylor and Coleman-Derr 2018). Actinomycetota directly enhance plant fitness through the production of phytohormones, primarily IAA, and to a lesser extent gibberellins and cytokinins (Aldesuquy et al. 1998; Anwar et al. 2016; Boukhatem et al. 2022; Kang et al. 2012, 2014; Katznelson et al. 1962; Yandigeri et al. 2012). They also produce compounds with the ability to reduce damage caused by reactive oxygen species (Lee et al. 2005; Leirós et al. 2014) and combat inhibitory ethylene levels that are produced by the plant as a response to stress (Glick 2005; Nascimento et al. 2014). Many bacteria, including Actinomycetota, can counteract rising ethylene levels that would otherwise hinder plant growth by excreting the enzyme 1-aminocyclopropane-1-carboxylate deaminase, and bacteria producing this enzyme have been shown to immediately enhance plant root elongation and shoot growth upon inoculation (Boukhatem et al. 2022; Nascimento et al. 2014). Nevertheless, we hesitate to make too many inferences from reference Actinomycetota genomes, as studies have shown that the

TABLE 2
Diversity of Actinomycetota/Actinobacteriota and Patescibacteria detected in *Panicum hallii* rhizosphere samples under late-stage control ($n = 56$ for amplicon, $n = 13$ for shotgun) and drought treatment ($n = 54$, $n = 12$ for shotgun) from amplicon and shotgun datasets through various methods of analysis and at different levels of classification[a]

Dataset	Analytical method	Treatment	Classification level	Fraction unique Actinomycetota/ Actinobacteriota	Fraction unique Patescibacteria
Amplicon	ASVs[b] (16S rRNA)	Control	ASVs	4.7%	1.9%
			Genera	9.1%	4.3%
		Drought	ASVs	7.6%	1.8%
			Genera	12.0%	3.6%
Shotgun	Bins[c]	Control	Unique bin ID	1.6%	8.9%
			Genera	1.9%	10.2%
		Drought	Unique bin ID	1.6%	8.9%
			Genera	1.9%	10.2%
	Metagenome-assembled genomes	Control	Unique bin ID	1.3%	18.8%
			Genera	1.7%	24.6%
		Drought	Unique bin ID	1.3%	18.8%
			Genera	1.7%	24.5%
	Average nucleotide identity-based detection by k-mer containment (sylph)	Control	Species	19.9%	0.3%
			Genera	17.1%	0.6%
		Drought	Species	25.6%	0.3%
			Genera	20.1%	0.6%
	Short, 20 amino-acid stretches within single-copy marker genes (SingleM)	Control	Species	20.1%	0.6%
			Genera	9.0%	11.4%
		Drought	Species	21.2%	1.0%
			Genera	9.0%	12.3%

[a] Whereas sylph will only classify a read if it can assign taxonomy to the species level, SingleM will classify operational taxonomic units to which it could not assign species-level taxonomy to the genus level or higher.
[b] Amplicon sequence variants (ASVs) filtered to retain only those that could be classified as archaea or bacteria at the domain level. ASVs classified as "mitochondria" or "chloroplast" also removed from total.
[c] Bins dereplicated as part of the CoverM pipeline. Original set of 1,198 bins, dereplicated to 1,194.

metabolic repertoire of *Streptomyces*, including the presence of drought-tolerance genes, can vary dramatically among isolates and that enrichment is highly strain specific, partially due to high rates of recombination and HGT increasing the heterogeneity of populations (Antony-Babu et al. 2017; Doroghazi and Buckley 2010; Fonseca-Garcia et al. 2025). For this reason, taxonomic classification is not a conclusive determinant of drought tolerance or functional capabilities (Fonseca-Garcia et al. 2025).

Although Actinomycetota MAGs are limited in this dataset, differential abundance analysis of the MQ/HQ MAGs recovered identified several closely associated bacteria that can be explored for features associated with stress tolerance or plant growth promotion. MAG analysis revealed a large proportion of Patescibacteria to be enriched under prolonged drought stress. Often referred to as "microbial dark matter," Patescibacteria are widespread, yet most representatives remain uncultivated (Hu et al. 2024; Marcy et al. 2007; Rinke et al. 2013; Wang et al. 2023). Although this phylum comprises diverse members, they share the common features of having very small, reduced genomes and limited biosynthetic capabilities, both of which are characteristic of a symbiotic/host-dependent lifestyle (Brown et al. 2015; Castelle et al. 2018; Kantor et al. 2013; Lemos et al. 2019; Rinke et al. 2013; Wang et al. 2023). In fact, as previously mentioned, two of the drought-enriched Patescibacteria MAGs belong to the class Saccharimonadia, which are known obligate epibionts of Actinomycetota (He et al. 2015; Murugkar et al. 2020; Wang et al. 2023). Interestingly, there is a similar discrepancy between the proportion of ASVs versus MAGs recovered for Patescibacteria as for Actinobacteriota (Actinomycetota), albeit in the opposite direction (1.8% of species-level ASVs in late-stage droughted samples versus 18.8% of dereplicated MQ/HQ MAGs classified as Patescibacteria; Table 2). Their very small genome size (usually <1 Mb) facilitates easy assembly from shotgun data, but frequent intron insertions in the 16S rRNA region of Patescibacteria make this gene fragment difficult to amplify with the typical V4 region 515F/806R primers used, as in this study (Brown et al. 2015; Castelle et al. 2018; Eloe-Fadrosh et al. 2016; Nicolas et al. 2021; Schulz et al. 2017; Zhao et al. 2022). Most studies that describe Patescibacteria from 16S rRNA gene amplification require the use of modified primers (Hu et al. 2024; McNichol et al. 2021) or an enrichment step prior to amplification (Lemos et al. 2019; Nicolas et al. 2021).

Metabolic analysis of annotated KO terms and PFAMs revealed an overrepresentation of ortholog groups and domains associated with stress response, signaling, metabolism of complex carbohydrates, amino acids, and lipids, as well as molecular salvaging, HGT, and chemotaxis/motility in drought-enriched MAGs, all of which may have implications for selection under drought conditions. Chemotaxis and cell motility enable root colonization by allowing microbes to respond to and move toward roots (Trivedi et al. 2020), and microbes with the ability to degrade a wider range of compounds will be better adapted to dynamic substrate availability. Under drought stress, switchgrass has been found to increase starch and sucrose metabolism and accumulate the osmoprotectant trehalose, with accumulation levels correlating with drought tolerance (Liu et al. 2015; Zhang et al. 2018b). Pathway enrichment of maltose alpha-D-glucosyltransferase and trehalase genes in drought-enriched MAGs confers a selective advantage for microbes with the ability to metabolize these disaccharides. One hydrolase in particular, 2-phosphosulpholactate phosphatase, is involved in the synthesis of the coenzyme M. Coenzyme M is a cofactor involved in alkene metabolism in bacteria (Partovi et al. 2018), which, in theory, could modulate ethylene levels in a manner similar to 1-aminocyclopropane-1-carboxylate deaminase. Furthermore, as a putative magnesium cofactor-requiring enzyme (Graham et al. 2001), this hydrolase may support plant growth and improve water retention under drought (Rodrigues et al. 2021; Santos et al. 2023; Senbayram et al. 2015). Additionally, molecular salvaging abilities in both plants and microbes enable the recycling of essential molecular building blocks, thus reducing energy expenditure (Glowacki et al. 2020; Konrad et al. 2012; Kovaľ et al. 2016; Li et al. 2025). This is of particular importance for Patescibacteria, as studies have shown that rhizosphere-associated Patescibacteria recycle DNA from soil necromass or other microbes that feed on plant exudates (Castelle et al. 2018; Starr et al. 2018). These salvaging processes are further facilitated by an increase in multiple signaling and transport membrane proteins in the drought-enriched MAGs, especially in Bacteroidota (Fairman et al. 2011).

Pathway enrichment for KO terms and PFAMs linked with carotenoid biosynthesis indicates enhanced abilities to accumulate antioxidants, which can alleviate oxidative stress caused by drought exposure. The GH3 family domain comprises the amino acid-conjugating component of enzymes involved in regulating the levels of IAA and jasmonate, phytohormones with critical roles in conferring drought tolerance (Zhang et al. 2018a; Zhang et al. 2020). These phytohormones confer resilience partially through protecting against reactive oxygen species by enhancing antioxidant accumulation and quenching activities (Qiu et al. 2014; Verma et al. 2022). Jasmonate has been shown to directly impact *Streptomyces* growth and development, as well as stimulate antibiotic production in these bacteria (van der Meij et al. 2023). Genetic features associated with conjugation and competence indicate HGT capabilities, on which Patescibacteria heavily rely (Gios et al. 2025).

Other late-stage drought-enriched taxa of note include *Sphingomonas* (phylum Pseudomonadota), which are known to possess multiple plant growth-promoting features, including genes required for phosphorus solubilization, nitrogen fixation, abiotic stress tolerance, and the production of phytohormones such as IAA (Khan et al. 2014; Lombardino et al. 2022; Luo et al. 2019; Yang et al. 2014), all of which strongly support enrichment of this taxa in the *P. hallii* rhizosphere under drought stress. *Pedobacter* (phylum Bacteroidota), a known plant growth-promoting microbe, along with *Dyadobacter* (phylum Bacteroidota), were identified as members of a perennial wheat line rhizosphere and found to exhibit significant plant growth-promoting traits, namely IAA and siderophore production (Giannelli et al. 2024; Morais et al. 2019; Yin et al. 2021). Previously described as "environmental superbugs" (Viana et al. 2018), *Pedobacter* have extensive resistance mechanisms that may promote their resilience under stress and against competitors. Another study identified *Dyadobacter* and *Sphingomonas* spp. to be enriched in the rhizosphere of grafted watermelon and found that these two genera cooperate as part of a metabolic interaction network. The study detected higher abundances of gene sequences associated with glycan biosynthesis and metabolism and terpene and polyketide metabolism in *Dyadobacter* MAGs and a higher potential in *Sphingomonas* MAGs for xenobiotics biodegradation and metabolism, carbohydrate metabolism, amino acid metabolism, and terpene and polyketide metabolism (Zhang et al. 2024). Known to utilize a wide range of carbon sources (Chelius and Triplett 2000; Dong et al. 2007), the generalist nature of *Dyadobacter* may facilitate their adaptability to diverse habitat conditions/stressors. *Niastella* (phylum Bacteroidota) has previously been implicated in plant growth promotion (Ali et al. 2022), and the production of compounds with antimicrobial activity by mangrove-associated *Ohtaekwangia* (phylum Bacteroidota) suggests a role in plant defense (Octaviana et al. 2022)

Several of the other late-stage drought-enriched taxa have been previously isolated from plant or soil environments, yet there has

been little investigation into their functional capacities as they relate to plant growth promotion (Alegria Terrazas et al. 2022; Fan et al. 2023; Li et al. 2010; Liu et al. 2021; McDonald et al. 2024; Ni et al. 2023; Obermeier et al. 2020; Reinhold-Hurek and Hurek 2000). Prior studies have correlated *Flavisolibacter* (phylum Bacteroidota) abundance with improved plant growth (Lin et al. 2021) and *Azospira* (phylum Pseudomonadota) abundance with resilience against plant diseases (Shang et al. 2021). *Chryseosolibacter* spp. (phylum Bacteroidota) have been shown to play a role in nitrogen cycling and maintaining soil fertility (Lin et al. 2025; Zhao et al. 2025), and JAICXS01 (phylum Nitrospirota) is capable of oxidizing nitrate and solubilizing phosphorus (GenBank accession: JAICXS000000000.1). Proteomic and comparative genomic analysis of related strains of the class Nitrososphaeria revealed biofilm formation, cell surface modification, carbohydrate conversion, and detoxification as essential adaptations of soil-dwelling *Nitrososphaera* strains (phylum Thermoproteota) (Kerou et al. 2016; Zhou et al. 2020). Pyrinomonadaceae are capable of surviving drought and nutrient limitation, evident by their isolation from savanna and tundra soils (Ivanova et al. 2020; Wüst et al. 2016), and they have shown enrichment in soil treated with a combination of N-fixing and phosphate-solubilizing bacteria, which correlated with improved crop yields (Ni et al. 2023). Metadata provided by GenBank (Clark et al. 2016) and Sandpiper (Woodcroft et al. 2026) link species of *Defluviilinea* (phylum Chloroflexota) (Singleton et al. 2021; GenBank accessions: JADJWS000000000.1, JADKIW000000000.1), *Nibricoccus* (phylum Verrucomicrobiota) (GenBank accessions: CBINDC000000000.1, JASGHG-000000000.1, JBFZFC000000000.1), JAEUUG01 (phylum Bacteroidota) (GenBank accession: JAEUUG000000000.1), 2-12-FULL-35-15 (phylum Bacteroidota), and the family SIBH01 (phylum Verrucomicrobiota) to plant and soil environments, including rhizosphere communities, but it remains to be explored if and how these late-stage drought-enriched taxa may support plant growth promotion. Although our analysis of functional capabilities provides clues as to the plant growth-promoting features of these microbes, more investigation into their metabolism is needed to elucidate the mechanisms behind their selection under water-limiting conditions.

Understanding how adaptable *P. hallii* and closely related biofuel crops are to climate change-related weather events will facilitate further studies that manipulate the associated microbial communities to improve their resiliency under stress. Cultivation of isolates from experimental samples in subsequent studies will enable whole-genome sequencing of microbes of interest, providing a means to unequivocally link bacterial lineages with plant growth-promoting genes. Transcriptomics will further elucidate the expression levels of these genes of interest under varying water treatments. It has been shown that growing plants of the same genotype with different sets of selected microbes can modulate plant traits (Compant et al. 2019; de Souza et al. 2020; Herrera Paredes et al. 2018; Khasanova et al. 2023). Future studies should test the effects of inoculating *P. hallii* or switchgrass with a synthetic microbial community that includes taxa highlighted in this study, through both amplicon and shotgun analysis, on plant growth under drought stress. Additionally, extending the duration of drought exposure will provide a more comprehensive understanding of how drought severity will impact the rhizosphere microbiome (Naylor and Coleman-Derr 2018). For this experiment, *P. hallii* was planted in a field in a region that has previously experienced prolonged drought events (https://www.drought.gov). Expanding this study to additional field sites with varied drought history will reveal how pre-exposure impacts the shifts in microbial community composition and diversity that occur under drought stress (Bouskill et al. 2013; Göransson et al. 2013; Naylor and Coleman-Derr 2018). Although it was not feasible for this study, experimenting on a larger field with additional plots will enable randomization of control and drought-treated beds within a plot, reducing spatial bias and strengthening the validity of our conclusions. These follow-up studies will aid in characterizing the stability of the drought-selected microbial community and inform how soil water history impacts the ability of rhizosphere-associated microbial communities to impart stress tolerance to *P. hallii* and related grasses.

Through this field experiment, we have shown that the drought-exposed *P. hallii* rhizosphere microbiome undergoes similar shifts in taxonomic composition to that of switchgrass and other biofuel crops and confirmed that the drought-enriched taxa have an enhanced capacity for plant growth-promoting functions. Their abilities to metabolize diverse and complex substrates and produce compounds that modulate plant stress hormone levels, osmoregulate, and protect against oxidative stress, among other threats, facilitate increased nutrient and water uptake, as well as root and shoot growth, in their plant hosts. In addition to confirming the enrichment of Actinobacteriota (Actinomycetota) under drought stress, our results highlight the recruitment of two lesser studied taxa (Patescibacteria and one particular Bacteriodota genus, *Chryseosolibacter*), both of which merit further consideration in future crop improvement and stress tolerance studies and emphasize the importance of a dual amplicon and shotgun-based approach to microbial community analysis.

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