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Intraspecific Variability in Root Traits and Edaphic Conditions Influence Soil Microbiomes Across 12 Switchgrass Cultivars

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ABSTRACT

Microbial communities help plants access nutrients and tolerate stress. Some microbiomes are specific to plant genotypes and, therefore, may contribute to intraspecific differences in plant growth and be a promising target for plant breeding. Switchgrass (*Panicum virgatum*) is a potential bioenergy crop with broad variation in yields and environmental responses; recent studies suggest that associations with distinct microbiomes may contribute to variation in cultivar yields. We used a common garden experiment to investigate variation in 12 mature switchgrass cultivar soil microbiomes and, furthermore, to examine how root traits and soil conditions influence microbiome structure. We found that average root diameter varied up to 33% among cultivars and that the cultivars also associated with distinct soil microbiomes. Cultivar had a larger effect on the soil bacterial than fungal community but both were strongly influenced by soil properties.

Root traits had a weaker effect on microbiome structure but root length contributed to variation in the fungal community. Unlike the soil communities, the root bacterial communities did not group by cultivar, based on a subset of samples. Microbial biomass carbon and nitrogen and the abundance of several dominant bacterial phyla varied between ecotypes but overall the differences in soil microbiomes were greater among cultivars than between ecotypes. Our findings show that there is not one soil microbiome that applies to all switchgrass cultivars, or even to each ecotype. These subtle but significant differences in root traits, microbial biomass, and the abundance of certain soil bacteria could explain differences in cultivar yields and environmental responses.

Keywords: bioenergy, microbiome, *Panicum virgatum*, root traits, switchgrass

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Plants associate with microbial communities that help them access resources and tolerate stress (Jiang et al. 2017; Pérez-Jaramillo et al. 2016). Some microbial communities are associated with specific plant genotypes (Adam et al. 2018; Emmett et al. 2017; Jiang et al. 2017; Pérez-Jaramillo et al. 2017) and, therefore, have the potential to be targets of plant breeding programs and inform crop choices (Busby et al. 2017; Mueller and Sachs 2015). Switchgrass (*Panicum virgatum* L.), a leading candidate for low-input bioenergy feedstock, exhibits broad phenotypic and genotypic variations that contribute to its ability to tolerate a diverse range of environments (Casler et al. 2017; Yang et al. 2009). However, genotypic differences only explain roughly 30% of the variation in cultivar yield responses across different regions, years, and fertilizer

rates (Casler et al. 2019). Recent studies suggest that the unexplained variability in cultivar yields and environmental responses may be driven, in part, by their associations with distinct microbial communities (Rodrigues et al. 2017; Sawyer et al. 2019; Singer et al. 2019a).

Switchgrass cultivars are broadly classified as upland and lowland ecotypes. Lowland ecotypes originate from southern, warm, and mesic regions, and upland ecotypes originate from northern, cold, and drier regions. Although there are distinct traits across ecotypes such as earlier flowering and senescence in upland cultivars (Casler 2012), there is also physiological and phenotypic variation within ecotypes, including in aboveground and belowground traits, drought tolerance, yields, and responses to fertilizer (Aimar et al. 2014; de Graaff et al. 2013; Stahlheber et al. 2020). Multiple recent studies also suggest that switchgrass cultivars belonging to upland and lowland ecotypes have distinct soil microbiomes (Revillini et al. 2019; Rodrigues et al. 2017; Sawyer et al. 2019; Singer et al. 2019a) but see Emery et al. (2018). However, most previous studies only focused on one or two of the most common cultivars, making it hard to identify general patterns or to determine whether soil microbiomes vary consistently by switchgrass ecotype. Furthermore, with one notable exception (Emery et al. 2018), most studies were conducted on young, immature plants even though switchgrass is a long-lived perennial that reaches stand maturity and peak yields after 3 years. Given reported ontogenetic differences in plants' microbial communities (Chaparro et al. 2014; Zhalnina et al. 2018), it seems likely that young and mature switchgrass plants will recruit distinct microbiomes that may have different effects on growth or other aspects of plant health such as nutrient acquisition.

Root and soil microbiomes are influenced by plant traits and soil conditions (Fierer 2017; Saleem et al. 2018). Plants, particularly long-lived perennials, can also alter soil properties, which then lead to differences in microbial communities (DuPont et al. 2014; Liang et al. 2012; Zhang et al. 2017). Switchgrass cultivars differ in their root exudate profiles (An et al. 2013), architecture, and tissue chemistry (de Graaff et al. 2013; Stewart et al. 2017), and these differences may lead to distinct microbiomes. For instance, cultivars with high specific root length (SRL) have a greater relative proportion of thin, high-quality (low carbon/nitrogen ratio) roots that provide more labile carbon (C) to microbes (Adkins et al. 2016; de Graaff et al. 2013; Stewart et al. 2017). This influences microbial community C acquisition, soil fungal/bacterial ratios (de Graaff et al. 2013; Roosendaal et al. 2016; Stewart et al. 2017), and the amount of C allocated belowground (Adkins et al. 2016; Stewart et al. 2017). These studies show that differences in root traits and consequent C provisioning likely contribute to variation in switchgrass cultivar microbiomes; however, few studies have measured variation in switchgrass root traits and microbial communities simultaneously (Roosendaal et al. 2016; Stewart et al. 2017).

Although root traits and soil conditions drive microbial community structure, the strength of these drivers may differ for root- and soil-associated microbial communities (Bulgarelli et al. 2013; Yu and Hochholdinger 2018). Plant signaling, exudation, and altered abiotic conditions filter and recruit bulk soil microbes to different microhabitats such as the rhizosphere (soils closely adhering to roots) and endosphere (internal root tissues). Soil-associated microbes are influenced by changes in root exudates and soil conditions, while root microbes are assembled through a two-step process whereby the previously filtered rhizosphere microbes are recruited to the roots through genotype-specific signaling (Bulgarelli et al. 2013). Therefore, although soil conditions affect both root and soil communities, root communities are often a less diverse but more host-associated subset of the surrounding soil

microbes (Bulgarelli et al. 2013). It is also predicted that root-associated communities have greater heritable variation than soil communities (Reinhold-Hurek et al. 2015); however, more research is needed to assert this claim. Knowing how microbiomes differ among cultivars' soils and roots as well as what influences microbiome structure will help us understand how microbes may contribute to cultivar and ecotype variation in the field and, furthermore, how microbes could be incorporated into switchgrass production.

We hypothesize that root traits and microbial communities will differ among switchgrass cultivars. Furthermore, we expect that a combination of root traits and soil conditions will drive soil microbiome structure, while root microbiome structure will be less diverse but more distinct among cultivars. We predict that root architectural traits known to increase belowground plant-derived C inputs (e.g., SRL or root diameter) will be an important driver of microbial community structure and biomass. In this study, we address these hypotheses by measuring root traits and microbiomes across 12 mature switchgrass cultivars, asking two primary questions. First, does microbial biomass and community structure vary across switchgrass cultivars? Second, what soil conditions and root traits influence microbial community structure and biomass?

MATERIALS AND METHODS

Site description. We conducted this study in southwest Michigan, United States, at the Great Lake Bioenergy Research Center's Switchgrass Variety Experiment (<https://lter.kbs.msu.edu/research/long-term-experiments/glbrc-switchgrass-variety-experiment/>) located at the W.K. Kellogg Biological Station Long-Term Ecological Research Site (42°23'47" N, 85°22'26" W). Mean annual precipitation is 100 cm and soils are moderately fertile sandy clay loam (<https://lter.kbs.msu.edu/research/>). In 2009, 12 switchgrass cultivars, including 8 upland and 4 lowland cultivars, were established in a complete randomized block design (4 cultivars with poor establishment were replanted in 2010). Details on seed source and breeding history are shown in Table 1. Cultivars were planted as live seed at a rate of 9 kg ha⁻¹ into 12 plots within four uniformly treated replicate blocks, in the same soil type, and within 80 m of one another ($n = 48$, plots = 4.6 × 12.2 m). The blocks were not irrigated and urea fertilizer was applied annually in the spring (nitrogen at 78 kg ha⁻¹). Preemergence weeds were controlled with Quinclorac Drive (1.1 kg ha⁻¹) and Atrazine (0.6 kg ha⁻¹) and postemergence weeds were treated with herbicides (Glyphosate, 2,4-D, or Dicamba) as needed.

Sampling and soil analyses. In June and July 2016, we collected soil cores (2 cm in diameter by 20 cm deep) from the rhizome (within 10 cm from the rhizome center) of three randomly chosen switchgrass plants from either end and the center of each block (3 replicate cores × 4 blocks = 12 cores/cultivar). All instruments were sterilized with 70% ethanol between sampling. Because plant phenological stage can affect microbial communities (Chaparro et al. 2014; Zhalnina et al. 2018), we sampled each cultivar at the same developmental stage: flowering (Emmett et al. 2017). The 12 cultivars flowered over a 4-week period and, at each sampling date, we sampled at least two cultivars (Table 1). This controlled for the impact of phenology on microbiome structure but microbiome differences may have also been affected by variation in host residence time (Dombrowski et al. 2017) or soil conditions. We accounted for some of this temporal variation by including soil moisture content, the edaphic factor that varied most among dates, as a covariate in our analyses (see Analyses section).

After sampling, the soils were stored at 4°C and were frozen at -20°C within 48 h after sampling. Before freezing the soil cores,

we sieved (1 mm) a 30 g subset of the collected soils to remove roots and rocks and subsample for various assays, including chloroform fumigation and potassium sulfate extractions for microbial biomass, soil nitrate and ammonium (12 g soil), volumetric soil moisture content (5 g of soils dried at 60°C), and downstream DNA extractions (2 g of soil stored at –20°C). Microbial biomass carbon (MBC) and nitrogen (MBN) were analyzed on a TOC analyzer (Shimadzu TOC-VCPH) and calculated by subtracting the total carbon (C) and nitrogen (N) of unfumigated samples from fumigated samples (Vance et al. 1987). Unfumigated potassium sulfate extracts were used to determine soil inorganic ammonium (NH₄⁺) and nitrate (NO₃⁻) with colorimetric 96-well plate assays. Ammonium concentration was analyzed using ammonia salicylate and ammonia cyanurate as described by Sinsabaugh et al. (2000). Nitrate reductase enzyme (EC number 1.7.1.1) was used to reduce NO₃⁻ to NO₂⁻ and concentrations of NO₂⁻ were determined using sulfanilamide and *N*-(1-naphthyl)-ethylenediamine. Absorbance for NH₄⁺ and NO₃⁻ assays were read on a Synergy HTX plate reader (BioTek, Winooski, VT, U.S.A.) at 610 and 540 nm, respectfully. All roots collected during initial sieving and remaining soils were stored at –20°C until further root trait analysis and root DNA extractions.

Root sterilization and trait analysis. The previously frozen sieved roots and undisturbed soils were wet sieved (2 mm) with Nanopure (0.2 μM) water and all visible roots were separated with sterilized tweezers for an average of 30 min per sample. These roots were stored at 4°C in Nanopure water and scanned within 48 h. To maintain sterility and minimize microbial cross-contamination, we sterilized all equipment with 70% ethanol between scans. The roots were scanned (1,200-dpi resolution with Epson perfection V600 scanner) in a glass scanning bed with 200 ml of Nanopure water, exported as tiff files, manually edited to remove image artifacts, and compressed before analyzing root traits with GiA Roots software (Galkovskyi et al. 2012). Following scanning, 0.25 g of the scanned roots (<2 mm in diameter to standardize for root age) were

subsampled and sterilized for root-associated (endophyte) microbial characterization (details below). The remaining roots were weighed and dried at 60°C for 1 week to calculate the dry/wet root biomass ratio. Predicted total dry root weight was back calculated using the dry/wet ratio to estimate the dry weight of the 0.25 g subset. This back calculation of total dry root weight may underestimate actual root weight values if root water content varies with root diameter; an underestimation of root weight could contribute to miscalculations of other root traits such as mass-weighted specific root length (total root length/dry root biomass). Using GiA Roots, we calculated the following root traits: total root length (in centimeters), average root diameter (in centimeters), total root system volume (in cubic centimeters), and SRL. SRL was calculated in two ways: (i) mass-weighted SRL, which we calculated using the back-calculated dry/wet root ratios (centimeters of total root length per gram of total dry root biomass), and (ii) volume-weighted SRL (centimeters of total root length per cubic centimeters of total root volume).

To prepare the root tissues for DNA extractions, we first sterilized the 0.25 g of subsampled roots. Immediately after scanning, we sterilized the subset roots following Sun et al. (2008): roots were immersed in 70% ethanol for 3 min, sterilized with fresh household sodium hypochlorite solution (2.5% available Cl⁻) for 5 min, rinsed with 70% ethanol for 30 s, rinsed 10 times with sterile autoclaved water, blotted dry with Kimwipes (Kimberly-Clark, Roswell, GA, U.S.A.), and frozen at –20°C (Sun et al. 2008). To test root-surface sterilization, the final water rinse was plated on Luria-Bertani (LB) agar and incubated at 30°C for 7 days. A majority of the LB plates had bacterial growth after 1 week of incubation. Although the bacterial growth may suggest incomplete sterilization of the rhizoplane, because these samples were root segments, the cultured bacteria may have been endophytic bacteria that dispersed from the interior of the roots. Due to the thorough sterilization procedure, we believe the remaining microbes are strongly root associated but cannot conclude that they are obligate endophytes. Before DNA extraction, the frozen, surface-sterilized root samples were

TABLE 1
Details on cultivar origin, sampling date, and establishment year in the common garden experiment^a

Cultivar	Ecotype	Sampling date	Year	Breeding history (native seed source)
Alamo	Lowland	27 July	2009	Seed increase from native remnant prairie (southern Texas) ^b
EG1101	Lowland	13 July	2010	Improved Alamo-type bred for biomass yield (NA) ^c
EG1102	Lowland	27 July	2010	Improved Kanlow-type bred for biomass yield (NA) ^c
Kanlow	Lowland	27 July	2009	Seed collection from native remnant prairie, selected for leafiness, vigor, late-season greenness (northern Oklahoma) ^b
Blackwell	Upland	28 June	2009	Seed increase from native remnant prairie (northern Oklahoma) ^b
Cave-in-Rock	Upland	20 July	2009	Seed increase from native remnant prairie (southern Illinois) ^b
Dacotah	Upland	28 June	2009	Seed increase from native remnant prairie, selected for leafiness, color and winter hardiness (southern North Dakota) ^b
EG2101	Upland	13 July	2010	Improved Cave-in-Rock bred for biomass yield (NA) ^c
Nebraska 28	Upland	20 July	2009	Seed increase native remnant prairie (Nebraska) ^b
Shelter	Upland	13 July	2010	Seed increase from native prairie, selected for thick stems, less leafiness, early maturing (West Virginia) ^b
Southlow	Upland	20 July	2009	Seed increase from local remnant native stands to represent local germplasm (southwest Michigan) ^d
Trailblazer	Upland	20 July	2009	Seed increase from natural grassland, selected for high digestibility and forage (Kansas and Nebraska) ^b

^a Seed source location and breeding history details from Stahlheber et al. (2020). NA denotes not available.

^b Alderson and Sharp (1994).

^c Ceres, Inc. Blade seeds.

^d USDA-NRCS (2014).

submerged in liquid N and ground with a tissue lyser (Qiagen Tissue Lyser II, Valencia, CA, U.S.A.). If any root pieces >2 mm remained, sterilized scissors (10% bleach and 70% ethanol) were used to more finely cut the roots.

DNA extraction, sequencing, and bioinformatics. DNA was extracted similarly from soil and sterilized roots but only a subset of cultivars were processed for root-associated microbes. Soil DNA was extracted from 0.25 g of sieved and homogenized sample from all 12 cultivars ($n = 144$ samples: 12 cultivars \times 4 blocks \times 3 replicate cores). Root DNA was extracted from approximately 0.25 g of sterilized, ground root tissue from four commonly planted cultivars (upland: Cave-in-Rock and Southlow; and lowland: Alamo and Kanlow; $n = 48$ samples: 4 cultivars \times 4 blocks \times 3 replicate cores, notated with “+” in all figures). For both soils and roots, we used the MoBio PowerSoil DNA extraction kit and followed all kit-suggested protocols, with an added 10-min cell lysis step at 65°C before the bead-beating step (MoBio Laboratories, Carlsbad, CA, U.S.A.). The purity and quantity of the extracted DNA was examined using a Nanodrop 2000 (Thermo Scientific, U.S.A.) and via fluorometry with the Quanti-iT PicoGreen dsDNA kit (Thermo Fisher, U.S.A.). We targeted the bacterial V4 region of the 16S ribosomal RNA (rRNA) gene (primers 515f/806r) and the fungal ITS1 region (primers ITS1-F/ITS2) for library preparation. Bacterial communities were analyzed for all soil (12 cultivars) and root (4 cultivars) DNA, whereas fungal communities were only analyzed from the soil DNA (12 cultivars).

Bacterial and fungal PCR and MiSeq Illumina (V2) paired-end sequencing was conducted by the Research Technology Support Facility Genomics Core at Michigan State University (East Lansing, MI, U.S.A.). Briefly, for both ITS and 16S sequences, reads were assembled, and quality filtered (maxEE < 1.0 and base pairs < 250) using Usearch (version 10.0.240) (Edgar 2010). Sequences were dereplicated, clustered, chimera checked, filtered de novo, and clustered into unique operational taxonomic units (OTUs) based on 97% identity using the default settings with Usearch UPARSE function. Representative sequences were aligned and classified using the Silva (version 123) and Unite (7.2) reference databases for bacterial and fungal sequences, respectively (Nilsson et al. 2018; Quast et al. 2012). Soil- and root-associated bacterial sequences were also aligned to the Greengenes (version 13.8) database using Usearch closed-reference (closed_ref) for downstream PICRUSt analysis (DeSantis et al. 2006; Langille et al. 2013). Nonbacterial and nonfungal sequences, singleton OTUs, and samples with poor sequence coverage were removed from the reference-based OTU tables (Supplementary Table S1). A bacterial phylogenetic tree was generated using an iterative maximum-likelihood approach with PASTA R package (Mirarab et al. 2015). Phylogenetic-based Weighted Unifrac distance was used for all bacterial community composition analyses. It is challenging to map the variable ITS region to a trustworthy phylogenetic tree (Nilsson et al. 2008); therefore, we used a nonphylogenetic community metric, Bray-Curtis, for the fungal community analyses.

Due to large variation (>10-fold) in library sizes within and among the root and soil samples, we rarefied our datasets using the ‘rarefy_even_depth’ function in the Phyloseq R package (McMurdie and Holmes 2014) to control for sequencing depth differences and minimize false discovery rates (FDRs) (McKnight et al. 2019; Weiss et al. 2017). The soil bacterial and fungal datasets for 12 cultivars were filtered and rarefied to 4,694 and 4,153 reads, respectively. We compared root and soil bacterial communities for four cultivars on a combined dataset that was rarefied to 2,026 reads. We confirmed that our results were robust to normalization techniques and not biased by rarefaction (McMurdie and Holmes 2014) by comparing community matrices normalized with rarefaction and Deseq2’s “variance

stabilizing transformation” (Love et al. 2014) with a Protest analysis in the Vegan R package (Oksanen et al. 2018). All Protest comparisons were significantly correlated ($P < 0.001$) (Supplementary Table S1) but the combined root and soil dataset had the weakest correlation ($r = 0.41$), likely due to the 27-fold difference in the sample library sizes. However, because rarefaction is the preferred method for normalizing for large variation in library depth (Weiss et al. 2017), we used the bacterial (Silva-referenced) and fungal (Unite-referenced) rarefied datasets for all community composition and diversity analyses. The rarefied Greengenes-referenced bacterial dataset was used to predict metagenome functions with PICRUSt. Fasta files (NCBI Sequence Read Archive, accession number PRJNA577732) and sequencing pipeline are publicly available (<https://github.com/TaylerUlbrich/SwitchgrassCultivarMicrobiomeStudy>).

Data analysis: Univariate statistics. Prior to all data analysis, we ensured that all univariate data met assumptions of normality (see Supplementary Materials for details). Univariate statistics were conducted using one-factor analyses of variance (ANOVA) models and type 3 sum of squares (Satterthwaite’s method) with the lmer4 and lmerTest packages in R (Bates et al. 2015; Kuznetsova et al. 2017). To differentiate the effect of cultivar and ecotype, all variables were analyzed with either cultivar or ecotype as a fixed effect, with a random, nested block factor. Because we sampled the cultivars across 4 weeks to control for phenology-driven variation in microbiomes (Chaparro et al. 2014; Zhalmirina et al. 2018), date was confounded with cultivar and ecotype. Due to this collinearity, the model was rank deficient when both date and cultivar or ecotype were included. Therefore, instead of date, we included soil moisture content, which varied up to 47% across sampling dates (ANOVA, $P < 0.001$; correlation with Julian date $P < 0.001$, $r = 0.52$) as a covariate when it improved model fit (i.e., lower Akaike information criteria evaluation [AIC]). Soil moisture content also correlated with soil nitrate ($r = 0.46$, $P < 0.002$), which varied by date ($P < 0.001$). However, we decided to include soil moisture content, not soil nitrate, as a covariate because soil moisture content also varied across blocks (ANOVA, $P < 0.001$), allowing us to account for both temporal and spatial heterogeneity. Two extreme outliers that were three times the interquartile range were removed from the soil moisture data; thus, cultivars EG1102 and Blackwell had only 11 replicates for any model that included soil moisture as a covariate. Several univariate models were improved with soil moisture as a covariate—fungal community richness and evenness, soil and root bacterial richness, MBN and MBC, and root length—but soil moisture was only a significant predictor variable ($P < 0.05$) for MBC. Posthoc comparisons (P values adjusted with Benjamini-Hochberg FDR, $\alpha = 0.05$) were conducted using the multcomp and emmeans R packages (Lenth 2019; Hothorn et al. 2008). Fungal Shannon diversity and Pielou’s evenness did not meet normality assumptions; therefore, we used nonparametric Kruskal-Wallis and Wilcoxon tests (no block factor included). Pearson correlations were used to determine relationships between edaphic conditions, root traits, and MBC using the cor.test in R (R Core Team 2018).

Data analysis: Microbiome community composition. Microbial community data were visualized and analyzed using the Vegan, Phyloseq, and ggplot2 R packages (McMurdie and Holmes 2013; Oksanen et al. 2018; Wickham 2016). We examined overall variation in the cultivars’ microbiome composition using permutation-based ANOVA (PERMANOVA) and β -dispersion tests with type 1 sum of squares. PERMANOVAs, β -dispersion, and posthoc pairwise comparisons (FDR-adjusted) were evaluated on the rarefied datasets using the previously described one-factor, blocked model, with soil moisture as a covariate, with the PRIMER-e software (version 6 with PERMANOVA+) (Anderson et al. 2008). After removing samples with poor sequence coverage and

samples with two extreme outliers for the soil moisture covariate, all cultivars had at least nine replicates for microbiome analyses (Supplementary Table S2). As in the univariate models, date and cultivar were confounded; thus, including sampling date in the model did not improve model fit (based on AIC evaluation). However, because the permutational null model can still be calculated for a rank-deficient design, we used supplemental PERMANOVAs with date as a covariate to evaluate the cultivar-level effects when controlling for date. Models with date used instead of soil moisture content were qualitatively similar but the significance was lower (Supplementary Tables S3 and S4). Within sampling date, PERMANOVAs were used to further evaluate cultivar-level differences not driven by confounding date effects (e.g., cultivars sampled on the same date in one model) (Table 1). All ordinations were made with the Phyloseq R package ‘ordinate’ function with `set.seed = 2` for reproducibility (McMurdie and Holmes 2013).

To further characterize differences in microbial community structure across cultivars, we evaluated the proportion of shared and indicator taxa among the cultivars. We defined shared taxa as those OTUs present in at least 75% of the samples within each cultivar (e.g., 9 of 12 sample units/cultivar) and across all cultivars. Indicator taxa were identified (after removing singleton OTUs) using the ‘multiplat’ function in the indicspecies R package (Cáceres and Legendre 2009) and defined as OTUs present in at least 25% of the samples (3 of 12 sample units, or indicspecies specificity parameter = 0.25). Rarefied datasets are biased against rare taxa; thus, it is possible that we identified fewer indicator taxa because less dominant, rare taxa were lost during rarefaction (McMurdie and Holmes 2014). We also characterized phyla-level differences among cultivars and ecotypes using the ‘manyglm’ function in the MVabund R package and ANOVA posthoc pairwise comparisons (FDR-adjusted), with either cultivar or ecotype as a fixed effect and soil moisture content as a covariate when it improved model fit (based on AIC) (details in Supplementary Materials) (R Core Team 2018; Wang et al. 2012).

We were also interested in whether compositional differences based on 16S rRNA were likely to lead to differences in cultivar N fixation, a function recently identified in switchgrass soils and roots and relevant to cultivar survival in low-nutrient environments (Roley et al. 2018, 2019, 2021). We assessed this by (i) calculating variation in the relative abundance of common N-fixing orders Rhizobiales and Burkholderiales and (ii) using PICRUSt to predict the relative proportion of putative N-fixing taxa (Langille et al. 2013) (details in Supplementary Materials). Both approaches have limitations but we intended for findings to generate further hypotheses, not to provide definitive assessments of N-fixing potential. The same univariate statistics described above were used to analyze proxies of functional differences among cultivars and ecotypes for the soil and root communities.

We further evaluated differences in cultivar microbiomes by determining how edaphic conditions and root traits affect microbiome structure and individual OTU- and order-level abundances. Differences in OTU- and order-level abundance with root traits were evaluated using the ‘manyglm’ and ‘anova’ functions in the MVabund R package (details in Supplementary Materials) (Wang et al. 2012). At the community level, we determined which variables (average root diameter, total root length, soil nitrate, soil ammonium, and soil moisture content) significantly contributed ($\alpha = 0.05$) to microbiome structure when controlling for spatial heterogeneity (block) with a partial distance-based redundancy analysis for each dataset: soil bacterial (Weighted Unifrac) and fungal (Bray-Curtis) communities for 12 cultivars and combined root and soil bacterial dataset for 4 cultivars (Weighted Unifrac).

We used the ‘dblda’ function in Vegan with a conditional matrix for block to determine the relative contribution of block and predictor variables to community structure, as well as the independent, “marginal” effects of each term (Oksanen et al. 2018). Specific root length (volume and mass weighted) and total dry root weight were removed from all analyses because they significantly correlated with average root diameter and total root length ($-0.50 < r > 0.50$, $P < 0.05$).

RESULTS

Root traits. Total dry root biomass (estimated from dry/wet root calculations), total root length, and mass-weighted SRL (total root length/root biomass) did not significantly differ by cultivar or ecotype ($P > 0.05$) (Supplementary Table S5). Mass- and volume-weighted SRL were significantly correlated ($r = 0.70$, $P < 0.001$) and, unlike mass-weighted SRL, volume-weighted SRL (total root length/root volume) significantly differed among cultivars ($P < 0.01$) but not by ecotype ($P > 0.05$) (Fig. 1A; Supplementary Table S5). The cultivar differences in volume-weighted SRL were likely driven by average root diameter, which significantly differed by cultivar ($P < 0.001$) (Fig. 1B), and was used to calculate root network volume. There was a 30% difference between the cultivars with the thickest (e.g., Cave-in-Rock and EG2101) and thinnest (e.g., Kanlow and NE28) roots.

Microbial biomass. MBC and MBN significantly differed among cultivars (MBC: $P < 0.001$, and MBN: $P < 0.001$) and ecotypes (MBC: $P < 0.01$, and MBN: $P < 0.001$) (Fig. 1C and D), even after controlling for soil moisture content which influenced MBC (soil moisture covariate with MBC: $P < 0.001$, and with MBN: $P > 0.05$) and varied by date ($P < 0.05$). Lowland MBC and MBN were 25 and 65% greater than upland ecotypes, respectively.

Soil- versus root-associated bacterial communities. For a subset of four commonly planted cultivars (Cave-in-Rock, Southlow, Alamo, and Kanlow), we found that root and soil bacterial communities differed in diversity, composition, and the extent to which they were affected by cultivar identity. Microhabitat (soil or root) explained 59% of the overall variance in community composition (Table 2; Fig. 2A), and the root community had five and three times lower bacterial richness and Shannon diversity, respectively, than the soil communities (Supplementary Table S6). The differences in β diversity between roots and soils were mirrored in their dominant phyla. The most abundant bacterial phyla in the roots ($n = 4$ cultivars) were Proteobacteria (70%), Actinobacteria (11%), and Bacteroidetes (5%), while the soil communities ($n = 4$ cultivars) were dominated by Acidobacteria (30%), Proteobacteria (29%), and Verrucomicrobia (11%) (Fig. 2B). The same phyla were most abundant in the soil communities when analyzed across all 12 cultivars (data not shown). Roots and soils also differed in the relative abundance of common N-fixing orders (Burkholderiales and Rhizobiales), with roots having approximately three times greater relative abundance than soils (Kruskal-Wallis: $P < 0.001$) (data not shown).

The degree of cultivar effect also differed for the root and soil bacterial communities ($n = 4$ cultivars). Cultivar explained 15% of the variation in the soil community but did not significantly influence the root communities (Table 2). The two upland cultivars’ soil communities significantly differed from the two lowland cultivars’ soil bacterial communities (data not shown) but this may have been driven by differences in soil conditions across sampling dates, which differed for the subset of two ecotypes (Supplementary Table S4). There was also no cultivar effect on root or soil bacterial α diversity (Supplementary Table S6) and there were fewer differences in the relative abundance of dominant soil phyla for these

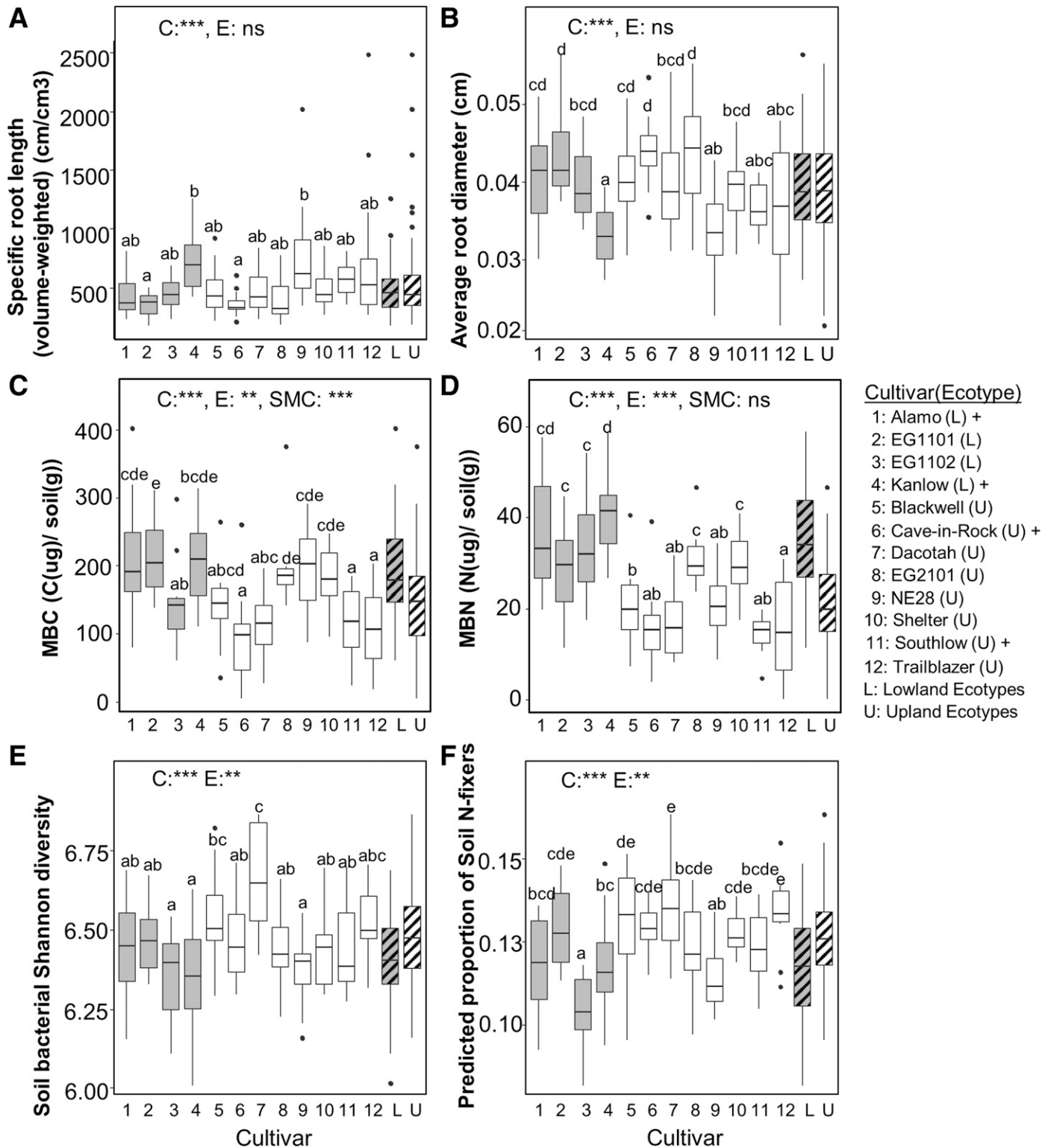


Fig. 1. Variation in cultivar and ecotype **A**, volume-weighted specific root length (SRL); **B**, average root diameter; **C**, microbial biomass carbon (MBC); **D**, microbial biomass nitrogen (MBN); **E**, soil bacterial Shannon diversity; and **F**, predicted proportion of putative nitrogen (N) fixers in soil. The last two bars represent means for lowland ($n = 4$; gray boxes) and upland ($n = 8$; white boxes) ecotypes. The central line is the median value for each cultivar, vertical bars represent the first and third interquartiles of the data, and points are outliers outside the interquartile range. Symbol + denotes subset of cultivars analyzed for root-associated bacterial communities. Different letters denote significant differences among cultivars (false discovery rate, $P < 0.05$). Analysis of variance results with fixed cultivar (C) or ecotype (E) term, nested block term, and soil moisture content (SMC) included as a covariate when it improved model fit (based on Akaike information criteria evaluation). Significance values: ns, *, **, and *** indicate $P > 0.05$, 0.05, and 0.01 and $P < 0.001$, respectively.

four cultivars, suggesting that there was less variation among these four commonly planted cultivars' microbiomes compared to the remaining eight cultivars.

Soil bacterial communities. When evaluated across all 12 cultivars, we found that the soil-associated bacterial communities significantly differed in composition and diversity. Soil bacterial richness, Shannon diversity, and Pielou's phylogenetic evenness differed among cultivars and was 1 to 3% higher for upland ecotypes for all diversity metrics ($P < 0.05$) (Fig. 1E; Supplementary Table S7). However, these differences were driven by Dacotah, which had the highest bacterial richness and Shannon diversity (Supplementary Table S8). Dacotah is a low-yielding upland cultivar that had greater weed invasion, which may have contributed to greater bacterial diversity. Even when controlling for sampling date (Supplementary Table S3) and soil moisture content (Table 3), soil bacterial community composition differed among cultivars. When controlling for soil moisture content, block (32%) and cultivar (21%) explained the most variation in community composition, whereas ecotype only explained 3% of the variation (Fig. 3A; Table 3). The bacterial communities of three cultivars—Alamo (lowland), EG1102 (lowland), and NE28 (upland)—

were more dissimilar from all other cultivars (pairwise comparisons, $P < 0.10$) (Supplementary Table S9). When assessed within sampling date, cultivar explained a significant proportion of variation in the bacterial community composition within one date (16%, $P < 0.05$) (Supplementary Table S10): cultivar NE28 had a significantly different soil bacterial community than the other three upland cultivars (Southlow, Cave-in-Rock, and Trailblazer) sampled on the same date.

The cultivars' soil bacterial communities also differed at the phyla level and were comprised of many shared and few unique taxa. Eight soil bacterial phyla (74.3% of all reads) significantly differed among cultivars (Fig. 4). Several of these phyla also differed by ecotype; specifically, Bacteroidetes, Planctomycetes, and Verrucomicrobia are more abundant in lowland cultivars, while Actinobacteria, δ -Proteobacteria, and Gemmatimonadetes are more abundant in upland cultivars. At the OTU level, we found that 160 OTUs (out of 14,590 total) were shared across all cultivars (present in 75% of samples units within and among cultivars). These shared OTUs make up 45% of the total sequences and are dominated by three classes: Acidobacteria (39%), α -Proteobacteria (17%), and Spartobacteria (12%). In contrast, indicator bacterial OTUs of the

TABLE 2 Percent variability (permutation-based analysis of variance [PERMANOVA R^2]) in bacterial community composition explained by habitat (soil or root) and cultivar ^a			
Habitat effect ^b	PERMANOVA R^2 (P) per factor		
	Soil and root bacteria (4 cultivars)	Soil bacteria (12 cultivars)	Root bacteria (4 cultivars)
Cultivar	2.59*	15.06**	ns
Block (cultivar)	6.56*	29.72***	ns
Habitat	58.64***	NA	NA
Cultivar \times habitat	ns	NA	NA
Habitat \times block (cultivar)	6.73*	NA	NA
Soil moisture	ns	4.41*	ns

^a Significance values: ns indicates $P > 0.05$ and asterisks signify the interaction between factors; * indicates $P \leq 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.001$. NA denotes not applicable for the model.

^b Nested factors shown in parentheses.

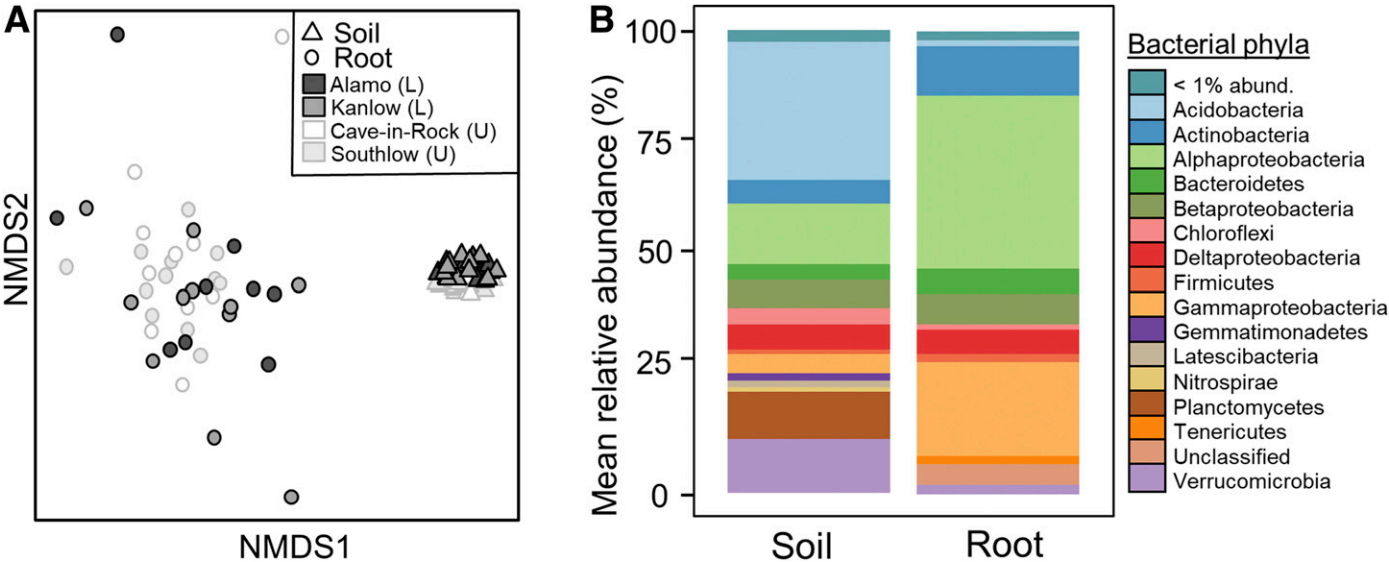


Fig. 2. A, Nonmetric multidimensional scaling ordination of combined soil and root bacterial community ($n = 4$ cultivars, Weighted Unifrac, stress: 0.08). Soil (triangles) and roots (circles) represent two lowland cultivars (L, dark gray points) and two upland cultivars (U, light gray points). **B,** Mean relative abundance (percentage) of bacterial phyla and proteobacteria classes in roots or soils among four cultivars.

12 cultivars include 683 OTUs and make up 21% of the total sequences dominated by classes Acidobacteria (33%), α -Proteobacteria (10%), and δ -Proteobacteria (7%).

We used PICRUST to test whether cultivars' soil and root bacterial communities might have different abilities to fix N₂. We first used NSTI scores to assess whether PICRUST accurately approximated bacterial function for our sequences. Larger NSTI scores (>0.15) are expected for highly diverse and largely uncharacterized environments such as soils and indicate less phylogenetic relatedness between the predicted OTUs and reference genomes (Langille et al. 2013). The average NSTI score for the soil samples was 0.23, which is within the typical range for soil samples (Langille et al. 2013) but indicates that results should be interpreted with caution due to weak phylogenetic relatedness. Root NTSTI (0.32) indicated low relatedness with reference genomes which,

therefore, were not analyzed. We found that cultivar soil bacterial communities varied in the proportion of OTUs with putative N-fixation genes ($P < 0.001$) (Fig. 1F). On average, upland ecotypes had a greater proportion of predicted soil N-fixers than lowland ecotypes ($P < 0.05$). Predicted soil N-fixer abundance negatively correlated with soil nitrate availability ($r = -0.33$, $P < 0.001$) but did not correlate with soil N-fixation rates ($P > 0.05$) that were measured in a paired study (Roley et al. 2021) (data not shown). We also compared the relative abundance of common N-fixing orders (Burkholderiales and Rhizobiales) and found no differences among cultivars ($P > 0.05$).

Soil fungal communities. When controlling for soil moisture content, the primary drivers of soil fungal community composition were similar to the bacterial community: block explained the most variation (33%), followed by cultivar (12%) and ecotype (1%) (Table 3; Fig. 3B). However, unlike the bacterial communities, the cultivar-level effects on fungal communities were not robust to variation across (Supplementary Table S3) or within (Supplementary Table S10) sampling dates. Fungal community diversity (richness, Shannon, and evenness) also did not differ by cultivar or ecotype ($P > 0.05$) (Supplementary Table S7).

Only one fungal phylum, Rozellomycota, significantly differed in abundance among the cultivars (MVabund 9, $P < 0.01$), and no phyla differed by ecotype (MVabund, $P > 0.05$). OTUs identified as Rozellomycota made up only 0.73% of the reads and, therefore, likely did not contribute much to variation in cultivar microbiomes. The dominant fungal phyla were Ascomycota (32%), Basidiomycota (17%), Mortierellomycota (14%), and Glomeromycota (9%) but 25% of the fungal OTUs were unclassifiable at phyla level. Among fungal OTUs (4,064 total), 37 were shared across all cultivars (present in 75% of samples units within and among cultivars). These shared OTUs made up 35% of the total sequences and were dominated by classes Mortierellomycetes (28%) and Sordariomycetes (23%), and those that were unclassified (29%). Indicator fungal OTUs of the 12 cultivars made up 25% of the total fungal sequences and included 213 OTUs dominated by classes Sordariomycetes (19%) and Dothideomycetes (17%), and 27% were unclassified at class level.

TABLE 3 Percent variability (permutation-based analysis of variance [PERMANOVA R^2]) in microbial community composition explained by cultivar or ecotype ^a		
Effect ^b	PERMANOVA R^2 (P) per factor (12 cultivars each)	
	Soil fungi	Soil bacteria
Cultivar effect		
Cultivar	11.95*	21.20***
Block (cultivar)	32.71***	31.94***
Soil moisture	1.85***	3.49***
Ecotype effect		
Ecotype	1.34*	3.43**
Plot (ecotype)	43.31***	49.70***
Soil moisture	1.85***	3.49***

^a Significance values: asterisks signify the interaction between factors; * indicates $P \leq 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.001$.
^b Nested factors shown in parentheses.

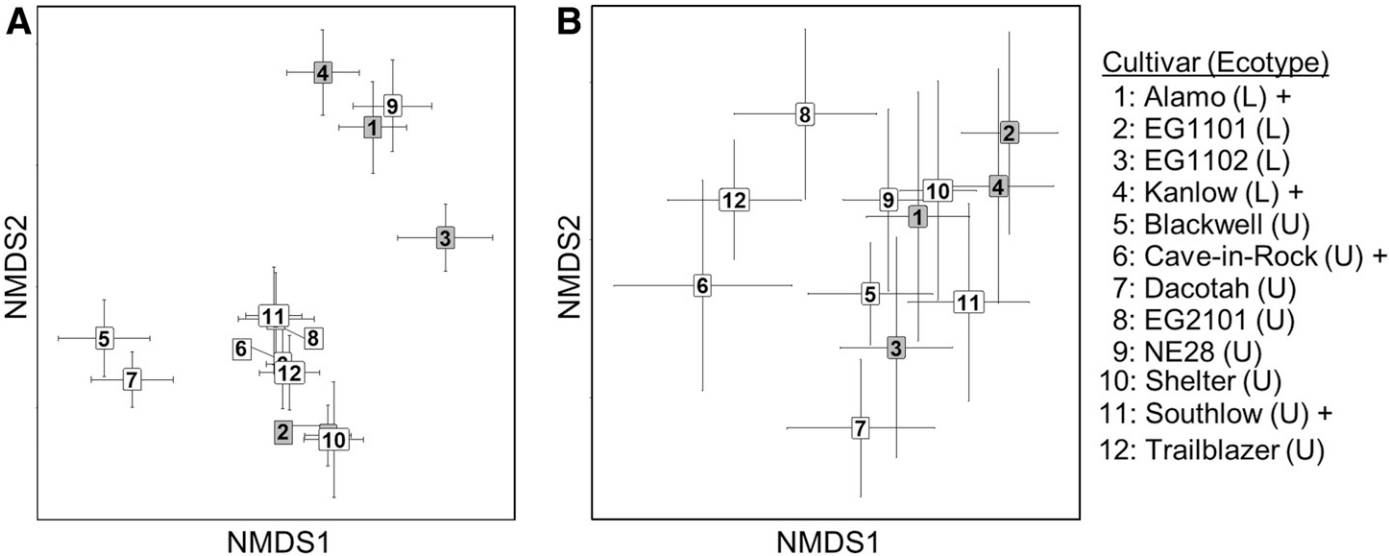


Fig. 3. Nonmetric multidimensional scaling (NMDS) ordination of **A**, soil bacterial community (Weighted UniFrac, stress: 0.18) and **B**, soil fungal community (Bray-Curtis, stress: 0.26) across four lowland (L, gray points) and eight upland (U, white points) cultivars. Numbers indicate centroid of sample replicates and horizontal and vertical bars represent ± 1 standard error from the centroid. Names followed by + denote subset of cultivars analyzed for root-associated bacterial communities. See Supplementary Figure S1 for NMDS with all sample replicates.

Effect of edaphic properties and root traits on microbiome.

To further understand variation in cultivar microbiomes, we investigated how root traits and edaphic conditions (N and water content) impact community structure. Across all 12 cultivars, the five predictor variables (average root diameter, root length, soil moisture content, soil nitrate, and soil ammonium) explained more variation for the soil bacterial (10%) than the soil fungal (5%) communities (Table 4). Mirroring the PERMANOVA results, spatial heterogeneity (conditional block variance) explained a significant portion of community dissimilarity for the soil bacteria and fungi. While controlling for variance due to spatial heterogeneity, variance in the bacterial community structure was still explained by soil nitrate (6%) and soil moisture content (2%) while the fungal community was explained by soil nitrate (1%) and root length (1%). Within the four cultivars evaluated for soil and root bacterial community composition, nitrate explained 6% of the variation in the soil community; however, no edaphic conditions or root traits contributed to variation in the root communities (Table 4).

We also investigated whether the relative abundance of bacteria or fungal taxa (at the order and OTU level) or microbial biomass

correlated with root traits (average root diameter and root length). We did not identify any bacterial orders that correlated with root traits but identified one fungal order, Mortierellales, that negatively correlated with root length (MVabund $P < 0.05$, correlation: $r = -0.41$, $P < 0.001$). Furthermore, MBC negatively correlated with root length ($r = -0.23$, $P < 0.01$) but not with average root diameter ($P > 0.05$).

DISCUSSION

We examined bacterial and fungal microbiomes, soil variables, and root traits across 12 mature switchgrass cultivars grown in a common garden experiment. Overall, we found that cultivars vary in their average root diameter, have different soil microbial biomass, and associate with distinct soil but not root bacterial communities. Differences in the soil microbiomes were driven by variation in root traits, phenology, and soil properties, and were more pronounced at the cultivar level than across ecotypes. Still, cultivar was a weaker driver of soil communities than among-plot soil heterogeneity, and we saw less overall variation in fungal

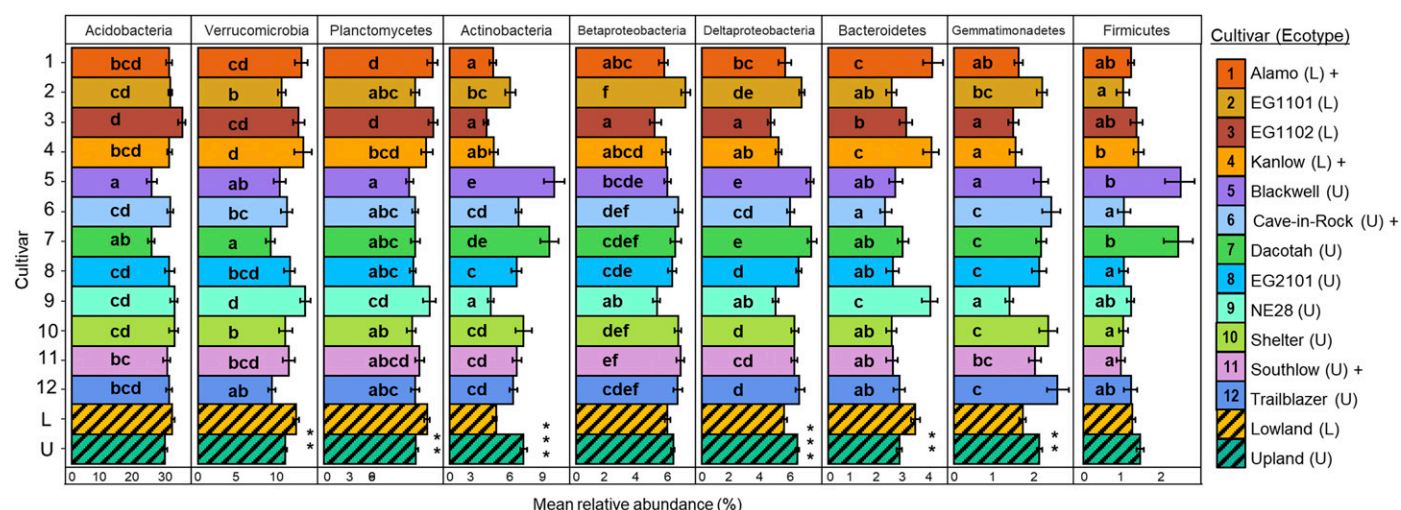


Fig. 4. Mean relative abundance of bacterial phyla (and proteobacteria classes) that significantly vary among cultivars (MVabund by cultivar: MVabund Dev (11/126) = 1,105.8, $P = 0.001$; each phyla, $P < 0.05$). Bars represent standard error. Phyla are ordered by relative abundance (left = most abundant) and, in each phyla, the bars are ordered by cultivar (1 to 12), followed by means for lowland (L; $n = 4$) and upland (U; $n = 8$) ecotypes. Names followed by + denote subset of cultivars analyzed for root-associated bacterial communities. Asterisks (*) above ecotypes indicate statistically significant differences among ecotypes (analysis of variance: *, **, and *** indicate $P < 0.05$, 0.01, and 0.001, respectively).

TABLE 4
Percent variability (R^2) of microbiome structure explained by soil conditions and root traits using db-RDA analysis^a

Conditions and traits	Soil bacteria (12 cultivars)	Soil fungi (12 cultivars)	Soil bacteria (4 cultivars)	Root bacteria (4 cultivars)
Nitrate (N in $\mu\text{g/g}$ of dry soil)	6.36***	1.17**	5.72**	NA
Ammonium (N in $\mu\text{g/g}$ of dry soil)	ns	ns	ns	NA
Soil moisture content (g/g of dry soil)	1.86**	ns	ns	NA
Average root diameter (cm)	ns	ns	ns	NA
Root length (cm)	ns	1.06*	ns	NA
Model significance	***	***	**	ns
Conditional variance	7.67	6.23	9.83	NA
Constrained variance	10.12	5.03	15.31	NA
Unconstrained variance	82.22	88.75	74.86	NA

^a Percent explained partitioned by conditional (block), constrained (all predictor variables), and unconstrained (residuals) factors; ns indicates $P > 0.05$, * indicates $P \leq 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.001$. NA denotes not applicable for models that were not significant ($P > 0.05$).

communities. These subtle but significant differences in root traits and soil bacterial communities that we observed may contribute to variation in cultivar yields, environmental responses, or ability to provide beneficial ecosystem services (e.g., soil C sequestration).

Cultivars have a greater effect on soil bacterial than root bacterial or soil fungal communities. Traditionally, ecotypes are used to classify differences among switchgrass cultivars; however, we found greater differences in switchgrass microbiomes across cultivars than between ecotypes. We found that cultivar explained 10 to 20% of the variance in soil microbiome β diversity, whereas ecotype explained less than 5% of the variation; these stronger cultivar effects were also found in a previous study on switchgrass cultivar soil bacterial and fungal communities (Singer et al. 2019a) but Emery et al. (2018) observed no cultivar effects on arbuscular mycorrhizal fungi (AMF) in the same common garden experiment. Our findings show that, at this site, the weak effect of cultivar on AMF is true for a broader assessment of fungi as well (assessed via the ITS region). Despite overall weak effects of ecotype on OTU-level composition, ecotypes differed in the relative abundance of several dominant bacterial phyla. This may suggest that higher-level taxonomic differences are conserved across ecotypes whereas finer, OTU-level differences occur among cultivars. Although we did not examine specific functions in this study, OTU-level differences among cultivars could contribute to variation in their nutrient cycling or yields. In fact, in the same common garden experiment, Stahlheber et al. (2020) found that aboveground traits and yields varied more among cultivars than between ecotypes, a pattern that could have been influenced by microbiome differences.

On a subset of four cultivars, we predicted that there would be a greater cultivar effect on root-associated than soil bacterial communities but, in fact, the soil bacterial communities differed more among cultivars. The weak cultivar effect on the root communities could have been influenced by our cultivar selection, such that the other eight cultivars—which had greater variation in soil communities—may have also had more distinct root microbiomes. Furthermore, it is also possible that we undersampled the root bacterial diversity, because many chloroplast and mitochondrial sequences reduced microbiome sampling. Despite these potential caveats, other studies conducted on a similar number of cultivars also report greater cultivar-level differences among soil than root microbiomes in switchgrass (Singer et al. 2019a, $n = 4$ cultivars) and rice (Edwards et al. 2015, $n = 6$ cultivars); therefore, we posit that our observation of greater cultivar effects on soil than root communities is biologically relevant. The soil communities also had less within-cultivar variation than the root communities. This has been observed previously (Edwards et al. 2015) and may suggest that there is greater intraspecific variation in traits that affect microbial recruitment to the rhizosphere (e.g., root structure, exudation, or diffuse signaling) than in traits that regulate microbial entry into the root (e.g., physical and immune system interactions). In fact, it may be that plant traits associated with root microbiome assembly are conserved at even higher taxonomic levels, because Singer et al. (2019b) found that two *Panicum* spp. have similar endophyte bacterial communities. The role of genotype on microbiome structure remains unclear but it could be clarified with surveys of microbiome variation across multiple genotypes and species. Additionally, it seems that the proximity of the microbiome to the plant may not be a good predictor of the influence of plant genotype on microbiome structure but finer-scale sampling (e.g., soil, rhizosphere, rhizoplane, and endosphere) would help confirm this (Edwards et al. 2015).

Edaphic conditions and plant traits influence soil community structure. Soil water and N content influenced switchgrass cultivar soil but not root microbiomes, while root traits only affected the soil

fungal community. Soil nitrate availability explained the most variation in the cultivars' soil microbiomes but no edaphic or root traits influenced the root community composition. Similar patterns were observed by Singer et al. (2019b): rhizosphere soil communities of *Panicum* spp. were more affected by soil type than endosphere communities. These edaphic conditions are considered to have larger effects on soil microbiomes than plant identity (Fierer 2017); however, the observed differences in soil N in this study could be driven by the cultivars' differential effects on N cycling (Roley et al. 2021) which could, in turn, influence the microbiome (Revillini et al. 2019). Contrary to our prediction, we did not observe any effect of root traits on bacterial community structure but found that fungal community structure was affected by root length. Root length may be a particularly important trait for root-colonizing fungi (e.g., AMF) because root system size determines the amount of niche space available for colonization. Few studies simultaneously evaluate fungal community structure and root length but, in the same common garden experiment, AMF root colonization correlated with root biomass (Emery et al. 2018). Our results support this finding because root length significantly correlated with root biomass ($r = 0.75$, $P < 0.001$). In these conclusions, we are presuming that root traits drive bacterial and fungal communities; however, the observed correlation could also describe microbes driving root traits (Petipas et al. 2020; Verbon and Liberman 2016).

We found that spatial variability (block factor) also explained a surprisingly large percentage (>30%) of variation in the soil microbiomes. Although our blocks were the same soil type and within 80 m of one another, they differed in soil moisture and N content (also in paired study Roley et al. 2021). Our analysis of microbiome composition and edaphic conditions controlled for this block effect; however, it is difficult to disentangle the relative contribution of cultivar traits, spatial heterogeneity, and sampling date on these edaphic conditions and, in turn, microbiome structure. Furthermore, it is possible that the variation across blocks contributed to greater plasticity in the cultivars' traits, thus making it more challenging to identify correlations between traits and microbiome structure. Overall, although the primary drivers of switchgrass microbiome structure are challenging to disentangle, our results suggest that heterogeneous soil conditions, plant traits, and feedbacks between plant traits and soil conditions all likely contribute to microbiome variability among switchgrass cultivars.

The strength of relationships between root traits and soil microbiomes can also be influenced by soil fertility and sampling techniques. Our study was conducted on productive, annually fertilized soils, and cultivar differences and plant-microbe associations may be stronger in less-fertile, marginal soils, when plants and microbes are more dependent on one another (Bell et al. 2014; Sawyer 2017). Sawyer (2017) found that switchgrass cultivar microbiomes were more distinct in less fertile soils. It is also possible that cultivars that were grown outside of their native range (e.g., not from the north-central United States) had weaker effects on their microbiomes because they could not associate with their native, potentially coevolved microbial communities. Studies of cultivars in common gardens across many sites could elucidate the contribution of native range or seed source on plant-microbe interactions. Furthermore, because we did not sample the soils directly adhering to the roots or use primers to target root-colonizing microbes (e.g., AMF), we may not have captured the microbes most influenced by root traits and exudates. Finally, we found that cultivars vary in average root diameter and, therefore, soils beneath each cultivar likely differ in the amount of root turnover and development. Microbial composition and function has been shown to vary with root age, type (e.g., seminal or nodal root), and location

(e.g., root branch or tip) (de Graaff et al. 2013; Kawasaki et al. 2016; Marschner and Baumann 2003); however, sampling with soil cores made it challenging to identify the effects of root age, type, or location on soil microbial communities. Therefore, future studies should use methods that standardize root age (e.g., use of root-in-growth cores) or root type and location (e.g., visualizing root differences and sampling within rhizoboxes) to better understand how root traits influence microbiome structure (Yu and Hochholdinger 2018).

Plant developmental stage (e.g., phenology and maturity) also contributes to microbiome variability (Edwards et al. 2018; Na et al. 2019; Zhalnina et al. 2018). We sampled cultivars at the same stage (flowering) to control for this variation but sampling on different dates may have increased differences in edaphic conditions that influence the microbiome. Yet, when we controlled for variation among sampling dates, cultivar still contributed to variation in the soil bacterial but not fungal communities. This suggests that the fungal communities were more influenced by variation in abiotic conditions across dates, or that cultivars with different phenology and, thus, sampling dates had more dissimilar fungal communities. In contrast, bacterial community structure was more strongly influenced by cultivar identity, which explained a significant percent (16%) of the variation in bacterial community structure within one of the four sampling dates. We hypothesize that greater differences were not observed within the other three sampling dates because cultivars with comparable phenology (e.g., flowering at the same time) likely have other similar traits and, thus, more similar microbial communities than cultivars with different phenology. However, to better understand the effect of similar phenology and traits on cultivar microbiomes, future studies should evaluate the switchgrass cultivar microbiomes across multiple phenological stages (Na et al. 2019; Qiao et al. 2017; Wagner et al. 2016) because both the microbiome structure and the magnitude of cultivar effects may change with phenological stage (İnceoğlu et al. 2010; Na et al. 2019).

Functional implications and conclusions. Differences in cultivar root traits and microbial biomass could contribute to variability in the cultivars' soil C-cycling and C-sequestration potential. We found differences in microbial biomass and root diameter but not root biomass across cultivars. Another study conducted in the same common garden experiment, however, did find differences in root biomass among cultivars (Emery et al. 2018). These differences in average root diameter have the potential to drive variation in the cultivars' C-cycling and microbial community structure. Root systems with high SRL, corresponding to long, thin roots, positively correlate with switchgrass-derived soil C (Adkins et al. 2016; Stewart et al. 2017), decomposition (de Graaff et al. 2013, 2014), bacterial/fungal ratios (de Graaff et al. 2013), and microbial biomass (PLFA-C) (Stewart et al. 2017). Greater rhizodeposition from thin roots can directly contribute to soil C pools, as well as indirectly influence soil C by supporting the growth and turnover of microbial communities which, in turn, contributes to greater soil C and aggregate stability (Grandy and Neff 2008; Tiemann et al. 2015). Therefore, the cultivars we identified with thinner roots (Kanlow and NE28) or with higher microbial biomass C (many lowland cultivars) may have greater potential to increase soil C in marginal soils and improve C sequestration.

The observed differences in microbial communities and root traits could also influence cultivar nutrient cycling and tolerance to different environmental conditions, in turn affecting yield. We found that the predicted N-fixer abundance in soil communities varied among cultivars and ecotypes. A paired study (same location and sampling dates) found that the rate of soil N fixation also varies among cultivars (Roley et al. 2021) but our PICRUST-inferred

functional potentials did not correlate to the measured rates (data not shown). Still, our results suggest that functional differences are likely, and future studies should investigate N fixation and other functions with more targeted approaches, because microbiome function may influence the suitability of various cultivars for surviving under different soil conditions.

In summary, we found that root traits, microbial biomass, and soil bacterial community composition differ among switchgrass cultivars, and that this variation could contribute to differences in their potential as bioenergy crops. Despite ecotype being the most common way to group cultivars, soil microbiome structure and root traits differed more among cultivars than ecotype. Future research on switchgrass-microbe interactions should examine multiple cultivars rather than relying on results from one model cultivar to make ecotype-level assumptions. Understanding how cultivar traits influence microbial communities can improve our ability to select and breed cultivars with optimal microbiome-mediated traits such as high N fixation or C sequestration. We also observed larger cultivar effects on bacterial than fungal soil communities, suggesting that there may be greater heritable variation and, thus, breeding potential for switchgrass bacterial than fungal microbiomes. This study shows that differences in switchgrass cultivars that have been documented aboveground also exist belowground and have the potential to influence the future success and ecosystem service provisioning of switchgrass as a bioenergy crop.

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