Intraspecific variability in root traits and edaphic conditions influence soil microbiomes across 12 switchgrass cultivars

Tayler C. Ulbrich,[1,2*] Maren L. Friesen,[4,5] Sarah S. Roley,[2,3,6] Lisa K. Tiemann,[7] Sarah E. Evans[1,2]

[1]Department of Integrative Biology, Michigan State University, East Lansing MI, USA.

[2]W.K. Kellogg Biological Station, Michigan State University, Hickory Corners MI, USA.

[3]Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI, USA.

[4]Department of Crop and Soil Sciences, Washington State University, Pullman WA, USA.

[5]Department of Plant Pathology, Washington State University, Pullman WA, USA.

[6]School of the Environment, Washington State University, Richland WA, USA.

[7]Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing MI, USA.

*Corresponding author: T.C. Ulbrich, E-mail: ulbrichtayler@gmail.com

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

Funding: Support for this research was provided by the Great Lakes Bioenergy Research Center, U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research (Awards DE-SC0018409 and DE-FC02-07ER64494), by the NSF Long-term Ecological Research Program (DEB 1832042 and 1637653) at the Kellogg Biological Station, by an NSF FSML grant (1722621), and by Michigan State University AgBioResearch.

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* L.) 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, further, 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 they 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: *Panicum virgatum,* switchgrass, microbiome, root traits

Introduction

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 so 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 variation 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. Further, 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 three 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 C:N) 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 contributes to variation in switchgrass cultivar microbiomes, but few studies have measured variation in switchgrass root traits and microbial communities simultaneously (but see Roosendaal et al. 2016; Stewart et al. 2017).

While 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

& 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), but 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, further, how microbes could be incorporated into switchgrass production.

We hypothesize that root traits and microbial communities will differ among switchgrass cultivars. Further, 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?

Methods

Site description

We conducted this study in southwest Michigan, USA, 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 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 eight upland and four lowland cultivars, were established in a complete randomized block design (four cultivars with poor establishment were replanted in 2010) (Table 1 for details on seed source and breeding history). Cultivars were planted at a rate of 9 kg live seed 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 x 12.2 m). The blocks were not irrigated and urea fertilizer was applied annually in the spring (78 kg N ha^{-1}). Pre-emergence weeds were controlled with Quinclorac Drive (1.1 kg ha^{-1}) and Atrazine (0.6 kg ha^{-1}) and post-emergence 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 diameter x 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 x 4 blocks = 12 cores per cultivar). All instruments were sterilized with 70% ethanol in 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 (simliar to Emmett et al. 2017). The

12 cultivars flowered over a four-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 hours 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 soils dried at 60°C), and downstream DNA extractions (2 g 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 (NH_4^+) and nitrate (NO_3^-) 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 (E.C #1.7.1.1) was used to reduce NO_3^- to NO_2^- and concentrations of NO_2^- were determined using sulfanilamide and *N*-(1-naphthyl)-ethylenediamine. Absorbance for NH_4^+ and NO_3^- assays were read on a Synergy HTX plate reader (BioTek, Winooski, Vermont, USA) at 610 nm 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.

171 *Root sterilization and trait analysis*

172 The previously frozen sieved roots and undisturbed soils were wet-sieved (2 mm) with nanopure
173 (0.2 µM) water and all visible roots were separated with sterilized tweezers for an average of 30
174 minutes per sample. These roots were stored at 4°C in nanopure water and scanned within 48
175 hours. To maintain sterility and minimize microbial cross-contamination, we sterilized all
176 equipment with 70% ethanol in between scans. The roots were scanned (1200 dpi resolution with
177 Epson perfection V600 scanner) in a glass scanning bed with 200 mL nanopure water, exported
178 as tiff files, manually edited to remove image artifacts, and compressed before analyzing root
179 traits with GiA Roots software (Galkovskyi et al. 2012, details in supplemental). Following
180 scanning, 0.25 g of the scanned roots (< 2 mm in diameter to standardize for root age) were
181 subsampled and sterilized for root-associated (endophyte) microbial characterization (details
182 below). The remaining roots were weighed and dried at 60°C for one week to calculate the
183 dry:wet root biomass ratio. Predicted total dry root weight was back-calculated using the dry:wet
184 ratio to estimate the dry weight of the 0.25 g subset. This back-calculation of total dry root
185 weight may underestimate actual root weight values if root water content varies with root
186 diameter; an underestimation of root weight could contribute to miscalculations of other root
187 traits, such as mass-weighted specific root length (total root length/dry root biomass). Using GiA
188 Roots, we calculated the following root traits: total root length (cm), average root diameter (cm),
189 total root system volume (cm^3), and specific root length (SRL). SRL was calculated in two ways:
190 1) mass-weighted SRL which we calculated using the back-calculated dry:wet root ratios (cm
191 total root length/ g total dry root biomass) and 2) volume-weighted SRL (cm total root length/
192 cm^3 total root volume).

193 To prepare the root tissues for DNA extractions, we first sterilized the 0.25 g of subsampled
194 roots. Immediately after scanning, we sterilized the subset roots following Sun et al. (2008):
195 roots were immersed in 70% ethanol for 3 minutes, sterilized with fresh household sodium
196 hypochlorite solution (2.5% available Cl^-) for 5 minutes, rinsed with 70% ethanol for 30 seconds,
197 rinsed ten times with sterile autoclaved water, blotted dry with Kimwipes (Kimberly-Clark,
198 Roswell GA, USA) and frozen at -20°C (Sun et al., 2008). To test root-surface sterilization, the
199 final water rinse was plated on Luria-Bertani agar and incubated at 30°C for 7 days. A majority
200 of the LB plates had bacterial growth after one week of incubation. Although the bacterial
201 growth may suggest incomplete sterilization of the rhizoplane, because these samples were root
202 segments, the cultured bacteria may have been endophytic bacteria that dispersed from the
203 interior of the roots. Due to the thorough sterilization procedure, we believe the remaining
204 microbes are strongly root-associated but cannot conclude they are obligate endophytes. Before
205 DNA extraction, the frozen, surface-sterilized root samples were submerged in liquid N and
206 ground with a tissue lyser (Qiagen Tissue Lyser II, Valencia, California, USA). If any root pieces
207 > 2 mm remained, sterilized scissors (10% bleach and 70% ethanol) were used to more finely cut
208 the roots.

209 *DNA extraction, sequencing, and bioinformatics*

210 DNA was extracted similarly from soil and sterilized roots, but only a subset of cultivars were
211 processed for root-associated microbes. Soil DNA was extracted from 0.25 g of sieved and
212 homogenized sample from all 12 cultivars (n = 144 samples: 12 cultivars x 4 blocks x 3 replicate
213 cores). Root DNA was extracted from approximately 0.25 g of sterilized, ground root tissue from
214 four commonly-planted cultivars (Upland: Cave-in-Rock, Southlow; Lowland: Alamo, Kanlow;
215 n = 48 samples: 4 cultivars x 4 blocks x 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-minute cell lysis step at 65°C before the bead-beating step (MOBIO Laboratories, Carlsbad, California, USA). The purity and quantity of the extracted DNA was examined using a Nanodrop 2000 (Thermo Scientific, USA) and via fluorometry with the Quanti-iT PicoGreen dsDNA kit (Thermo Fisher, USA). We targeted the bacterial V4 region of the 16S 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, while 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, Michigan, USA). 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 Greengenes (version 13.8) database using Usearch closed-reference (closed_ref) for downstream PICRUSt analysis (DeSantis et al. 2006; Langille et al. 2013). Non-bacterial and non-fungal sequences, singleton OTUs, and samples with poor-sequence coverage were removed from the reference-based OTU tables (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), so we used a non-phylogenetic 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 (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 & 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 ($p < 0.001$, 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 (https://github.com/TaylerUlbrich/SwitchgrassCultivarMicrobiomeStudy) are publicly available.

Data analysis: univariate statistics

Prior to all data analysis, we assured that all univariate data met assumptions of normality (see supplemental 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 lm4 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. Since we sampled the cultivars across four weeks to control for phenology-driven variation in microbiomes (Chaparro et al. 2014; Zhalnina 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 (ANVOA, $p < 0.001$), 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, so 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, microbial biomass nitrogen and carbon, root length – but soil moisture was only a significant predictor variable ($p < 0.05$) for microbial biomass carbon. Post-hoc comparisons (p values adjusted with Benjamini–Hochberg false discovery rate, 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, so we used non-parametric Kruskal-Wallis and Wilcox tests (no block factor included). Pearson correlations were used to determine relationships between edaphic conditions, root traits, and microbial biomass carbon 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 & Holmes, 2013; Oksanen et al., 2018; Wickham, 2016). We examined overall variation in the cultivars' microbiome composition using permutation-based ANOVA (PERMANOVA) and betadispersion tests with type 1 sum of squares. PERMANOVAs, betadispersion, and post-hoc 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 & 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 9 replicates for microbiome analyses (Table S2). As in the univariate models, date and cultivar were confounded, so 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 (Tables S3, 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 & 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., 9/12 sample units per cultivar) and across all cultivars. Indicator taxa were identified (after removing singleton OTUs) using the 'multiplatt' function in the indicspecies R package (Caceres & Legendre, 2009) and defined as OTUs present in at least 25% of the samples (3/12 sample units, or indicspecies specificity parameter = 0.25). Rarefied datasets are biased against rare taxa, so it is possible that we identified fewer indicator taxa because less dominant, rare taxa were lost during rarefaction (McMurdie & Holmes, 2014). We also characterized phyla-level differences among cultivars and ecotypes using the 'manyglm' function in the MVAbund R package and ANOVA post-hoc 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 supplemental) (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. 2020, 2019, 2018). We assessed this by 1) calculating variation in the relative abundance of common N-fixing orders Rhizobiales and Burkholderiales and 2) using PICRUSt to predict the relative proportion of putative N-fixing taxa (Langille et al., 2013) (details in supplemental). 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 difference 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 supplemental) (Wang et al., 2012). At the community level, we determined which variables (average root diameter, total root length, soil nitrate, soil ammonium, 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 'dbrda' 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 as 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 ($p > 0.05$, 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)

353 significantly differed among cultivars ($p < 0.01$) but not by ecotype ($p > 0.05$, Figure 1A, Table
354 S5).The cultivar differences in volume-weighted SRL were likely driven by average root
355 diameter which significantly differed by cultivar ($p < 0.001$, Figure 1B), and was used to
356 calculate root network volume. There was a 30% difference between the cultivars with the
357 thickest (e.g., Cave-in-Rock and EG2101) and thinnest (e.g., Kanlow and NE28) roots.

358 *Microbial biomass*

359 Microbial biomass carbon (MBC) and nitrogen (MBN) significantly differed among cultivars
360 (MBC: $p < 0.001$, MBN: $p < 0.001$) and ecotypes (MBC: $p < 0.01$, MBN: $p < 0.001$)
361 (Figure 1C, D), even after controlling for soil moisture content which influenced MBC (soil
362 moisture co-variate with MBC: $p < 0.001$, with MBN: $p > 0.05$) and varied by date ($p < 0.05$).
363 Lowland MBC and MBN were 25% and 65% greater than upland ecotypes, respectively.

364 *Soil vs. root associated bacterial communities*

365 For a subset of four commonly-planted cultivars (Cave-in-Rock, Southlow, Alamo, Kanlow), we
366 found that root and soil bacterial communities differed in diversity, composition, and the extent
367 to which they were affected by cultivar identity. Microhabitat (soil or root) explained 59% of the
368 overall variance in community composition (Table 2, Figure 2A), and the root community had
369 five and three times lower bacterial richness and Shannon diversity than the soil communities,
370 respectively (Table S6). The differences in beta diversity between roots and soils were mirrored
371 in their dominant phyla. The most abundant bacterial phyla in the roots (n = 4 cultivars) were
372 Proteobacteria (70%), Actinobacteria (11%) and Bacteroidetes (5%), while the soil communities
373 (n = 4 cultivars) were dominated by Acidobacteria (30%), Proteobacteria (29%), and
374 Verrucomicrobia (11%)(Figure 2B). The same phyla were most abundant in the soil
375 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 (Table S4). There was also no cultivar-effect on root or soil bacterial alpha diversity (Table S6) and there were fewer differences in the relative abundance of dominant soil phyla for these four cultivars (Figure 4), 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 1-3% higher for upland ecotypes for all diversity metrics ($p < 0.05$, Figure 1E, Table S7). However, these differences were driven by Dacotah, which had the highest bacterial richness and Shannon diversity (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 (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

399 the most variation in community composition, while ecotype only explained 3% of the variation
400 (Figure 3A, Table 3). The bacterial communities of three cultivars – Alamo (lowland), EG1102
401 (lowland), and NE28 (upland) – were more dissimilar from all other cultivars (pairwise
402 comparisons, $p < 0.10$, Table S9). When assessed within sampling date, cultivar explained a
403 significant proportion of variation in the bacterial community composition within one date (16%,
404 $p < 0.05$, Table S10): cultivar NE28 had a significantly different soil bacterial community than
405 the other three upland cultivars (Southlow, Cave-in-Rock, Trailblazer) sampled on the same date.

406 The cultivars' soil bacterial communities also differed at the phyla level and are comprised of
407 many shared and few unique taxa. Eight soil bacterial phyla (74.3% of all reads) significantly
408 differed among cultivars (Figure 4). Several of these phyla also differed by ecotype; specifically,
409 Bacteroidetes, Planctomycetes, and Verrucomicrobia are more abundant in lowland cultivars,
410 while Actinobacteria, Deltaproteobacteria, and Gemmatimonadetes are more abundant in upland
411 cultivars. At the OTU-level, we found that 160 OTUs (out of 14,590 total) were shared across all
412 cultivars (present in 75% of samples units within and among cultivars). These shared OTUs
413 make up 45% of the total sequences and are dominated by three classes –Acidobacteria (39%),
414 Alphaproteobacteria (17%) and Spartobacteria (12%). In contrast, indicator bacterial OTUs of
415 the 12 cultivars include 683 OTUs and make up 21% of the total sequences dominated by classes
416 Acidobacteria (33%), Alphaproteobacteria (10%) and Deltaproteobacteria (7%).

417 We used PICRUSt to test whether cultivars' soil and root bacterial communities might have
418 different abilities to fix N_2. We first used NSTI scores to assess whether PICRUSt accurately
419 approximated bacterial function for our sequences. Larger NSTI scores (> 0.15) are expected for
420 highly diverse and largely uncharacterized environments like soils and indicate less phylogenetic
421 relatedness between the predicted OTUs and reference genomes (Langille et al. 2013). The

average NSTI scores for the soil samples was 0.23, which is within the typical range for soil samples (Langille et al. 2013) but indicates results should be interpreted with caution due to weak phylogenetic relatedness. Root NTSI (0.32) indicated low relatedness with reference genomes, and therefore were not analyzed. We found that cultivar soil bacterial communities varied in the proportion of OTUs with putative N-fixation genes ($p < 0.001$, Figure 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., 2020, 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, Figure 3B). However, unlike the bacterial communities, the cultivar-level effects on fungal communities were not robust to variation across (Table S3) or within sampling dates (Table S10). Fungal community diversity (richness, Shannon, evenness) also did not differ by cultivar or ecotype ($p > 0.05$, 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 only made up 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%), Sordariomycetes (23%), and those Unclassified (29%). Indicator fungal OTUS of the 12 cultivars make up 25% of the total fungal sequences and include 213 OTUs dominated by classes Sordariomycetes (19%), Dothideomycetes (17%), and 27% were unclassified at class level.

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, soil ammonium) explained more variation for the soil bacterial (10%) than the soil fungal (5%) communities (Table 4). Mirroring the PERMAONVA 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 most explained by soil nitrate (6%) and soil moisture content (2%) while the fungal community was most 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, but 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, 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, $p < 0.001$). Further, microbial biomass carbon 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 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, but we found greater differences in switchgrass microbiomes across cultivars than between ecotypes. We found that cultivar explained 10-20% of the variance in soil microbiome beta diversity, while 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, while 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. Further, it is also possible that we under-sampled the root bacterial diversity, as 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, as Singer et al. (2019b) found that two *Panicum* species 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 (e.g., Edwards et al. 2015).

Edaphic conditions and plant traits influence soil community structure

Soil water and nitrogen 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) – *Panicum* species' rhizosphere soil communities 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), but the observed differences in soil N in this study could be driven by the cultivars' differential effects on N cycling (Roley et al., 2020) 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), since 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 supports 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, but the observed correlation could also describe microbes driving root traits (Petipas et al., 2020; Verbon & Liberman, 2016).

We found that spatial variability (block factor) also explained a surprisingly large percent (> 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 nitrogen content (also in paired study, Roley et al. 2020). Our analysis of microbiome composition and edaphic conditions controlled for this block effect, yet 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. Further, 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 et al. 2017). Sawyer et al. (2017) found that switchgrass cultivar microbiomes were more distinct in less fertile soils. It is also possible that cultivars that were grown outside of

559 their native range (e.g. not from the north-central United States) had weaker effects on their
560 microbiomes because they could not associate with their native, potentially co-evolved microbial
561 communities. Studies of cultivars in common gardens across many sites could elucidate the
562 contribution of native range or seed source on plant-microbial interactions. Further, because we
563 did not sample the soils directly adhering to the roots or use primers to target root-colonizing
564 microbes (e.g., AMF) we may not have captured the microbes most influenced by root traits and
565 exudates. Finally, we found that cultivars vary in average root diameter and, therefore, soils
566 beneath each cultivar likely differ in the amount of root turnover and development. Microbial
567 composition and function has been shown to vary with root age, type (e.g., seminal or nodal
568 root), and location (e.g., root branch or tip) (de Graaff et al. 2013; Kawasaki et al. 2016;
569 Marschner and Baumann 2003), but sampling with soil cores made it challenging to identify the
570 effects of root age, type, or location on soil microbial communities. Therefore, future studies
571 should use methods that standardize root age (e.g., use of root-in-growth cores) or root type and
572 location (e.g., visualizing root differences and sampling within rhizoboxes) to better understand
573 how root traits influence microbiome structure (Yu and Hochholdinger 2018).

574 Plant developmental stage (e.g., phenology, maturity) also contributes to microbiome variability
575 (Edwards et al. 2018; Na et al. 2019; Zhalnina et al. 2018). We sampled cultivars at the same
576 stage (flowering) to control for this variation, but sampling on different dates may have increased
577 differences in edaphic conditions that influence the microbiome. Yet, when we controlled for
578 variation among sampling dates, cultivar still contributed to variation in the soil bacterial, but not
579 fungal communities. This suggests that the fungal communities were more influenced by
580 variation in abiotic conditions across dates, or that cultivars with different phenology and, thus,
581 sampling dates, had more dissimilar fungal communities. In contrast, bacterial community

582 structure was more strongly influenced by cultivar identity, which explained a significant percent
583 (16%) of the variation in bacterial community structure within one of the four sampling dates.
584 We hypothesize that greater differences were not observed within the other three sampling dates
585 because cultivars with comparable phenology (e.g., flowering at the same time) likely have other
586 similar traits and, thus, more similar microbial communities than cultivars with different
587 phenology. However, to better understand the effect of similar phenology and traits on cultivar
588 microbiomes, future studies should evaluate the switchgrass cultivar microbiomes across
589 multiple phenological stages (e.g., Na et al. 2019; Qiao et al. 2017; Wagner et al. 2016) as both
590 the microbiome structure and the magnitude of cultivar effects may change with phenological
591 stage (Inceoglu et al. 2010; Na et al. 2019).

592 *Functional implications and conclusions*

593 Differences in cultivar root traits and microbial biomass could contribute to variability in the
594 cultivars' soil C-cycling and C sequestration potential. We found differences in microbial
595 biomass and root diameter, but not root biomass, across cultivars. Another study conducted in
596 the same common garden experiment, however, did find differences in root biomass among
597 cultivars (Emery et al. 2018). These differences in average root diameter have the potential to
598 drive variation in the cultivars' C-cycling and microbial community structure. Root systems with
599 high SRL, corresponding to long, thin roots, positively correlate with switchgrass-derived soil C
600 (Adkins et al., 2016; Stewart et al., 2017), decomposition (de Graaff et al. 2013, 2014),
601 bacterial:fungal ratios (de Graaff et al. 2013), and microbial biomass (PLFA-C) (Stewart et al.
602 2017). Greater rhizodeposition from thin roots can directly contribute to soil C pools, as well as
603 indirectly influence soil C by supporting the growth and turnover of microbial communities
604 which, in turn, contributes to greater soil C and aggregate stability (Grandy & 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. 2020), 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, as 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 differs 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, like 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.

Acknowledgements

Support for this research was provided by the Great Lakes Bioenergy Research Center, U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research (Awards DE-SC0018409 and DE-FC02-07ER64494), by the NSF Long-term Ecological Research Program (DEB 1832042 and 1637653) at the Kellogg Biological Station, by an NSF FSML grant (1722621), and by Michigan State University (MSU) AgBioResearch. TCU was supported by and MSU Plant Sciences Fellowship and an NSF Graduate Research Fellowship. We thank Phil Robertson for helpful comments on earlier versions of this manuscript, Cody Bekkering, Jordan Priebe, Zhenyao Ye, Christin Parr, and YJ Su for help with sample processing, Lukas Bell-Dereske for statistical guidance, and Joe Simmons for long-term maintenance of the LTER plots.

References

Adam, E., Bernhart, M., Müller, H., Winkler, J., & Berg, G. (2018). The Cucurbita pepo seed microbiome: genotype-specific composition and implications for breeding. *Plant and Soil*, *422*(1–2), 35–49. https://doi.org/10.1007/s11104-016-3113-9

Adkins, J., Jastrow, J. D., Morris, G. P., Six, J., & de Graaff, M. A. (2016). Effects of switchgrass cultivars and intraspecific differences in root structure on soil carbon inputs and accumulation. *Geoderma*, *262*, 147–154. https://doi.org/10.1016/j.geoderma.2015.08.019

Aimar, D., Calafat, M., Andrade, A. M., Carassay, L., Bouteau, F., Abdala, G., & Molas, M. L.

(2014). Drought effects on the early development stages of Panicum virgatum L.: Cultivar differences. *Biomass and Bioenergy*, *66*, 49–59. https://doi.org/10.1016/j.biombioe.2014.03.004

An, Y., Ma, Y., & Shui, J. (2013). Switchgrass root exudates have allelopathic potential on lettuce germination and seedling growth. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, *63*(6), 497–505. https://doi.org/10.1080/09064710.2013.810770

Anderson, M. L., Gorley, R. N., & Clarke, K. R. (2008). *PERMANOVA+ for PRIMER* (p. Plymouth UK).

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bell, T. H., El-Din Hassan, S., Lauron-Moreau, A., Al-Otaibi, F., Hijri, M., Yergeau, E., & St-Arnaud, M. (2014). Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny. *The ISME Journal*, *8*(2), 331–343. https://doi.org/10.1038/ismej.2013.149

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E. V. L., & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*, *64*(1), 807–838. https://doi.org/10.1146/annurev-arplant-050312-120106

Busby, P. E., Soman, C., Wagner, M. R., Friesen, M. L., Kremer, J., Bennett, A., … Leach, J. E. (2017). Research priorities for harnessing plant microbiomes in sustainable agriculture. *PLoS Biology*, (March), 1–14. https://doi.org/10.1371/journal.pbio.2001793

671 Caceres, D., & Legendre, P. (2009). Associations between species and groups of sites: indices
672 and statistical inference. *Ecology,* 90(12), 3566-3574.

673 Casler, M. D. (2012). Chapter 2: Switchgrass Breeding, Genetics, and Genomics. In *Switchgrass,*
674 *Green Energy and Technology* (pp. 29–53). https://doi.org/10.1007/978-1-4471-2903-5

675 Casler, M. D., Sosa, S., Boe, A. R., & Bonos, S. A. (2019). Soil quality and region influence
676 performance and ranking of switchgrass genotypes. *Crop Science*, *58*(0), 1-12.
677 https://doi.org/10.2135/cropsci2018.06.0409

678 Casler, M. D., Sosa, S., Hoffman, L., Mayton, H., Ernst, C., Adler, P. R., … Bonos, S. A. (2017).
679 Biomass yield of switchgrass cultivars under high- versus low-input conditions. *Crop*
680 *Science*, *57*(2), 821-832. https://doi.org/10.2135/cropsci2016.08.0698

681 Chaparro, J. M., Badri, D. V., & Vivanco, J. M. (2014). Rhizosphere microbiome assemblage is
682 affected by plant development. *ISME Journal*, *8*(4), 790–803.
683 https://doi.org/10.1038/ismej.2013.196

684 de Graaff, M. A., Jastrow, J. D., Gillette, S., Johns, A., & Wullschleger, S. D. (2014).
685 Differential priming of soil carbon driven by soil depth and root impacts on carbon
686 availability. *Soil Biology and Biochemistry*, *69*, 147–156.
687 https://doi.org/10.1016/j.soilbio.2013.10.047

688 de Graaff, M. A., Six, J., Jastrow, J. D., Schadt, C. W., & Wullschleger, S. D. (2013). Variation
689 in root architecture among switchgrass cultivars impacts root decomposition rates. *Soil*
690 *Biology and Biochemistry*, *58*(March), 198–206.
691 https://doi.org/10.1016/j.soilbio.2012.11.015

692 DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., … Andersen, G.
693 L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench
694 compatible with ARB. *Appl. Environ. Microbiol.*, *72*(7), 5069–5072.

695 Dombrowski, N., Schlaeppi, K., Agler, M. T., Hacquard, S., Kemen, E., Garrido-Oter, R., …
696 Schulze-Lefert, P. (2017). Root microbiota dynamics of perennial Arabis alpina are
697 dependent on soil residence time but independent of flowering time. *ISME Journal*, *11*(1),
698 43–55. https://doi.org/10.1038/ismej.2016.109

699 DuPont, S. T., Beniston, J., Glover, J. D., Hodson, A., Culman, S. W., Lal, R., & Ferris, H.
700 (2014). Root traits and soil properties in harvested perennial grassland, annual wheat, and
701 never-tilled annual wheat. *Plant and Soil*, *381*(1-2), 405–420.
702 https://doi.org/10.1007/s11104-014-2145-2

703 Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST.
704 *Bioinformatics*, *26*(19), 2460–2461.

705 Edwards, J., Santos-Medellín, C. M., Liechty, Z. S., Nguyen, B., Lurie, E., Eason, S., …
706 Sundaresan, V. (2018). Compositional shifts in root-associated bacterial and archaeal
707 microbiota track the plant life cycle in field-grown rice. *PLoS Biology*, *16*(2), 1–28.
708 https://doi.org/10.1371/journal.pbio.2003862

709 Edwards, J., Johnson, C., Santos-medellín, C., Lurie, E., & Kumar, N. (2015). Structure,
710 variation , and assembly of the root-associated microbiomes of rice. *Proceedings of the*
711 *National Academy of Sciences*, *112*(8), E911–E920.
712 https://doi.org/10.1073/pnas.1414592112

713 Emery, S. M., Kinnetz, E. R., Bell-Dereske, L., Stahlheber, K. A., Gross, K. L., & Pennington,

714 D. (2018). Low variation in arbuscular mycorrhizal fungal associations and effects on
715 biomass among switchgrass cultivars. *Biomass and Bioenergy*, *119*(April), 503–508.
716 https://doi.org/10.1016/j.biombioe.2018.10.012

717 Emmett, B. D., Youngblut, N. D., Buckley, D. H., & Drinkwater, L. E. (2017). Plant phylogeny
718 and life history shape rhizosphere bacterial microbiome of summer annuals in an
719 agricultural field. *Frontiers in Microbiology*, *8*(December), 1–16.
720 https://doi.org/10.3389/fmicb.2017.02414

721 Fierer, N. (2017). Embracing the unknown: Disentangling the complexities of the soil
722 microbiome. *Nature Reviews Microbiology*, *15*(10), 579–590.
723 https://doi.org/10.1038/nrmicro.2017.87

724 Galkovskyi, T., Mileyko, Y., Bucksch, A., Moore, B., Symonova, O., Price, C. A., & Topp, C.
725 N. (2012). GiA Roots: software for the high-throughput analysis of plant root system
726 architecture. *BMC Plant Biology*, *12*(116).

727 Grandy, A. S., & Neff, J. C. (2008). Molecular C dynamics downstream: The biochemical
728 decomposition sequence and its impact on soil organic matter structure and function.
729 *Science of the Total Environment*, *404*(2–3), 297–307.
730 https://doi.org/10.1016/j.scitotenv.2007.11.013

731 Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric
732 models. *Biometrical Journal*, *50*(3), 346–363.

733 Inceoglu, O., Salles, J. F., Van Overbeek, L., & van Elsas, J. D. (2010). Effects of Plant
734 Genotype and growth stage on the betaproteobacterial communities associated with
735 different potato cultivars in two fields. *Applied and Environmental Microbiology*, *76*(11),

3675–3684. https://doi.org/10.1128/microbe.8.242.1

Jiang, Y., Li, S., Li, R., Zhang, J., Liu, Y., Lv, L., … Li, W. (2017). Plant cultivars imprint the rhizosphere bacterial community composition and association networks. *Soil Biology & Biochemistry*, *109*, 145–155. https://doi.org/10.1016/j.soilbio.2017.02.010

Kawasaki, A., Donn, S., Ryan, P. R., Mathesius, U., Devilla, R., Jones, A., … Shen, Q. (2016). Microbiome and exudates of the root and rhizosphere of *Brachypodium distachyon*, a model for wheat. *PLoS ONE*, *11*(10), e0164533. https://doi.org/10.1371/journal.pone.0164533

Kuznetsova, A., & Brockhoff, P.B., Christensen, R. H. . (2017). lmerTest Package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13

Langille, M. G. I., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., a Reyes, J., … Huttenhower, C. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, *31*(9): 813-821. https://doi.org/10.1038/nbt.2676

Lenth, R. V. (2019). emmeans: Estimated marginal means, aka least-squares means. *R package version 1.4*. https://doi.org/https://CRAN.R-project.org/package=emmeans

Liang, C., Jesus, E. da C., Duncan, D. S., Jackson, R. D., Tiedje, J. M., & Balser, T. C. (2012). Soil microbial communities under model biofuel cropping systems in southern Wisconsin, USA: Impact of crop species and soil properties. *Applied Soil Ecology*, *54*, 24–31. https://doi.org/10.1016/j.apsoil.2011.11.015

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and

757 dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550.

758 Marschner, P., & Baumann, K. (2003). Changes in bacterial community structure induced by
759 mycorrhizal colonisation in split-root maize. *Plant and Soil*, *251*(2), 279–289.
760 https://doi.org/10.1023/A:1023034825871

761 Mcknight, D. T., Huerlimann, R., Bower, D. S., Schwarzkopf, L., Alford, R. A., & Zenger, K. R.
762 (2019). Methods for normalizing microbiome data: an ecological perspective. *Methods in*
763 *Ecology and Evolution, 10*(3), 389-400. https://doi.org/10.1111/2041-210X.13115

764 McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R package for reproducible interactive
765 analysis and graphics of microbiome census data. *PloS One*, *8*(4).
766 https://doi.org/https://doi.org/10.1371/journal.pone.0061217

767 McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: why rarefying microbimoe data is
768 inadmissible. *PLoS Computational Biology*, *10*(4), 1-12.
769 https://doi.org/10.1371/journal.pcbi.1003531

770 Mirarab, S., Nguyen, N., Guo, S., Wang, L.-S., Kim, J., & Warnow, T. (2015). PASTA: Ultra-
771 Large Multiple Sequence Alignment for Nucleotide and Amino-Acid Sequences. *Journal of*
772 *Computational Biology, 22*(5), 377–386. https://doi.org/10.1089/cmb.2014.0156

773 Mueller, U. G., & Sachs, J. L. (2015). Engineering microbiomes to improve plant and animal
774 health. *Trends in Microbiology*, *23*(10), 606–617. https://doi.org/10.1016/j.tim.2015.07.009

775 Na, X., Cao, X., Ma, C., Ma, S., Xu, P., Liu, S., … Qiao, Z. (2019). Plant stage, not drought
776 stress, determines the effect of cultivars on bacterial community diversity in the rhizopshere
777 of Broomcorn Millet (*Panicum miliaceum* L.). *Frontiers in Microbiology*, *10*(April), 1–11.

https://doi.org/10.3389/fmicb.2019.00828

Nilsson, R. H., Kristiansson, E., Ryberg, M., Hallenberg, N., & Larsson, K. H. (2008). Intraspecific ITS variability in the Kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary Bioinformatics*, (4), 193–201.

Nilsson, R. H., Larsson, R. H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., … Tedersoo, L. (2018). The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, *47*(D1), D259–D264.

Ogle, D. H., Wheeler, P., & Dinno, A. (2019). *FSA: Fisheries Stock Analysis. R.package*. https://doi.org/https://github.com/droglenc/FSA

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Solymos, P. (2018). Vegan: Community Ecology Package. *R package version 2.5-2.*

Pérez-Jaramillo, J. E., Carrión, V. J., Bosse, M., Ferrão, L. F. V., De Hollander, M., Garcia, A. A. F., … Raaijmakers, J. M. (2017). Linking rhizosphere microbiome composition of wild and domesticated Phaseolus vulgaris to genotypic and root phenotypic traits. *ISME Journal*, *11*(10), 2244–2257. https://doi.org/10.1038/ismej.2017.85

Pérez-Jaramillo, J. E., Mendes, R., & Raaijmakers, J. M. (2016). Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Molecular Biology*, *90*(6), 635–644. https://doi.org/10.1007/s11103-015-0337-7

Petipas, R. H., Bowsher, A. W., Bekkering, C. S., Jack, C. N., McLachlan, E. E., White, R. A.,

799 … Friesen, M. L. (2020). Interactive effects of microbes and nitrogen on *Panicum Virgatum*
800 root functional traits and patterns of phenotypic selection. *International Journal of Plant*
801 *Sciences*, *181*(1), 20–32. https://doi.org/10.1086/706198

802 Qiao, Q., Wang, F., Zhang, J., Chen, Y., Zhang, C., Liu, G., … Zhang, J. (2017). The variation in
803 rhizosphere microbiome of cotton with soil type, genotype, and developmental stage.
804 *Scientific Reports*, *7*(1), 1–10. https://doi.org/10.1038/s41598-017-04213-7

805 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., … Glöckner, F. O. (2012).
806 The SILVA ribosomal RNA gene database project: improved data processing and web-
807 based tools. *Nucleic Acids Research*, *41*(D1), D590–D596.

808 R Core Team. (2018). R: A language and environment for statistical computing. R Foundation
809 for Statistical Computing. Vienna, Austria. https://www.R-project.org/.

810 Reinhold-Hurek, B., Bünger, W., Burbano, C. S., Sabale, M., & Hurek, T. (2015). Roots shaping
811 their microbiome: global hotspots for microbial activity. *Annual Review of Phytopathology*,
812 *53*(1), 403–424. https://doi.org/10.1146/annurev-phyto-082712-102342

813 Revillini, D., Wilson, G. W. T., Miller, R. M., Lancione, R., & Johnson, N. C. (2019). Plant
814 diversity and fertilizer management shape the belowground microbiome of native grass
815 bioenergy feedstocks. *Frontiers in Plant Science*, *10*(August), 1–18.
816 https://doi.org/10.3389/fpls.2019.01018

817 Rodrigues, R. R., Moon, J., Zhao, B., & Williiams, M. A. (2017). Microbial communities and
818 diazotrophic activity differ in the root-zone of Alamo and Dacotah switchgrass feedstocks.
819 *GCB Bioenergy*, *9*, 1057–1070. https://doi.org/10.1111/gcbb.12396

Roley, S. S., Duncan, D. S., Liang, D., Garoutte, A., Jackson, R. D., Tiedje, J. M., & Robertson, G. P. (2018). Associative nitrogen fixation (ANF) across a nitrogen input gradient. *PLoS one 13*(6), e0197320. 1–37. https://doi.org/10.1371/journal.pone.0197320

Roley, S. S., Xue, C., Hamilton, S. K., Tiedje, J. M., & Robertson, G. P. (2019). Isotopic evidence for episodic nitrogen fixation in switchgrass (*Panicum virgatum* L.). *Soil Biology and Biochemistry*, *129*, 90–98. https://doi.org/10.1016/j.soilbio.2018.11.006

Roley, S. S, Ulbrich, T. C., & Robertson, G. P. (2020). Nitrogen fixation and resorption efficiency differences among twelve upland and lowland switchgrass cultivars. *Phytobiomes*. https://doi.org/10.1094/PBIOMES-11-19-0064-FI

Roosendaal, D., Stewart, C. E., Denef, K., Follett, R. F., Pruessner, E., Comas, L. H., … Soundararajan, M. (2016). Switchgrass ecotypes alter microbial contribution to deep soil C. *Soil*, *2*(2), 185–197. https://doi.org/10.5194/soil-2015-92

Saleem, M., Law, A. D., Sahib, M. R., Pervaiz, Z. H., & Zhang, Q. (2018). Impact of root system architecture on rhizosphere and root microbiome. *Rhizosphere 6*, 47–51. https://doi.org/10.1016/j.rhisph.2018.02.003

Sawyer, A., Lamb, John, & Rosen, C. (2017). Switchgrass yield, nutrient uptake, and rhizosphere microbial community composition as affected by cultivar and soil fertility. *University of Minnesota Dissertation*. Retrieved from http://www.mdpi.com/2223-7747/6/4/46

Sawyer, A., Staley, C., Lamb, J., Sheaffer, C., Kaiser, T., Gutknecht, J., … Rosen, C. (2019). Cultivar and phosphorus effects on switchgrass yield and rhizosphere microbial diversity. *Applied Microbiology and Biotechnology*, *103*(4), 1973–1987.

https://doi.org/10.1007/s00253-018-9535-y

Singer, E., Bonnette, J., Kenaley, S. C., Woyke, T., & Juenger, T. E. (2019a). Plant compartment and genetic variation drive microbiome composition in switchgrass roots. *Environmental Microbiology Reports*, *11*(2), 185–195. https://doi.org/10.1111/1758-2229.12727

Singer, E., Bonnette, J., Woyke, T., & Juenger, T. E. (2019b). Conservation of endophyte bacterial community structure across two panicum grass species. *Frontiers in Microbiology*, *10*(September). https://doi.org/10.3389/fmicb.2019.02181

Sinsabaugh, R. L., Reynolds, H., & Long, T. M. (2000). Rapid assay for amidohydrolase (urease) activity in environmental samples. *Soil Biology & Biochemistry*, *32*(14), 2095–2097.

Stahlheber, K. A., Lindquist, J., Drogosh, P. D., Pennington, D., Gross, K. L., Station, W. K. K. B., & Corners, H. (2020). Predicting productivity : A trait-based analysis of variability in biomass yield among switchgrass feedstock cultivars. *Agriculture, Ecosystems and Environment*, *300*(October), 106980. https://doi.org/10.1016/j.agee.2020.106980

Stewart, C. E., Roosendaal, D., Denef, K., Pruessner, E., Comas, L. H., Sarath, G., … Soundararajan, M. (2017). Seasonal switchgrass ecotype contributions to soil organic carbon, deep soil microbial community composition and rhizodeposit uptake during an extreme drought. *Soil Biology and Biochemistry*, *112*, 191–203. https://doi.org/10.1016/j.soilbio.2017.04.021

Sun, L., Qiu, F., Zhang, X., Dai, X., Dong, X., & Song, W. (2008). Endophytic bacterial diversity in rice (Oryza sativa L.) roots estimated by 16S rDNA sequence analysis. *Microbial Ecology*, *55*(3), 415–424. https://doi.org/10.1007/s00248-007-9287-1

Tiemann, L. K., Grandy, A. S., Atkinson, E. E., Marin-Spiotta, E., & Mcdaniel, M. D. (2015). Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecology Letters*, *18*(8), 761–771. https://doi.org/10.1111/ele.12453

Vance, E. D., Brookes, P. C., & Jenkinson, D. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry*, *19*(6), 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Verbon, E. H., & Liberman, L. M. (2016). Beneficial microbes affect endogenous mechanisms controlling root development. *Trends in Plant Science*, *21*(3), 218–229. https://doi.org/10.1016/j.tplants.2016.01.013

Wagner, M. R., Lundberg, D. S., del Rio, T. G., Tringe, S. G., Dangl, J. L., & Mitchell-Olds, T. (2016). Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nature Communications*, *7*(12151), 1–15. https://doi.org/10.1038/ncomms12151

Wang, Y., Naumann, U., Write, S., & Warton, D. J. (2012). mvabund: an R package for model-based analysis of multivariate data. *Methods in Ecology and Evolution*, *3*, 472–474.

Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., … Knight, R. (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, *5*(1), 27. https://doi.org/10.1186/s40168-017-0237-y

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis.* (p. Springer-Verlag, New York). p. Springer-Verlag, New York.

Yang, J., Worley, E., Wang, M., Lahner, B., Salt, D. E., Saha, M., & Udvardi, M. (2009). Natural variation for nutrient use and remobilization efficiencies in switchgrass. *Bioenergy*

Research, *2*(4), 257–266. https://doi.org/10.1007/s12155-009-9055-9

Yu, P., & Hochholdinger, F. (2018). The role of host genetic signatures on root-microbiome interactions in the rhizosphere and endospheres. *Frontiers in Plant Science*, *9*, 1896. https://doi.org/10.3389/fpls.2018.01896

Zhalnina, K., Louie, K. B., Hao, Z., Mansoori, N., Nunes da Rocha, U., Shi, S., … Brodie, E. L. (2018). Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nature Microbiology, 3*(4), 470–480. https://doi.org/10.1038/s41564-018-0129-3

Zhang, R., Vivanco, J. M., & Shen, Q. (2017). The unseen rhizosphere root–soil–microbe interactions for crop production. *Current Opinion in Microbiology*, *37*, 8–14. https://doi.org/10.1016/J.MIB.2017.03.008

Table 1. Details on cultivar origin, sampling date, and establishment year in the common garden experiment. Seed source location and breeding history details from Stahlheber et al. (2020); 'NA' denotes not available.

Cultivar	Ecotype	Sampling date	Establishment year	Breeding history (Native seed source)
Alamo	Lowland	July 27	2009	Seed increase from native remnant prairie[1] (Southern Texas)
EG1101	Lowland	July 13	2010	Improved Alamo-type bred for biomass yield[2] (NA)
EG1102	Lowland	July 27	2010	Improved Kanlow-type bred for biomass yield[2] (NA)
Kanlow	Lowland	July 27	2009	Seed collection from native remnant prairie, selected for leafiness, vigor, late-season greenness[1] (Northern Oklahoma)
Blackwell	Upland	June 28	2009	Seed increase from native remnant prairie[1] (Northern Oklahoma)
Cave-in-Rock	Upland	July 20	2009	Seed increase from native remnant prairie[1] (Southern Illinois)
Dacotah	Upland	June 28	2009	Seed increase from native remnant prairie, selected for leafiness, color and winter hardiness[1] (Southern North Dakota)
EG2101	Upland	July 13	2010	Improved Cave-in-Rock bred for biomass yield[2] (NA)
Nebraska 28	Upland	July 20	2009	Seed increase native remnant prairie[1] (Nebraska)
Shelter	Upland	July 13	2010	Seed increase from native prairie, selected for thick stems, less leafiness, early maturing[1] (West Virginia)
Southlow	Upland	July 20	2009	Seed increase from local remnant native stands to represent local germplasm[3] (Southwest Michigan)
Trailblazer	Upland	July 20	2009	Seed increase from natural grassland, selected for high digestibility and forage[1] (Kansas & Nebraska)

[1]Alderson, J., and W. C. Sharp. 1994. Grass varieties in the United States. USDA, Agriculture Handbook 170. Washington,D.C.
[2]Ceres, Inc. Blade® seeds (www.bladeseeds.com)
[3]Release Brochure for Southlow Michigan Germplasm switchgrass (*Panicum virgatum*). USDA-Natural Resources Conservation Service, Rose Lake Plant Materials Center, East Lansing, MI 48823. Published September 2001, April 2014

Table 2. Percent variability (PERMANOVA R^2) in bacterial community composition explained by habitat (soil or root) and cultivar. Significance values: ns $p > 0.05$, *$p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$. '()' signifies nested factors, '*' signifies the interaction between factors, and 'NA' denotes not applicable for the model.

Factor	Soil & root bacteria (4 cultivars)	Soil bacteria (4 cultivars)	Root bacteria (4 cultivars)
Habitat Effect	%R^2 (p)	%R^2 (p)	%R^2 (p)
Cultivar	2.59 *	15.06**	ns
Block (Cultivar)	6.56*	29.72***	ns
Habitat	58.64***	NA	NA
Cultivar*habitat	ns	NA	NA
Habitat*Block(Cultivar)	6.73*	NA	NA
Soil moisture	ns	4.41*	ns

Table 3. Percent variability (PERMANOVA R^2) in microbial community composition explained by cultivar or ecotype. Significance values: ns $p > 0.05$, *$p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$. '()' signifies nested factors and '*' signifies the interaction between factors.

Factor	Soil fungi (12 cultivars)	Soil bacteria (12 cultivars)
Cultivar Effect	%R^2 (p)	%R^2 (p)
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***

Table 4. Percent variability (R^2) of microbiome structure explained by soil conditions and root traits using db-RDA analysis. Percent explained partitioned by conditional (block), constrained (all predictor variables), and unconstrained (residuals) factors; ns $p > 0.05$, * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$. 'NA' denotes not-applicable for models that were not significant ($p > 0.05$)

	Soil bacteria (12 cultivars)	Soil fungi (12 cultivars)	Soil bacteria (4 cultivars)	Root bacteria (4 cultivars)
Nitrate (µg N/ g dry soil g)	6.36***	1.17**	5.72**	NA
Ammonium (µg N/ g dry soil)	ns	ns	ns	NA
Soil Moisture Content (g/g 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

Figure 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 N-fixers in soil. The last two bars represent means for lowland (n = 4; gray boxes) and upland (n = 8; white boxes) ecotypes. 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. '+' denotes subset of cultivars analyzed for root-associated bacterial communities. Different letters denote significant differences among cultivars (FDR, $p < 0.05$). ANOVA 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 AIC evaluation). Significance values: ns $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure 2. A) NMDS 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 grey points) and two upland cultivars (U, light grey points). B) Mean relative abundance (%) of bacterial phyla and proteobacteria classes in roots or soils among four cultivars.

Figure 3. NMDS ordination of A) soil bacterial community (Weighted Unifrac, stress: 0.18) and B) soil fungal community (Bray-Curtis, stress: 0.26) across 4 lowland (L, grey points) and 8 upland (U, white points) cultivars. Numbers indicate centroid of sample replicates and horizontal and vertical bars represent ± 1 SE from the centroid. '+' denotes subset of cultivars analyzed for root-associated bacterial communities. See supplemental figure S1 for NMDS with all sample replicates.

Figure 4. Mean relative abundance of bacterial phyla (and proteobacteria classes) that significantly vary among cultivars (MVabund by cultivar: MVabund Dev(11/126) = 1105.8, 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 (1-12), followed by means for lowland (L; n = 4) and upland (U; n = 8) ecotypes. '+' denotes subset of cultivars analyzed for root-associated bacterial communities; '*' above ecotypes indicate statistically significant differences among ecotypes (ANOVA: * p <0.05, **p<0.01, ***p<0.001).

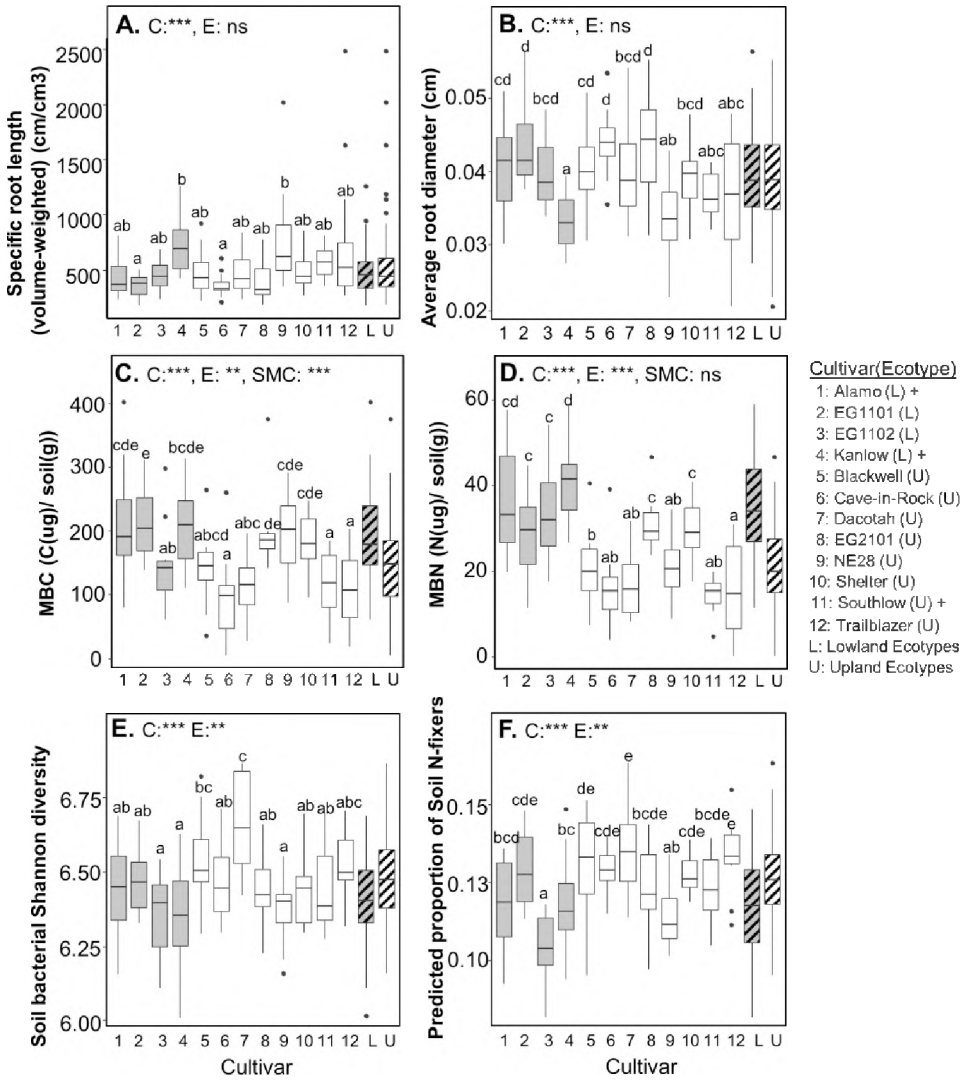

Figure 1. Variation in cultivar and ecotype A) volume-weighted specific root length (SRL), B) average root diameter, C) microbial biomass carbon (MBC), D) microbial biomass nitrogen (MBN), E) soil bacterial Shannon diversity, and F) predicted proportion of putative N-fixers in soil. The last two bars represent means for lowland (n = 4; gray boxes) and upland (n = 8; white boxes) ecotypes. 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. '+' denotes subset of cultivars analyzed for root-associated bacterial communities. Different letters denote significant differences among cultivars (FDR, $p < 0.05$). ANOVA 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 AIC evaluation). Significance values: ns $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

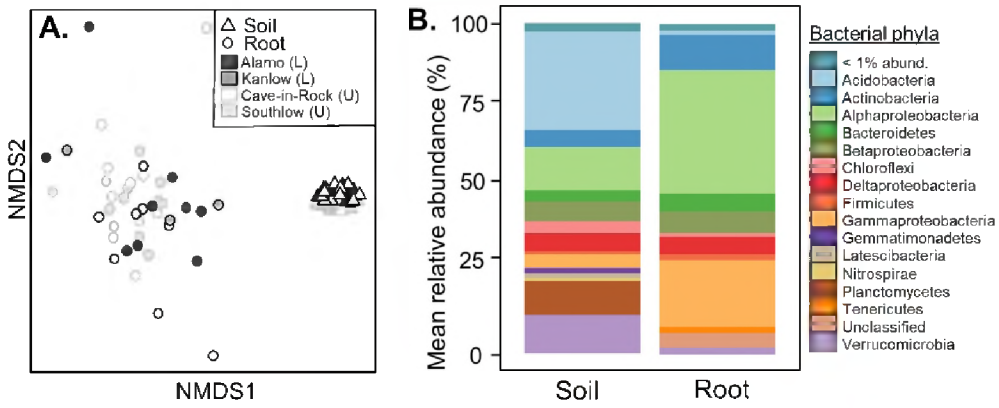

Figure 2. A) NMDS ordination of combined soil and root bacterial community (n = 4 cultivars, Weighted Unifrac, stress: 0.08). Soil (triangles) and roots (circles) represent two lowland cultivars (L, dark grey points) and two upland cultivars (U, light grey points). B) Mean relative abundance (%) of bacterial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

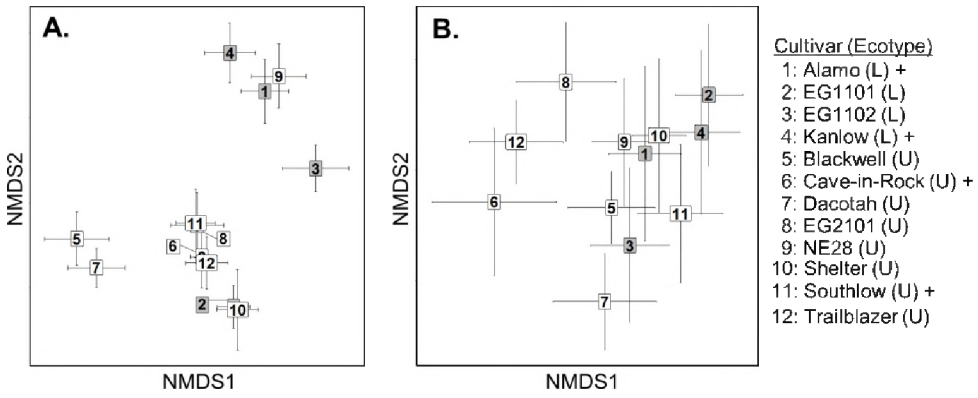

Figure 3. NMDS ordination of A) soil bacterial community (Weighted Unifrac, stress: 0.18) and B) soil fungal community (Bray-Curtis, stress: 0.26) across 4 lowland (L, grey points) and 8 upland (U, white points) cultivars. Numbers indicate centroid of sample replicates and horizontal and vertical bars represent ± 1 SE from the centroid. '+' denotes subset of cultivars analyzed for root-associated bacterial communities. See supplemental figure S1 for NMDS with all sample replicates.

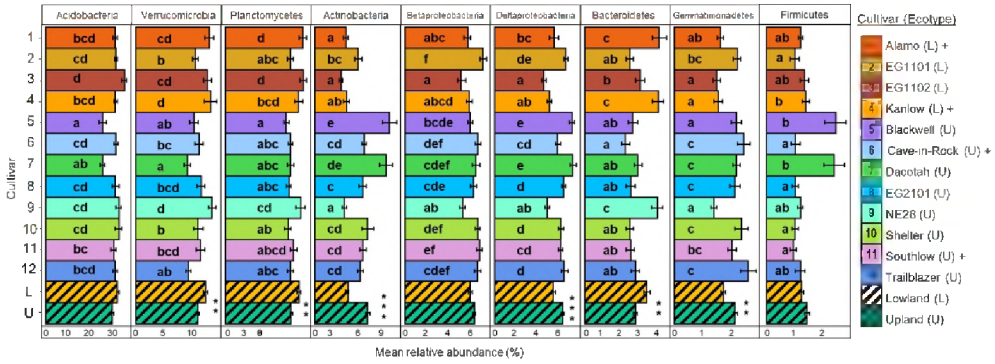

Figure 4. Mean relative abundance of bacterial phyla (and proteobacteria classes) that significantly vary among cultivars (MVabund by cultivar: MVabund Dev(11/126) = 1105.8, p = 0.001; each phyla p <0.05). Bars represent standard error. Phyla are ordered by relative abundance (left = most abundant) and, in each phyla, the bars are ordered by cultivar (1-12), followed by means for lowland (L; n = 4) and upland (U; n = 8) ecotypes. '+' denotes subset of cultivars analyzed for root-associated bacterial communities; '*' above ecotypes indicate statistically significant differences among ecotypes (ANOVA: * p <0.05, **p<0.01, ***p<0.001).

e-Xtra supplemental methods

Root morphology with GiA Roots

Root morphologies were quantified using a skeletonization algorithm applied to images of roots produced using an Epson perfection V600 scanner. After scanning roots at 1200 DPI, the images were edited manually with Adobe Photoshop Elements 16 to remove image artifacts and then resized to 300 DPI. GiA Roots (Galkovskyi et al. 2012) software algorithm masked roots against the image background using the adaptive image thresholding feature and a set of manually evaluated parameters (mean shift, minimum component size, block size). After masking and identifying roots in the image, GiA Roots trimmed imaged roots down to a diameter of a single pixel and measured root length by dividing total skeleton pixels by a known conversion factor established by a ruler image.

Univariate Data Analysis

Prior to all data analysis, we assured that all univariate data met assumptions of normality; transformations for normality included: predicted dry root biomass (square-root), root length density (square-root), GiA Roots specific root length (log), soil moisture content (log), and soil ammonium and nitrate (log +1), and root-bacterial evenness (squared). Soil fungal Shannon diversity and evenness indices were not able to be normalized, so we used non-parametric Kruskal-Wallis and Wilcox-tests with cultivar or ecotype as fixed effects (no block effect). However, although the data was non-normal, we confirmed that fungal Shannon diversity had the same results with a the mixed-effects model with a block factor included. Two extreme outliers that were three times the interquartile range were removed from soil moisture data and these two datapoints were also removed from soil nitrate and ammonium data, as soil moisture content data was used to normalize nitrogen values per unit of dry soil. Several datapoints for microbial biomass carbon were negative, likely because carbon values were lower than the instrument's standard error. These negative values were omitted from the analysis.

MVAbund analysis of taxa grouping and correlations with root traits

The 'manyglm' function in the MVAbund R-package was used to identify bacterial and fungal taxa that had significantly different relative abundance among cultivars, ecotypes, or plant compartments (Wang et al. 2012). Cultivar, ecotype, or soil type (root or soil) were treated as fixed effects in a "negative-binomial"-fit model. Block could not be included as a random factor due to unequal replication across blocks (because of samples removed for poor-sequence coverage). Taxa that significantly differed among groups ($p < 0.05$) were then analyzed with ANOVA tests (FDR adjustment to correct for multiple testing, $\alpha = 0.05$) with either cultivar or ecotype as a fixed effect. Soil moisture content was included as a covariate to account for variation across sample dates. Relative abundance data was log-transformed when it did not meet assumptions of normality. Further, we used the manyglm model to identify if the abundance of any fungal or bacterial groups (classes or orders) or individual OTUs (OTUs present in at least 80% of the samples) correlated with root length or diameter. Continuous root length and average root diameter data were fit with the negative-binomial manyglm model. Significant relationships

between root traits and microbial orders or OTUs found with MVAbund were confirmed with a linear regression analysis.

Nitrogen-fixation capacity estimates

The mean relative abundances of Burkholderiales and Rhizobiales were calculated using the rarefied soil (12 cultivars) and combined root and soil (4 cultivars) bacterial datasets, then analyzed with the non-parametric Kruskal-Wallis tests (R Core Team, 2018) with either cultivar, ecotype, or sample type as main effects. We also approximated the N-fixation capacity of the soil (12 cultivars) and root (4 cultivars) bacterial communities using PICRUSt (Langille et al. 2013). PICRUSt infers function based on phylogenetic relatedness to a database of reference genomes, so is only an approximation due to the tenuous and highly variable relationship between 16S rRNA sequence and function. We first calculated nearest sequenced taxon index (NSTI) scores, which provides a measure of phylogenetical distance between each OTU and the referenced metagenome and describes the confidence in functional assignment (Langille et al. 2013). We normalized all OTUs by their predicted 16S rRNA gene copy number, which provides a pseudo-abundance estimate for each OTU and then used 'metagenome_predictions' to obtain OTU-specific gene counts for N-fixation using the following KEGG pathway orthologs: K02588, K02586, K02591, K00531. We calculated each samples' predicted proportion of N-fixation genes by dividing the number of OTUs with at least one predicted N-fixation pathway for each sample by the normalized abundance of OTUS (e.g., the total 16S-gene normalized OTU counts).

e-Xtra supplemental figures & tables

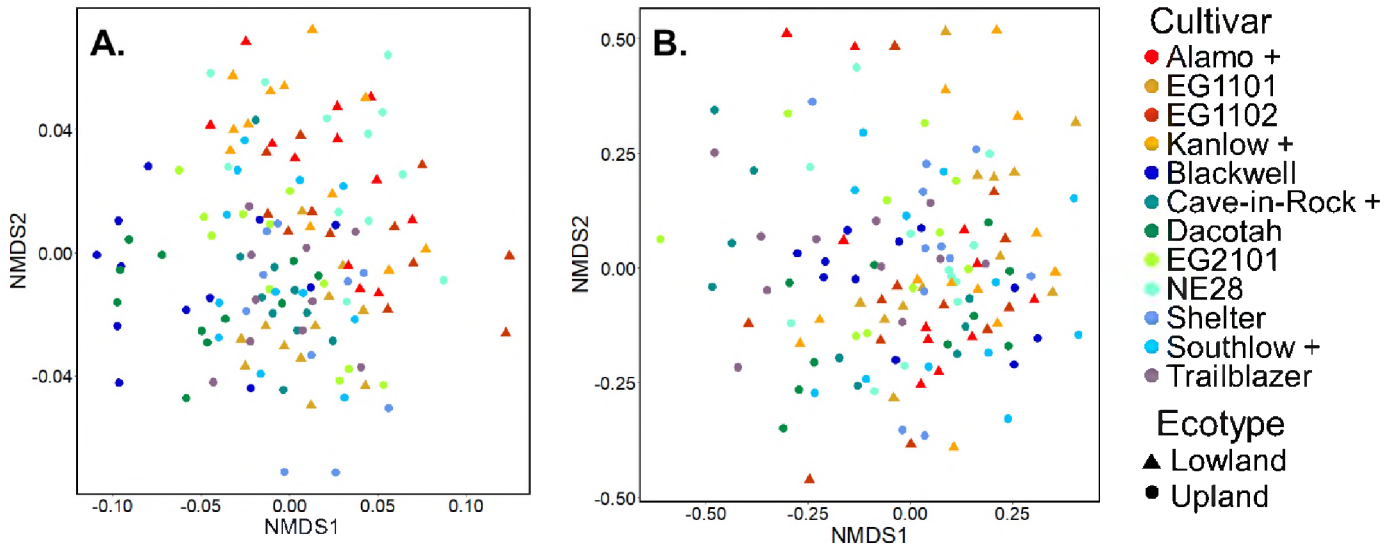

Figure S1. NMDS ordination of A) soil bacterial community (Weighted Unifrac, stress = 0.18) and B) soil fungal community (Bray-Curtis, stress = 0.26). Each point is a replicate soil core; final replicate number for each cultivar after removing poor sequence coverage samples in Table S2. Warm colors and triangles represent lowland ecotypes (n = 4), cool colors and circles represent upland ecotypes (n = 8). '+' denotes subset of cultivars analyzed for root-associated bacterial communities.

Table S1. Bioinformatics filtering details for bacterial (16S) and fungal (ITS) samples.

	Bacterial root and soil	Bacterial soil	Bacterial soil (PICRUSt analyses)	Fungal Soil
Reference Database	Silva (v.123)	Silva (v.123)	Greengenes (v.13.8)	Unite (v.7.2)
Total Read #	3,323,839	2,294,871	2,031,361	2,202,804
Total OTU # (97% similarity threshold)	20,972	20,278	11,931	4,736
% non-bacterial or fungal reads	19.07%	0.79%	0.72%	0%
# samples after removing poor-sequence coverage	182 (removed 10 samples)	138 (removed 5 samples)	138 (removed 6 samples)	135 (removed 9 samples)
Post-filtering Read #	2,680,275	2,267,356	2,009,262	2,196,278
Post-filtering OTU#	18,535	17,878	8,878	4,639
Rarefaction Read # cut-off	2,026	4,694	4,117	4,153
Post-Rarefaction Read #	368,732	647,772	568,146	560,655
Post-Rarefaction OTU#	12,197	14,590	7,905	4,064
Protest results (comparing rarefaction and Deseq2 VST normalization)	$p < 0.001$, r = 0.41	$p < 0.001$, r = 0.91	NA	$p < 0.001$, r = 0.82

Table S2. Fungal and bacterial sequencing final replicate number (out of 12 replicates; 4 blocks with 3 replicate soil cores) after removing samples with poor sequence coverage and samples with extreme outliers for soil moisture content covariate (n = 1 from EG1101 and n = 1 from Blackwell). 'NA' denotes not applicable; only 4 cultivars analyzed for root bacterial community.

	Alamo	EG1101	EG1102	Kanlow	Blackwell	Cave-in-Rock	Dacotah	EG2101	NE28	Shelter	Southlow	Trailblazer
Soil Bacterial Community (12 cultivars)	12	12	12	12	12	10	11	11	12	10	12	10
Combined Soil, Root bacterial community (4 cultivars)	12, 9	NA	NA	12, 11	NA	10, 10	NA	NA	NA	NA	12, 12	NA
Fungal Bacterial Community (12 cultivars)	11	12	12	12	11	9	10	10	12	11	12	11

Table S3. Percent variability (PERMANOVA R^2) in microbial community composition explained by cultivar or ecotype. Cultivar or ecotype treated as main effects with sampling date as a covariate and a nested block term. Significance values: ns $p >$ 0.05, *$p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$. '()' signifies nested factors and '*' signifies the interaction between factors.

Factor	Soil fungi (12 cultivars)	Soil bacteria (12 cultivars)
Cultivar Effect	%R^2 (p)	%R^2 (p)
Cultivar	ns	13.03**
Block (Cultivar)	34.23***	33.77***
Sampling Date	1.56**	9.13***
Ecotype Effect		
Ecotype	ns	ns
Plot (Ecotype)	43.31***	45.79***
Sampling Date	1.56***	9.13***

Table S4. Percent variability (PERMANOVA R^2) in bacterial community composition explained by habitat (soil or root) and cultivar. Cultivar and habitat treated as main effects with sampling date as a covariate and a nested block term. Cultivar-effect for a subset of soil and root communities also presented; NA indicates not applicable for the model. Significance values: ns $p > 0.05$, *$p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$. '()' signifies nested factors and '*' signifies the interaction between factors.

Factor	Soil & root bacteria (4 cultivars)	Soil bacteria (4 cultivars)	Root bacteria (4 cultivars)
Habitat Effect	$\%R^2$ (p)	$\%R^2$ (p)	$\%R^2$ (p)
Cultivar	ns	ns	ns
Block (Cultivar)	6.42 *	32.9 ***	ns
Habitat	58.72 ***	NA	NA
Cultivar*habitat	ns	NA	NA
Habitat*Block(Cultivar)	6.71 *	NA	NA
Sampling Date	1.67 **	10.34 ***	ns

Table S5. Root trait differences among switchgrass cultivars and ecotypes. ANOVA results with fixed cultivar or ecotype term, nested block term, and soil moisture content as a covariate when it improved model fit (based on Akaike information criteria). F-statistic and significance values: ns $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, 'NA' denotes not-applicable for the model.

	Dry Root Mass (g)	Average Root Diameter (cm)	Root Network Volume (cm^3)	Root Network Length (cm)	Volume-weighted SRL (cm/cm^3)	Mass-weighted SRL (cm/g)
Cultivar Effect						
Cultivar	1.61 (ns)	4.43***	1.99 (ns)	1.21 (ns)	3.61**	1.62 (ns)
Soil Moisture	NA	NA	NA	0.73 (ns)	NA	NA
Ecotype Effect						
Ecotype	2.47 (ns)	0.001(ns)	1.49 (ns)	3.32 (ns)	0.288 (ns)	2.43 (ns)
Soil Moisture	NA	NA	NA	0.99 (ns)	NA	NA

Table S6. Bacterial alpha diversity among root and soil habitats. ANOVA results with habitat and cultivar (n = 4) as fixed terms, a nested block term, and soil moisture content as a covariate when it improved model fit (lower Akaike information evaluation). Wilcox test with compartment as a fixed effect was used for non-parametric Pielou's evenness. F-statistic and significance values: ns $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, 'NA' denotes not-applicable for the model.

	Richness	Shannon Diversity	Pileou's Evenness
Habitat	3509.8 ***	1178.1 ***	W = 1 ***
Cultivar	0.16 (ns)	1.73 (ns)	NA
Habitat * Variety	1.06 (ns)	1.74 (ns)	NA
Soil Moisture	2.15 (ns)	NA	NA
Habitat Means (Soil, Root)	889, 171	38.1, 14.2	0.91, 0.73

Table S7. Alpha diversity statistics for soil bacterial and fungal communities. ANOVA results with either fixed cultivar or ecotype term, nested block term, and soil moisture content as a covariate when it improved model fit (lower Akaike information evaluation). Fungal Shannon diversity was analyzed with non-parametric Kruskal-Wallis and Wilcox Tests. F-statistic and significance values: ns $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, 'NA' denotes not-applicable for the model.

Community	Bacterial Soil Community (n = 12 cultivars)			Fungal soil community (n = 12 cultivars)		
Diversity Metric	Richness	Shannon Diversity	Pielou's Evenness	Richness	Shannon Diversity	Pielou's Evenness
Cultivar Effect						
Cultivar	2.17*	4.4***	4.71***	0.63 (ns)	$X^2 = 7.22$ (ns)	$X^2 = 8.98$ (ns)
Soil Moisture	0.65 (ns)	NA	NA	1.40 (ns)	NA	NA
Ecotype Effect						
Ecotype	2.18*	6.15*	5.41**	0.04 (ns)	W = 2177 (ns)	W = 2109 (ns)
Soil Moisture	0.65 (ns)	NA	NA	0.10 (ns)	NA	NA
Ecotype Means (Upland, Lowland)	1460,1416	6.49, 6.41	0.89,0.88	6.70, 9.26	4.47,4.42	0.76,0.75

Table S8. Alpha diversity statistics without Dacotah cultivar for soil bacterial community (n = 11). ANOVA results with either fixed cultivar or ecotype term, nested block term, and soil moisture content as a covariate when it improved model fit (lower Akaike information evaluation). Fungal Shannon diversity was analyzed with non-parametric Kruskal-Wallis and Wilcox Tests. F-statistic and significance values: ns $p > 0.05$ *$p < 0.05$, **$p< 0.01$, ***$p< 0.001$, 'NA' denotes not-applicable for the model.

Diversity Metric	Richness	Shannon Diversity	Pielou's Evenness
Cultivar Effect			
Cultivar	2.08 (ns)	2.14 (ns)	2.06 (ns)
Soil moisture	0.27 (ns)	NA	NA
Ecotype Effect			
Ecotype	2.47 (ns)	3.72 (ns)	2.96 (ns)
Soil moisture	4.18 *	NA	NA
Ecotype means (Upland, Lowland)	1448,1416	6.47,6.41	0.89,0.88

Table S9. Pairwise p-values (FDR adjusted) for soil bacterial community composition among lowland (L) and upland (U) cultivars. Model included cultivar as a fixed effect with a nested block term and soil moisture content as a covariate. Shading represents p value < 0.1 Final column denotes how many of the 11 comparisons for each cultivar were significant at $p < 0.10$.

	Alamo	EG1101	EG1102	Kanlow	Blackwell	Cave-in-Rock	Dacotah	EG2101	NE28	Shelter	Southlow	Trailblazer	# p < 0.1
Alamo (L)													7
EG1101 (L)	0.09												4
EG1102 (L)	0.61	0.09											6
Kanlow (L)	0.92	0.09	0.32										4
Blackwell (U)	0.09	0.13	0.09	0.17									3
Cave-in-Rock (U)	0.09	0.25	0.09	0.09	0.22								4
Dacotah (U)	0.09	0.15	0.09	0.09	0.82	0.19							4
EG2101 (U)	0.09	0.18	0.09	0.13	0.19	0.63	0.19						3
NE28 (U)	0.92	0.09	0.57	0.79	0.09	0.09	0.09	0.09					6
Shelter (U)	0.09	0.93	0.15	0.09	0.15	0.44	0.18	0.61	0.09				3
Southlow (U)	0.16	0.57	0.09	0.17	0.21	0.9	0.21	0.92	0.13	0.64			1
Trailblazer (U)	0.09	0.52	0.15	0.13	0.19	0.57	0.19	0.57	0.12	0.64	0.75		1

Table S10. Percent variability (PERMANOVA R^2) for cultivar effect on soil bacterial and fungal communities within sampling dates. Significance values: ns $p > 0.05$, *$p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$; R^2 = Factor SS/Total SS.

Sampling Date	June 28th	July 13th	July 20th	July 27th
Soil bacterial community	$\%R^2$ (p)	$\%R^2$ (p)	$\%R^2$ (p)	$\%R^2$ (p)
Cultivar	ns	ns	0.16**	ns
Block(Cultivar)	48***	36**	33***	41***
Soil fungal community	R^2 (p)	R^2 (p)	R^2 (p)	R^2 (p)
Cultivar	ns	ns	ns	ns
Block(Cultivar)	37***	36***	33**	36***
# cultivars sampled	2	3	4	3