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Plant compartment and genetic variation drive microbiome composition in switchgrass roots

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Summary

Switchgrass (*Panicum virgatum*) is a promising biofuel crop native to the United States with genotypes that are adapted to a wide range of distinct ecosystems. Various plants have been shown to undergo symbioses with plant growth-promoting bacteria and fungi, however, plant-associated microbial communities of switchgrass have not been extensively studied to date. We present 16S ribosomal RNA gene and internal transcribed spacer (ITS) data of rhizosphere and root endosphere compartments of four switchgrass genotypes to test the hypothesis that host selection of its root microbiota prevails after transfer to non-native soil. We show that differences in bacterial, archaeal and fungal community composition and diversity are strongly driven by plant compartment and switchgrass genotypes and ecotypes. Plant-associated microbiota show an enrichment in *Alphaproteobacteria* and *Actinobacteria* as well as *Sordariales* and *Pleosporales* compared with the surrounding soil. Root associated compartments display low-complexity communities dominated and enriched in *Actinobacteria,* in particular *Streptomyces,* in the lowland genotypes, and in *Alphaproteobacteria,* specifically *Sphingobium*, in the upland genotypes. Our comprehensive root analysis serves as a snapshot of host-specific bacterial and fungal associations of switchgrass in the field and confirms that host-selected microbiomes persist after transfer to non-native soil.

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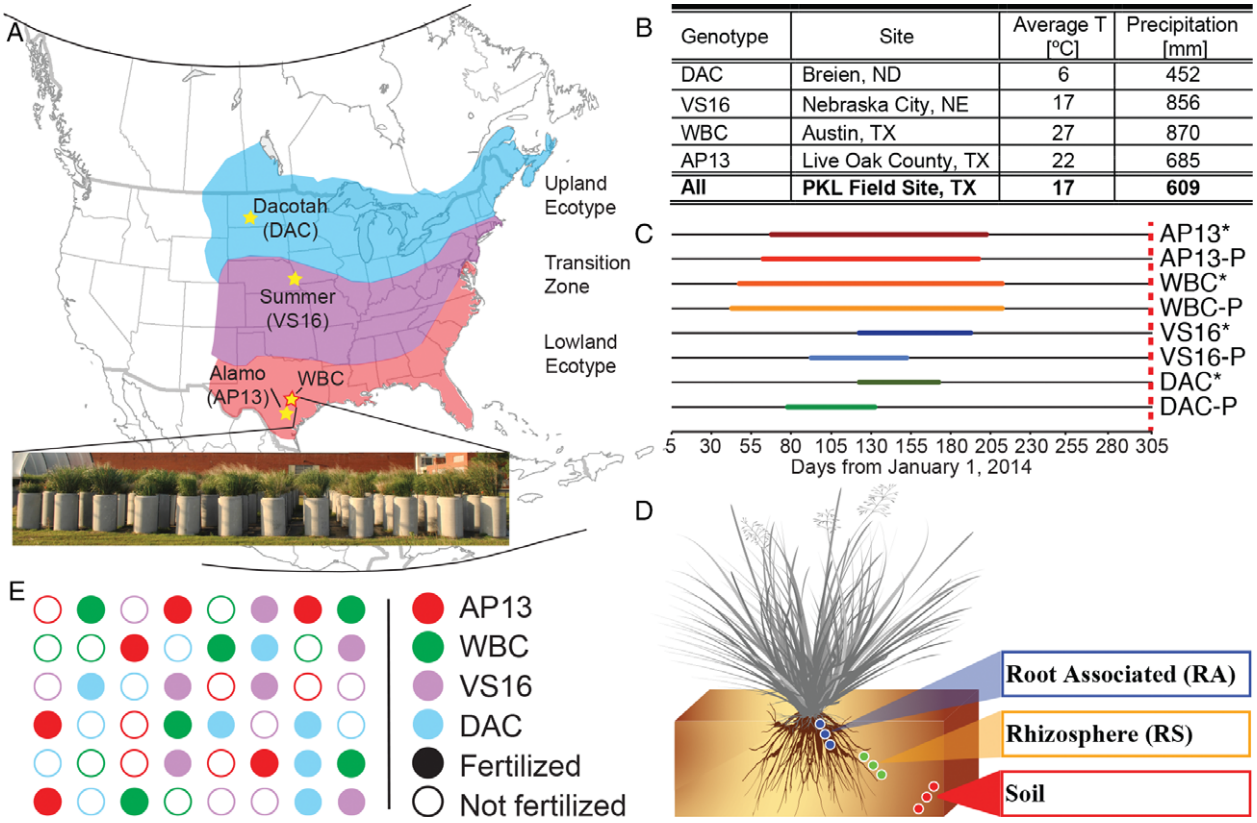
Introduction

Terrestrial plants are colonized by diverse communities of microorganisms that can differentially affect plant health and growth (Yeoh *et al*., 2017; Naylor and Coleman-Derr, 2018). The result of the interactions between plants and their microbiota can be regarded as an extended plant phenotype (Price *et al*., 2010; Vorholt, 2012; Wagner *et al*., 2016; Müller *et al*., 2016). Understanding plant-microbe interactions is motivated by the potential to predict and prevent plant disease, increase crop yield and correlate specific phenotypes to either environmental stimuli, microbial activity, plant physiology or a combination thereof. For example, microbes have been shown to increase resource uptake and provide novel nutritional and defence pathways thereby contributing to plant health (Berg, 2014; Wang *et al*., 2016). Plant-microbiome interactions appear to be cultivar-dependent, *i.e.* intraspecific plant genetic variation can alter root and rhizosphere (Bressan *et al*., 2009; Price *et al*., 2010; Vorholt, 2012; Bulgarelli *et al*., 2012; Lebeis, 2015; Müller *et al*., 2016; Wagner *et al*., 2016). Hence, it is important to study natural exogenous stimuli as well as host genetic variation driving the plasticity of complex plant-microbiome interactions.

Switchgrass is a perennial C4 grass native to the plains of North America exhibiting phenotypic variability and adaptation, especially across latitude and precipitation gradients (Casler *et al*., 2004; Cline *et al*., 2007; Casler *et al*., 2007a, b; Lowry *et al*., 2014) (Fig. 1). Switchgrass genotypes are broadly classified into lowland and upland ecotypes, which are estimated to have diverged approximately 1.5-1 Mio years ago and as a result each possess a great number of genetic varieties (Zhang *et al*., 2011). Lowland ecotypes, local to the Southwestern United States, are generally found in riparian areas and floodplain habitats, and show characteristic vigorous, tall, thick-stemmed phenotypes. Upland ecotypes originate from the Northern United States, prefer colder climates, and are adapted to drier conditions, while exhibiting short, rhizomatous, thin-stemmed phenotypes. The large genetic variation exhibited by switchgrass offers the possibility to investigate host-microbiome relationships as a function of plant genotype and ecotype. Furthermore, previous studies showed that switchgrass



Genotype	Site	Average T [°C]	Precipitation [mm]
DAC	Breien, ND	6	452
VS16	Nebraska City, NE	17	856
WBC	Austin, TX	27	870
AP13	Live Oak County, TX	22	685
All	**PKL Field Site, TX**	**17**	**609**

Fig. 1. Switchgrass genotype origin and sampling scheme.
A and B. Display information on switchgrass genotype origin and average annual temperature as well as average annual precipitation at each site of origin. Plant cultivars were then transferred to the Pickle (PKL) field site in Texas (red-framed star) and planted in cylinders according to the layout in (E). Soil samples were taken from within cylinders avoiding plant and root material. Colour bars in (C) compare days between green-up and flowering at original sites (*) and as observed after planting at the PKL field site (-P) for each genotype. Red dashed line marks time of sampling. Plant compartments sampled for this study include Rhizosphere (RS), Root-associated (RA) and soil samples from within the vicinity of selected plants (D). RA is defined as root endosphere and rhizoplane.

productivity is not directly affected by fertilizer treatments (Duran *et al*., 2016). Reasons for that could be that switchgrass is co-limited by other nutrients, that fertilizer treatments actually stimulate growth of competing weeds and/or that the associated microbial assemblage provides sufficient N to switchgrass plants. Studying the difference in microbial populations in fertilized and non-fertilized plants could provide insights in this context. Some bacterial and fungal isolates retrieved from switchgrass have been characterized (Gravert and Munkvold, 2002; Ghimire *et al*., 2010; Bourgue *et al*., 2013; Xia *et al*., 2013). However, few studies looked at microbial population differences associated with switchgrass genotype (Uppalapati *et al*., 2012) and habitats (Kaufman *et al*., 2007; Hesselsoe *et al*., 2009; Kleczewski *et al*., 2012; Willems, 2014; Zhang *et al*., 2016). The effect of plant compartment, host genotype and ecotype as well as fertilizer on the plant bacterial and fungal microbiota has not been addressed.

In this study, we quantify the measurable impact of host compartment and genetic variability observed across two ecotypes and four switchgrass genotypes on associated root microbial communities. The selected four genotypes represent grandparent plants to an F2 mapping population of 100 s of progeny that allow future mapping of quantitative trait loci (QTL) to plant phenotypic traits associated with ecosystem adaptation and correlated with microbiome communities. Our goals for this study were to quantitatively evaluate the relationships between plant compartment, ecotype and genotype, fertilizer and the taxonomic diversity and community structure of bacteria, archaea and fungi. For that purpose, we analysed proximal soil as well as rhizosphere and root endosphere of two-year old plants half of which were fertilized. Because switchgrass upland and lowland genotypes are deeply diverged, we were specifically interested to see whether host ecotype impacted microbiome variability more than host genotype and if this is affected by fertilizer treatment. We report major taxonomic clades unique to plant compartment and switchgrass genotypes and discuss the extent of core microbiomes. Using these analyses, we studied the impact that plant genetic variability and associated adaptive divergence to climate and

soil conditions have on plant-microbe associations, and thereby show the importance of cultivar-dependent microbiome interpretations.

Results and discussion

We analysed rhizosphere (RS) and root associated (RA) bacterial, archaeal and fungal communities from four switchgrass genotypes planted at one location and sampled at the end of the second growth season via Illumina iTag sequencing (Fig. 1D). Plant genotypes showed striking phenotypic differences at the time of sample collection (Supporting Information Figs S1, S2) likely due to phenological divergence to different growth season durations and earlier onset of senescence in the upland genotypes (Fig. 1C). Lowland ecotypes acclimated to warmer, wetter Southern climates were characterized by ~10-fold larger biomass compared with the upland ecotypes (Supporting Information Fig. S1 and Supporting Information Table S1) when grown in Austin.

Microbial community assembly is impacted by plant compartment and genotype

In order to gain insight into microbial community structure in switchgrass and drivers of microbiome assembly, we analysed the distribution of bacterial/archaeal and fungal OTUs correlated to factors considered in the experimental design, i.e. genotype, ecotype and fertilization level. We found that microbial community variability was explained to varying degrees by plant compartment, genotype, ecotype and fertilizer treatment (Table 1). Plant compartment explained the largest percent of community variance in both bacterial/archaeal (26.7%) and fungal datasets (28.1%) and shows distinct grouping of compartments with soil and RS bacterial communities clustering relatively closely together (Table 1A, Figs. 2A and B and Supporting Information Fig. S3, S4), which is in line with other plant-microbiome studies indicating that a significant portion of the rhizosphere microbial community is recruited by the plant from surrounding soil, *e.g.* (Wagner *et al*., 2016; Coleman-Derr *et al*., 2016). Differential recruitment of microbial communities in RS *vs.* RA plant compartments has been attributed to the functions that microbiomes fulfil in each compartment respectively. Root and soil communities are assumed to be primarily reliant on the protection and carbon supply from their plant host via exudates (Bais *et al*., 2006). The importance of the specific host in microbial community dynamics is supported by the relatively strong influence of genotype and ecotype on RS communities in our dataset: Genotype (nested within Ecotype) explained 16.3% and 14.0% of RS bacterial and fungal community variability respectively (Table 1B). Similar to genotype, switchgrass ecotype contribution to community variability in the RS was comparable in bacteria/archaea (5.1%) and fungi (4.5%) (Table 1B). Genotype neither significantly impacted bacterial/archaeal nor fungal RA communities, while ecotype explained 7.9% and 6.7% respectively (Table 1B). This indicates that there are notable differences between upland and lowland switchgrass ecotypes in the RA, however, differences in the microbial communities among switchgrass genotypes belonging to the same ecotype may be weaker or could not be resolved in this study due to the small number of genotypes per ecotype.

Interestingly, fertilizer treatment only significantly explained 3.6% of the bacterial/archaeal community variability in the RS and did not significantly increase plant biomass (Supporting Information Fig. S1 and Table 1). Fertilizer in interaction with other factors (compartment, genotype, ecotype) did not render any significant correlations. Fertilization was previously shown to not affect productivity in switchgrass monocultures (Duran *et al*., 2016; Fike *et al*., 2017). In our study, it appears that plants were either not N-limited due to sufficient N-supply via the microbiome or as given in the nutrient-rich potting soil used in this study or that plants were co-limited by other nutrients. While soil and fertilizer-induced nutrient shifts in the soil were previously correlated with microbial community variability (Bardgett *et al*., 1999; Marschner, 2003), plant-associated features rather than fertilization shape the microbial community structure in our study. Existing plant-recruited microorganisms may outperform

Table 1. Microbial community variability as described by (A) factors for all considered plant compartment samples (bacteria/archaea: RS, RA; fungi: RS, RA – rarefied to 5000 seqs), (B) individual factors per compartment for bacterial and archaeal as well as fungal communities.

A

Factor	Bacteria/archaea [%] (N = 233)	Fungi [%] (N = 177)
Compartment	26.7	28.1
Ecotype	8.3	–
Compartment* Ecotype	2.1	2.8
Genotype (Ecotype)	–	–
Fertilizer	–	–

B

Factor	Compartment	Bacteria/archaea [%]	Fungi [%]
Genotype	Rhizosphere	16.3	14.0
	Root Associated	–	–
Ecotype	Rhizosphere	5.1	4.5
	Root Associated	7.9	6.7
Fertilizer	Rhizosphere	–	–
	Root Associated	–	–

Values reported are statistically significant ($p < 0.05$).
'–' denotes no significant correlation, '*' signifies the interaction between factors.

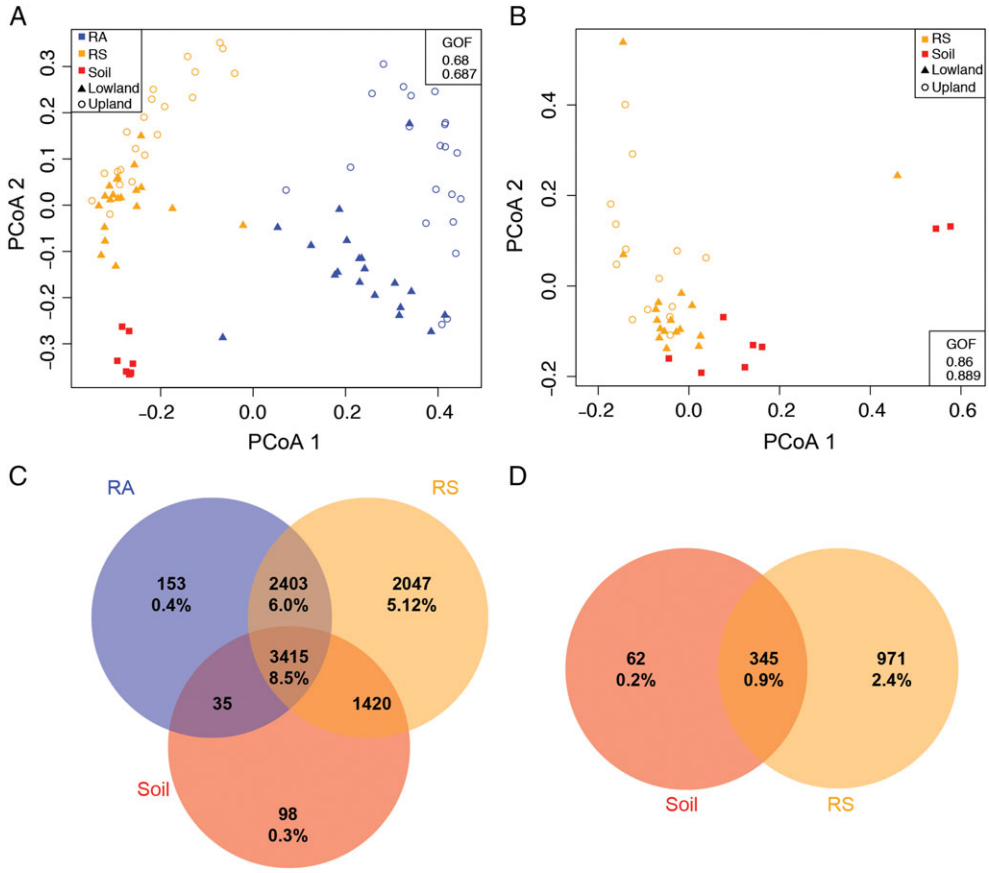


Fig. 2. Principal Coordinate Analysis of bacterial/archaeal (A) and fungal (B) communities at the genus level after rarefaction. Colours denote compartments considered. GOF = goodness of fit. Complementary analyses by genotype for bacterial/archaeal and fungal communities are displayed in Supporting Information Fig. S6 and S7. Venn diagrams of shared bacterial/archaeal (C) and fungal (D) genera across plant compartments. Most bacterial, archaeal and fungal soil OTUs were also present in a plant compartment. The majority of bacterial/archaeal OTUs associated with the RA was also present in the rhizosphere and surrounding soils.

microorganisms newly arriving with or stimulated by fertilizer additions and continued plant-microbe communication, e.g. via root exudation, as observed in various other plants (Ridl *et al*., 2016; Sasse *et al*., 2018).

Since plant compartment, genotype and ecotype displayed distinct clustering of microbial community diversity and composition, we continued our analyses with samples grouped according to these factors. We detected 12 bacterial classifiable phyla and 14 classifiable fungal orders primarily belonging to *Ascomycota* (at >1% relative abundance) across RS, RA (for bacteria/archaea) and soil (Fig. 4 and Supporting Information Fig. S5A). The most abundant archaeal phylum (*Crenarchaeota*) was present at an average relative abundance of 0.1% across RS, RA and soil (data not shown). The plant-associated sample types (RS and RA) displayed significantly higher relative abundances of *Actinobacteria* and *Alphaproteobacteria* in RS and RA as well as *Sordariales*, *Pleosporales*, *Hypocreales, Dothideales* and *Capnodiales* in RS (Supporting Information Table S2A and Supporting Information Fig. S6A-B). RS and soil compartments showed largest bacterial and fungal community complexity (Fig. 3). The number of observed bacterial and fungal OTUs in RA communities was about half of that in respective RS communities likely demonstrating highly selective mechanisms in the RA induced by the host and enhanced competition for resources compared with the soil as noted in other studies (Lundberg *et al*., 2012) (Fig. 3). In the following, we will look further into the phylogenetic distribution of these root microbiota.

Core root associated communities mimic core rhizosphere communities

While RS microbiome complexity was significantly higher compared with the RA compartment across all bacterial, archaeal and fungal communities (Supporting Information Table S4, S5) and despite distinct total microbial community structure differences between RS and RA (Fig. 2A and B), we observed the same core taxa of relatively high abundance in both RS and RA compartments. The switchgrass root core microbiome was dominated by

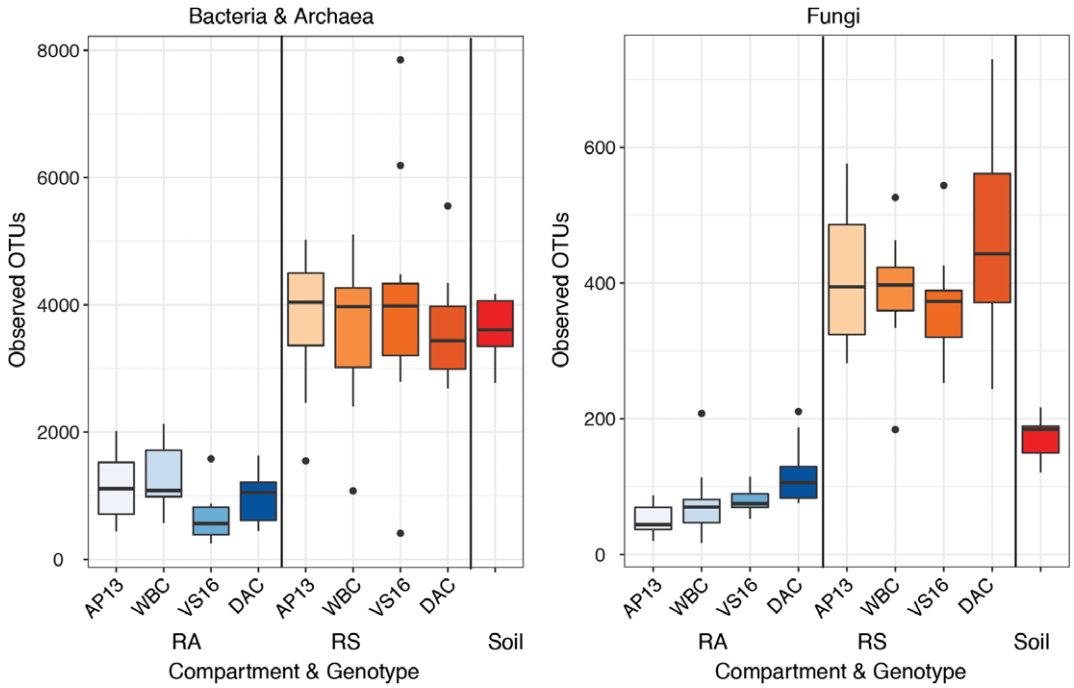


Fig. 3. Number of observed OTUs in bacterial/archaeal and fungal communities by compartment and genotype.
Microbial diversity and richness differed significantly among compartment types and across switchgrass genotypes and ecotypes in some compartments (Supporting Information Tables S2, S3). Bacterial and fungal RS and soil communities showed comparable diversity, whereas the RA compartment displayed roughly half as many OTUs in both bacterial/archaeal and fungal communities respectively. Interestingly, fungal diversity was higher in the RS than in the surrounding soil.

Streptomyces, *Sphingobium*, *Bacillaceae*, *Ralstonia* and *Olivibacter,* as well as *Aureobasidium* (*Dothideales*), *Alternaria* (*Pleosporales*) *and Cladosporium* (*Capnodiales*) (Table 2B-C). Fungal RS community of 70.6% was attributed to *Microascales, Sordariales* and *Pleosporales* (Fig. 4B). The remaining core taxa were composed of *Sordariales, Hypocreales* and *Pleosporales* at comparable relative abundances in RS and RA compartments (Table 2C). The RA was significantly enriched in *Streptomyces* (45.2%) and *Sphingobium* (10.6%) compared with all other studied plant and soil niches (Fig. 4A, Supporting Information Table S6) suggesting preferential colonization of switchgrass RA by these genera. Conserved archaeal OTUs were only found in the RS core and belonged to the genus *Candidatus Nitrososphaera* present at <0.02% (data not shown).

We further aimed to identify taxa that were distinct between switchgrass genotypes and ecotypes in order to link microbial community statistics with genotype- and ecotype-differentiation (Supporting Information Table S3B). In both, RS and RA, taxa belonging to the *Alphaproteobacteria* were enriched in the upland genotypes, while *Actinobacterial* taxa were indicative of lowland genotype communities (Supporting Information Table S3). All significantly enriched *Actinobacteria* in the lowland ecotypes belong to the *Actinomycetales* and include *Williamsiaceae*, *Glycomyces, Cryptosporangiaceae* and *Euzebia* (Supporting Information Table S3B). The enrichment of *Alphaproteobacteria* in the upland ecotype RS, and more specifically, the DAC genotype, is primarily driven by increased relative sequence abundances classified as *Sphingobium* (Supporting Information Table S3B-C). Similarly, in the RA *Sphingobium* and other *Alphaproteobacteria, including Novosphingobium,* unclassified *Sphingomonadales, Phenylobacterium* and *Phaeospirillum* were found to be indicator OTUs of the DAC RA. We observed significantly fewer occurrences of *Gemmatimonadetes*, *Solibacteres* and *Phycisphaerae* in the DAC RS (Supporting Information Table S3B). In the fungal RS communities, abundance difference across switchgrass genotypes were not significant at any taxon level, however, *Phaenonectriella, Nectria, Pseudallescheria, Phaenonectriella* and *Zopfiella* were on average at least an order of magnitude less abundant in the DAC genotype (Supporting Information Table S3). Similarly, *Curvularia, Peyronellaea* and *Didymostilbe* were enriched in the DAC compared with the other genotypes.

Summary

Our study provides a first and comprehensive snapshot of microbe-microbe and microbe-switchgrass genotype associations and interaction potential in switchgrass roots

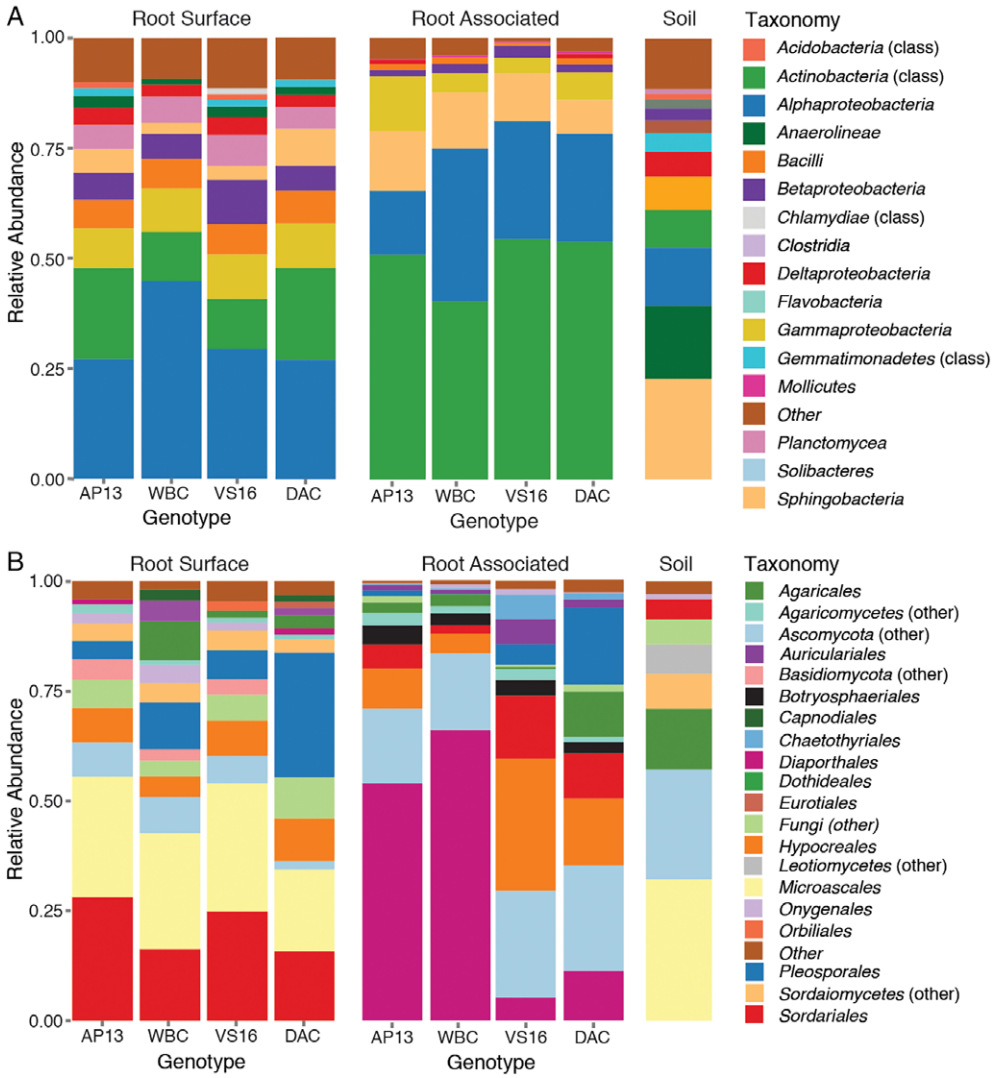


Fig. 4. Relative abundances of bacterial classes (A) and fungal orders (B) by compartment and genotype. 'Other' include taxa with <1% abundance per category.

A. While the bacterial/archaeal relative abundance profiles at the class level were mostly comparable similar across genotypes, while community composition differed substantially between compartments. OTUs belonging to the bacterial classes *Alphaproteobacteria, Actinobacteria, Gammaproteobacteria, Betaproteobacteria, Bacilli* and *Planctomycea* constitute ≥80% of the relative abundance in RS and RA plant compartments, whereas the most abundant soil classes include *Sphingobacteria* (22.8%), *Anaerolineae* (16.6%), *Alphaproteobacteria* (13.1%), *Actinobacteria* (8.7) and *Gammaproteobacteria* (7.5%). The RS and RA bacterial/archaeal communities were both dominated by *Alphaproteobacteria*, *Actinobacteria*, *Firmicutes*, *Gammaproteobacteria and Sphingobacteria.* The most abundant archaeal class of the *Thaumarchaeota* was most prevalent in the soil (0.2%) compared with the plant compartments (RS: 0.1%, RA: 0.01%). Compared with other plant compartments, the RS was predominantly distinct to the other niches due significantly increased abundance of OTUs belonging to *Bacillaceae, Ralstonia, Rhodospirillaceae* and *Sphingobacteriales* (Supporting Information Table S2B). OTUs significantly enriched in the RS compared with the soil furthermore include orders *Sordariales* and *Pleosporales* (Supporting Information Table S2). The only archaeal OTU present in all rhizosphere samples belongs to the *Nitrososphaerales* (*Cand. Nitrososphaera*). The RA was significantly enriched in *Streptomyces* (45.2%) and *Sphingobium* (10.6%) compared with all other studied plant and soil niches (Fig. 4A and Supporting Information Table S2) suggesting close interactions between these genera and the roots.

B. Very few members of the known arbuscular mycorrhizal fungi (AMF) phylum *Glomeromycota* (12 OTUs; 0.6%) were detected in the soil and rhizosphere due to the use of ITS primers. Detected AMF were exclusively members of the *Glomus* spp. and relative abundances did not correlate with switchgrass genotype.

by means of 16S rRNA gene and ITS amplicon sequencing in a field study. Results from this study are important for identifying key bacterial and fungal players and hypothesis generation with regards to their functionality on switchgrass roots under real-world conditions. Harnessing the native switchgrass microbiome for future inoculation treatments likely present cost-effective and sustainable strategies to improve or stabilize crop yield, e.g. under conditions of drought or enhanced pathogen stress. In this context, we report that bacterial/archaeal and fungal communities showed significant differences from soil to rhizosphere to the root associated compartment. Host genotype

Table 2. Core microbiome statistics (A) by plant compartment represented as number of taxa in shared in X% of samples according to bacterial/archaeal (16S) and fungal (ITS) community datasets.

A

	RS [# of OTUs]		RA [# of OTUs]	
Samples (%)	Bacteria/archaea	Fungi	Bacteria/archaea	Fungi
50	2066	177	252	13
60	1642	146	213	10
70	1274	117	169	8
80	936	90	125	7
90	774	63	80	4
100	171	7	24	1

B

Phyla/Class	RS [%]	Phyla/Class	RA [%]
Alphaproteobacteria	25.3	*Alphaproteobacteria*	31.3
Actinobacteria	15.8	*Actinobacteria*	20.0
Planctomycetes	14.1	*Firmicutes*	12.5
Firmicutes	10.1	*Gammaproteobacteria*	11.3
Chlorflexi	6.4	*Planctomycetes*	6.3
Acidobacteria	5.9	*Betaproteobacteria*	6.3
Gammaproteobacteria	5.6	*Bacteroidetes*	3.8
Deltaproteobacteria	5.3	*Acidobacteria*	2.5
Betaproteobacteria	3.8	*Deltaproteobacteria*	2.5
Gemmatimonadetes	2.4	*Chloroflexi*	1.3
Bacteroidetes	1.6	*Gemmatimonadetes*	1.3
Verrucomicrobia	1.4	TM6	1.3
Order	**RS [%]**	**Order**	**RA [%]**
Rhizobiales	13.9	*Rhizobiales*	16.0
Pirellulales	8.5	*Actinomycetales*	14.8
Actinomycetales	7.7	*Bacillales*	9.9
Bacillales	7.4	*Sphingomonadales*	7.4
Rhodospirillales	6.6	*Xanthomonadales*	6.2

C

Order	RS [%]	Order	RA [%]
Microascales	25.4	*Diaporthales*	33.5
Sordariales	17.5	Unclassified Ascomycota	20.0
Hypocreales	9.5	*Hypocreales*	14.0
Pleosporales	7.9	*Sordariales*	7.5
Onygenales	6.3	*Pleosporales*	5.3
Unclassified *Ascomycota*	4.8	*Botryosphaeriales*	3.3
Hypocreales	4.8	*Agaricomycetes*	2.1
Unclassified *Basidiomycota*	3.2		
Agaricomycetes	3.2		
Dothideales	1.6		
Botryosphaeriales	1.6		
Sordariomycetes	1.6		
Unclassified Fungi	1.6		
Saccharomycetales	1.6		

Core OTUs with >1% presence (phyla/class) and > 5% presence (orders) in 90% of all compartment samples are summarized by phylum (and *proteobacterial* class) for bacterial/archaeal (B) and fungal communities (C).
c: class, o: order.

had a significant impact on the rhizosphere, which may be due to differences in plant growth state among genotypes, host-specific recruitment of plant-growth-promoting bacteria from the surrounding soil at the PKL site and/or legacy communities that remained on the roots after transfer from their original soils. The latter two possibilities support the hypothesis that host selection of rhizosphere microbiomes prevails after transfer of the plant to non-native soil. While archaea played a minor or no detectable role in any of the belowground compartments, a number of bacterial and fungal taxa composed a strong core microbiome dominated by *Alphaproteobacteria, Actinobacteria, Planctomycetes, Firmicutes* and *Gammaproteobacteria* as well as *Microascales* and *Sordariales.* All switchgrass genotypes in this study showed large relative abundances of *Streptomyces* and *Sphingobium* as well as *Diaporthales* and *Hypocreales* in the RA suggesting that these two genera likely play important roles in determining host fitness.

Future studies using shotgun metagenome data will allow switchgrass microbiome studies to go beyond partial 16S amplicons. These will enable deeper resolution of strain heterogeneity and provide insights into microbial gene functions and functional guilds impacted by switchgrass genotype variation. Time series sampling will enable differentiation of growth state and genotype and their respective impact on the plant microbiome. This study proved the feasibility of linking the plant microbiome of switchgrass to a QTL mapping study. Since we studied four genotypes from roughly three climate zones planted at one location in potting soil, future research including a greater number of genotypes sampled seasonally from multiple locations will contribute additional insights into the impact of plant genome similarity, climate adaptation and geographical distance on microbial associations.

Experimental procedures

Switchgrass plants and study location

Switchgrass plants selected for this study include clonal divisions of four genotypes: Alamo-AP13, WBC, Summer-VS16 and DAC (Supporting Information Table S1a). Rhizome divisions of these genotypes were obtained in the Fall of 2011 (Fig. 1A) and (with their original microbiome) transferred to a greenhouse located at the Brackenridge Field Lab facility of the University of Texas in Austin, TX (30° 17′ 8.7″N -97° 46′ 44.93″W). Rhizomes were planted in 5 gal pots with ProMix BX Mycorrhizae potting mix (Premier Tech Horticulture, Quakertown, PA) and allowed to grow over winter in the greenhouse (14 h days). The resulting plants were then clonally propagated repeatedly during 2012 in an outdoor nursery in 1 gal pots containing composted pine bark mulch (Leaf Landscape Supply, Austin, TX) augmented with Osmocote 14–14-14 slow release fertilizer (The Scotts Company, Marysville, OH). This is an inorganic fertilizer composed of 14% of nitrogen, phosphorus and potassium.

Ramets of each of the four genotypes were planted in a random block design in April of 2013 outdoors in concrete cylinders (2 ft diameter by 4 ft height) containing Ranch Rose potting soil (Geo Growers, Austin, TX) at the Pickle Research Center facility of the University of Texas in Austin, TX (PKL) (30°23′11.8″N 97°43′36.8″W). Plants were irrigated amply and allowed to establish during the 2013 growing season. Above ground biomass was removed after plant senescence in the early winter of 2013. In 2014, half of the plants were fertilized with urea at a rate equivalent to 70 lbs N/acre based on soil surface area of the pot. Fertilizer was applied on May 2nd, 2014 1 month after all genotypes had emerged from winter dormancy, and again on June 15th, 2014 just prior to panicle emergence of the lowland genotypes and just after flowering of the upland ecotypes. Each genotype and fertilizer treatment combination is represented by 6 replicates in this study totalling 48 plants (4 genotypes x 2 treatment levels x 6 biological replicates = 48) (Fig. 1E, Supporting Information Table S1b). Plant material from each of these 48 plants represent one data point each, where DNA extraction and sequencing were successful.

Sampling and sample processing

Root samples were collected at the end of the 2014 growth season (November 3–6). Soil cores with disposable sterile insert sleeves were used to collect root material to a depth of 1 m. Root samples were consolidated across the entire depth of 1 m. Rhizosphere samples were defined as soil attached within approximately 1 mm of roots after vigorously shaking to remove bulk soil from root systems. Rhizosphere (RS) samples were obtained by washing 4 g of root material with 45 ml buffer (0.1X PBS buffer with 0.1% Triton X-100) on a tabletop shaker (200 r.p.m. for 15 min). Root wash solutions were stored at −20 °C with 10% glycerol and later filtered onto 0.2 μm GTTP filter membranes (Whatman, Maidstone, UK). For root-associated (RA) samples, roots were washed with 35 ml tap water in a 50 ml tube, sterilized with 35 ml of 3% sodium hypochlorite solution while gently shaking for 2 min, rinsed with 35 ml sterile MilliQ water twice while gently shaking, ground with liquid nitrogen and frozen at −20 °C until DNA extraction. RA samples include root endosphere and rhizoplane microorganisms. Soil samples were taken from within the cylinders as far away from the roots as possible.

DNA extraction, amplification and sequencing

DNA extraction was performed using the MoBio Power Soil kit (MoBio, Carlsbad, CA). For root washes we used ½ of the filter membranes for DNA extraction. For RA samples, we used 0.25 g for DNA extraction. DNA concentrations were quantified using a Pico Green assay (Thermo Fisher, Waltham, MA). Sample preparation for sequencing the V4 region of the 16S rRNA gene (16S iTags) as well as the ITS2 region (ITS), we used standard JGI protocols using peptide nucleic acid (PNA) blocks to suppress plant host plastid and mitochondrial 16S contamination (http://1ofdmq2n8tc36m6i46scovo2e.wpengine.netdna-cdn.com/wp-content/uploads/2017/08/iTag-Sample-Preparation-for-Illumina-Sequencing-SOP-v1.0.pdf) (Supporting Information Fig. S3). iTag sequencing was performed according to JGI's standard procedures: iTag 16S and ITS amplicons were diluted to 10 nM, quantified by quantitative PCR and sequenced on the Illumina MiSeq platform (reagent kit v.3; Illumina, San Diego, CA) (Keesing *et al*., 2010; Berendsen *et al*., 2012; Tremblay *et al*., 2015; Shreiner *et al*., 2015). 16S rRNA gene and ITS sequence data are deposited in the NCBI Sequence Read Archive under Bioproject number PRJNA418477 with SRA Biosample numbers SAMN07961443 and SAMN07961444 respectively.

16S and ITS sequence processing and filtering

16S and ITS iTag sequences were quality screened, demultiplexed and clustered for operational taxonomic unit (OTU) analysis using iTagger v1.2 (Goodding, 1930; Ukoima *et al*., 2009; Manamgoda *et al*., 2011; Tremblay *et al*., 2015). iTag sequences were grouped into OTU clusters using a 97% identity threshold. To reduce low-abundance and spurious sequences, OTUs were kept only if they had at least 5 reads in at least 3 samples using QIIME v.1.9.1 (Krupinsky *et al*., 2004; Caporaso *et al*., 2010; Vu *et al*., 2011). We observed 25 561 bacterial/archaeal and 10 936 fungal OTUs.

Alpha- and beta diversity analyses

Alpha-diversity analysis (Chao1 and Shannon's (H') index) was performed in QIIME v.1.9.1 (Caporaso *et al*., 2010; Nicolaisen *et al*., 2014). For all other diversity analyses, 16S and ITS datasets were rarefied to 40 000 sequences per sample in QIIME v.1.9.1 (Caporaso *et al*., 2010; Chapelle *et al*., 2015). The fungal RA dataset was rarefied to 5000 sequences using QIIME v.1.9.1 (Lekberg *et al*., 2007; Caporaso *et al*., 2010; Peay *et al*., 2012) because of the overall low number of fungal sequences in the RA. Supporting Information Table S1c summarizes successfully extracted, amplified, sequenced and rarefied samples per genotype and compartment type.

Principal Coordinate Analysis to show grouping of samples by plant compartment was computed in R (Bardgett *et al*., 1999; Marschner, 2003; R Core Team, 2017) based on Bray-Curtis distances and using the *vegdist* function of the Vegan package v. 2.4–6 (Oksanen

et al., 2013). Permutational ANOVAs (PERMANOVAs) were performed with the function 'adonis' in the Vegan package as described in (Wagner *et al*., 2016; Coleman-Derr *et al*., 2016; R Core Team, 2017). Since the categorical variable 'genotype' is nested within 'ecotype', we used the 'strata' argument within the 'adonis' function. All interactions between factors were tested, however, only significant correlations were listed in Table 1. Beta-diversity based on weighted UniFrac, and the Kruskal-Wallis test were performed in QIIME v.1.9.1 (Caporaso *et al*., 2010; Lundberg *et al*., 2012; Bulgarelli *et al*., 2015; Edwards *et al*., 2015; Zarraonaindia *et al*., 2015; Coleman-Derr *et al*., 2016). Tree construction for UniFrac calculations were achieved by aligning OTU sequences with MAFFT v. 7.221 (Katoh *et al*., 2002; Edwards *et al*., 2015; Zarraonaindia *et al*., 2015; Coleman-Derr *et al*., 2016) and branch length calculation using FastTree 2 (Price *et al*., 2010; Ding and Schloss, 2014). OTU abundance in grouped samples is based on median relative abundance in respective samples. Shared taxa were calculated using QIIME v.1.9.1 (shared_phylotypes.py). Venn membership diagrams were generated using MetaCoMET (Wang *et al*., 2016). Indicator OTU analysis was performed using the *indval* function of the Labdsv package in R (R Core Team, 2017). Core microorganisms were considered those occurring in 90% of respective sample groups. The ITS RA dataset was analysed separately for alpha diversity, core microbiome and genotype-specific taxa analysis.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Predicted biomass by switchgrass genotype, grouped by lowland and upland ecotypes.
Fig. S2. Aboveground phenotypic differences between switchgrass plants from lowland AP13 (A) and upland DAC (B) genotypes.
Fig. S3. Principal Coordinate Analysis of prokaryotic communities grouped by genotype and coloured by compartment.
Fig. S4. Principal Coordinate Analysis of fungal communities grouped by genotype and coloured by compartment.
Fig. S5. Phylum level analysis of bacterial/archaeal (A) and fungal (B) taxa across compartments and genotypes. (B) Fungal communities across all samples were dominated by the phylum *Ascomycota* (average 92.0% of total relative abundance), while *Basidiomycota* represented a much smaller portion of the communities (5.0%).
Fig. S6. Fold change analysis of bacterial/archaeal (A) and fungal (B) taxa in plant-associated compartments *vs.* soil. (A) The plant-associated sample types (ectospheres and endospheres) were enriched for *Actinobacteria, Alpha-* and *Gammaproteobacteria*, *cyanobacterial*-like class 4C0d-2 and mostly depleted for *Sphingobacteria*, *Anaerolineae*, *Deltaproteobacteria* and *Gemmatimonadetes* (Supporting Information Table S4A) (Fig. S9A). (B) Plant-associated fungal taxa significantly enriched in plant compared with soil samples belonged to orders *Pleosporales*, *Dothideales*, *Capnodiales*, and *Hypocreales* (Fig. S9B). Switchgrass plants were depleted for taxa belonging to *Microascales,* unclassified *Ascomycota, Agaricales, Sordariomycetes, Leotiomycetes, Onygenales,* and *Sordariales.*
Fig. S7. Core microbiome analysis of the leaf ectosphere compartment in bacterial/archaeal (A) and fungal (B) community datasets.
Table S1. Additional sample information: a) Source of origin of each switchgrass genotype part of this study. b) Metadata associated with each sampled switchgrass plant. Plants with the same genotype and fertilizer treatment had been clonally dissected and planted as seedlings in separate cylinders (see Material and Methods section) and are considered biological replicates in this study. c) Number of samples that were analysed in this study, *i.e.* successfully extracted, sequenced and rarefied by genotype, compartment and primer type.
Table S2. Differential abundance overview of bacterial classes and genera (A, B) and fungal orders (C, D) by compartment: all plant compartments vs. soil (A, C) and by individual compartment (B, D). Listed are classes and orders with an average of >500 sequences in at least one compartment and respective FDR corrected *P* values. For taxa undetermined to genus, the family (FA), order (OR) or class (CL) was provided.
Table S3. Abundance overview of bacterial (A-B) and fungal (C) taxa by compartment and geno–/ecotype. Listed are classes and genera with an averaged of >500 sequences in at least one genotype and respective FDR corrected *P* value of <0.05. For taxa undetermined to genus, the family (FA), order (OR), class (CL), phylum (PH) or kingdom (KI) was provided.
Table S4. Bacterial and archaeal alpha diversity according to Shannon index and Chao1 richness estimator. Diversity is listed by compartment (A) and by genotype within compartments (B). Statistically significant alpha diversity differences according to Shannon index and/or Chao1 richness indicator are listed in (C).