Title

Assembly and seasonality of core phyllosphere microbiota on perennial biofuel crops

Authors

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Perennial grasses are promising feedstocks for biofuel production. The phyllosphere (aerial surfaces) of these cellulosic crops provide a sizable surface area of microbial habitation. Leaf microbial communities may benefit the host plant, have consequence for biomass deconstruction, and interact with foliar pathogens. Here, we characterized the origins, diversity, and assembly of bacterial and archaeal communities of two perennial cellulosic feedstocks: switchgrass (*Panicum virgatum* L.) and miscanthus (*Miscanthus x giganteus*). We used 16S rRNA gene sequencing to assess microbiome structure on leaves and soils, following crop phenology every three weeks from pre-emergence through senescence for one miscanthus growing season and two consecutive switchgrass seasons. We found abundant and persistent core leaf taxa that originated in soil but were adapted for life on the leaf, rather than vagabonds that randomly disperse from air or soil. Seasonal and host-specific assembly suggested a functional relationship between the phyllosphere microbiomes and plants. This foundational knowledge advances goals to leverage native microbiomes to promote crop wellness and productivity in the field, and specifically in support of biofuels agriculture.

The phyllosphere represents the largest environmental surface area of microbial habitation on the planet (Peñuelas and Terradas 2014; S.E. Lindow and Brandl 2003; Vorholt 2012), and much of that surface area is cultivated agriculture. Eleven percent of the world's land is dedicated to arable and permanent agricultural crops (Bruinsma et al. 2002), including an estimated 1.5 x 10^7 km^2 of cropland (Foley et al. 2011). Biofuel crops like miscanthus and switchgrass are selected to have extended growing seasons, to produce ample phyllosphere biomass, and to maintain high productivity when grown on marginal lands that are not optimal

for food agriculture (Heaton, Dohleman, and Long 2008; Tornqvist et al. 2017; Robertson et al. 2017; Stoof et al. 2015). In the field, these crops provide the microbial equivalent of mansions to their phyllosphere microbiota that are, upon senescence, harvested for conversion to biofuels and related bioproducts. Improved understanding of the phyllosphere microbiome is expected to advance goals to predict or manage changes in biomass quality in response to abiotic stress like drought (Ong et al. 2016; Emerson et al. 2014) or biotic stress like foliar pathogens (Cox et al. 2013; Alexander et al. 2017; Sattler and Funnell-Harris 2013).

Phyllosphere microorganisms may provide numerous benefits to plants, including increased stress tolerance (Hamilton et al. 2012; Redman et al. 2002; Steven E Lindow and Leveau 2002), promotion of growth and reproduction (Canto and Herrera 2012; Doty et al. 2009; Taghavi et al. 2009), protection from foliar pathogens (Lee et al. 2014), and, with soil microbes, control of flowering phenology (Wagner et al. 2014). Phyllosphere microorganisms are also thought to play important roles in Earth's biogeochemical cycles by moderating methanol emissions from plants (Iguchi, Yamanaka, and Budhiono 2000; Galbally and Kirstine 2002) and contributing to global nitrogen fixation (Fürnkranz et al. 2008). Despite this importance, knowledge of phyllosphere microbiomes remains relatively modest, especially for agricultural crops (Weyens et al. 2009; Hacquard and Schadt 2015; Kinkel 1997; Vorholt 2012). However, to leverage plant microbiomes to support productivity and resilience both above and below ground (Lebeis 2014; Vandenkoornhuyse et al. 2015; Turner, James, and Poole 2013), there is a need to advance foundational knowledge of phyllosphere microbiome diversity and dynamics.

Leveraging the Great Lakes Bioenergy Research Center's Biofuel Cropping System Experiment (BCSE; a randomized block design established at Michigan State's Kellogg Biological Station in 2008), we identified core phyllosphere microbiome members for miscanthus and switchgrass, quantified drivers of their seasonal dynamics from measured weather, plant, and soil data, and assessed the contributions of soil microbes to the phyllosphere assembly.

Results and Discussion

Seasonal microbiome dynamics

With our sequencing efforts, we exhaustively sampled the phyllosphere communities, and also approached richness asymptotes with soils (**Figure S1A**). There were clear seasonal changes in the structures of switchgrass and miscanthus phyllosphere bacterial and archaeal communities (**Figure 1A**, PERMANOVA pseudoF for time = 40.79, $p < 0.001$), and these could be attributed to changes in both soil and leaf properties, as well as to weather (**Table S1**). Over the 2016 season, miscanthus and switchgrass phyllosphere communities were synchronous (changed at the same pace and to the same extent, Procrustes Sum of Squares = 0.398, Correlation = 0.776, $p = 0.015$), and community structure became less variable as the growing season progressed (**Figure S2).** Switchgrass 2016 and 2017 leaf communities were highly synchronous, suggesting a predictable, interannual assembly (Procrustes Sum of Squares: 0.1187, Procrustes rotation: 0.9388, Significance: 0.001). The switchgrass community structures were overall equivalent between 2016 and 2017, with the exception of the final time points that were collected post-senescence.

Phyllosphere communities were relatively simple in their within-sample (alpha) diversity, with 1,115 total taxa observed across both crops (**Figure S3**), observed at an exhaustive sequencing depth that was rarefied to 146 reads per sample (**Figure S1B**). At first, we were surprised that 146 reads could well-describe the leaf diversity (we had performed much deeper sequencing), but inclusion of additional reads did not alter analysis outcomes and rarefaction suggests that coverage is more than sufficient. Indeed, richness ranged from 30 to 77 average taxa per sampling time (range is 7 to 105 taxa per plot, n = 171, **Figure 2A-C**). In 2016, switchgrass and miscanthus phyllosphere communities were consistent in their richness, with the exception of one time point in early June that had a transient increase in richness (**Figure 2A**). This increase may be attributed to fertilizer application 17 days prior, though no overall impact of fertilizer was seen on community structure or richness (PERMANOVA PseudoF = 0.490, p = 0.926, t-test p = 0.714). Evenness had similar seasonal trends to richness (**Figure S4)**. Also, cumulative richness increased most between the two earliest time points, and then tapered gradually upward until senescence (**Figure 2D**), showing that the contributions of new taxa was low but consistent over time. Together, these data suggest that these phyllosphere communities are not stochastically assembled, nor are they a linear accumulation over seasonal leaf exposure to whatever taxa are dispersed. The communities instead follow a directional assembly over the growing season, and the assembly was highly consistent over two years in the switchgrass.

Contribution of soil microorganisms to phyllosphere assembly

The three sources of microorganisms to the phyllosphere are soils (S.E. Lindow and Brandl 2003), the vascular tissue of the plant or its seed (Barret et al. 2015), and the atmosphere (Vorholt 2012). We wanted to understand the potential for soil as a source of microorganisms for phyllosphere assembly, as several studies have shown that soil microbes contribute to the phyllosphere microbiome (Hamonts et al. 2018; Bodenhausen, Horton, and Bergelson 2013).

First, we interrogated the 2016 time series to determine the influence of soils on leaf microbial communities for both crops. The structures of leaf communities were very distinct from soils (**Figure 1C**, pseudoF = 72.81, $p < 0.001$). Though soil communities also changed seasonally (**Figure 1B**, pseudoF = 7.18, $p < 0.001$), they experienced less overall change than the phyllosphere (**Figure 1B and 1A**, 2% v. 29.4% var. explained by axis 1 for soils and leaves, respectively). While fertilization had no impact on phyllosphere communities ($p = 0.938$), it did have small but significant influence on soil communities (pseudo F = 4.85, $p < 0.001$). These seasonal and fertilization treatment patterns were reproduced in the 2017 switchgrass plots (Leaf psuedoF for time = 21.56 $p = 0.001$, Leaf pseudoF for fertilization = 0.72, $p = 0.638$, soil pseudoF for time = 3.03 $p = 0.002$, soil pseudoF for fertilization = 3.31 $p = 0.001$).

Next, we used the full soil dataset (not rarified) to explore whether any phyllosphere members were detected even among very rare soil members, and this also allowed us to characterize the seasonal nuances of soil contributions to the leaf. Surprisingly, 87.2% and 77.7% of taxa detected in the phyllosphere were also detected in soil in 2016 and 2017, respectively. A closer look at the relationships between abundance and occupancy (persistence in detection over time) across the leaf and soil environments further supports adaptation or

129 selection of leaf taxa to the phyllosphere (**Figure 3**). The most persistently detected leaf taxa
130 were inconsistently observed in soils (**Figure 3A**), and the most abundant leaf taxa were
131 relatively rare in soils (**Figure 3B**). This suggests host selection or adaptation of the abundant
132 leaf microbiome members to the leaf habitat. Finally, other studies have found that soil
133 microbes contribute more to early-season phyllosphere communities (Copeland et al. 2015),
134 and we observed similar patterns: the most abundant soil taxa that were also detected on
135 leaves were more prominent in the early season and then became rare and transient on leaves
136 in the late season.

137 We conclude from these results that soil is the most substantial reservoir of leaf
138 microorganisms for these perennial crops and note that deep sequencing was required of the
139 soils to observe many of the prominent leaf taxa. This is in contrast to the studies on other
140 plants that have suggested that the phyllosphere is comprised largely of passively dispersed
141 and stochastically assembled microbes from the atmosphere (e.g., (Ottesen et al. 2016;
142 Maignien et al. 2014; Vokou et al. 2012). Though we cannot assert that no phyllosphere
143 members were dispersed from the atmosphere, the vast majority of leaf microbes were
144 detectable in local soils and the consistent leaf trajectory across crops and seasons suggests
145 both determinism and habitat specificity.

146

147 *Core members of the switchgrass and miscanthus phyllosphere*

148 Though there was high overlap between the crops' phyllosphere community structures
149 early in the growing season, crops diverged to a distinct structure by late season (supported by
150 crop separation along PCoA2 in **Figure 1A**, overall miscanthus v. switchgrass community

151 structure PERMANOVA pseudoF = 6.69, p = 0.001). To define a core microbiome for each crop
152 and season, we considered both the occupancy and abundance patterns of these taxa (**Figure**
153 **S6**). We identified core phyllosphere taxa that were both persistent (occupancy > 0.4) and
154 abundant (mean $\log_{10}$ relative abundance > -2.5); these core taxa were generally detected on
155 both crops, while taxa that were uniquely detected on either crop were rare and not persistent
156 (**Figure S6**).

157 We then layered information about a core taxon's detection across replicated plots
158 within and across time, as a general indication of the variability in its detection and abundance
159 (**Figure 4**). These core taxa included several key Proteobacteria (*Methylobacterium*,
160 *Sphingomonas*, and *Pseudomonas* spp). Though these core taxa were selected because they
161 were very abundant in the miscanthus and switchgrass phyllospheres over time, their
162 taxonomic affiliations are consistent with the literature for other phyllosphere communities
163 (Knief et al. 2010; Kinkel 1997; Rastogi, Coaker, and Leveau 2013; S.E. Lindow and Brandl 2003;
164 Vorholt 2012; Bodenhausen, Horton, and Bergelson 2013), providing new support for their
165 temporal importance in the phyllosphere.

166 We next assessed potential interaction networks among the core phyllosphere
167 members for each crop (**Figure 5**). We used a co-occurrence algorithm that explicitly considers
168 replicated time series and investigates both linear and non-linear relationships (Xia et al. 2011).
169 All three networks were qualitatively similar: they had three major subnetworks that had
170 positive associations within each subnetwork, but negative associations across the
171 subnetworks. The subnetworks were taxonomically consistent across crops and years, which
172 suggests potential for redundancy in their core taxa because closely related taxa are

hypothesized to have substantial overlap in their functional repertoire (Martiny et al. 2015). The early-season subnetworks **(i)** included several *Gammaproteobacteria* with positive relationships to *Betaproteobacteria*, and these then negatively connected to the mid- and late-subnetworks. The late-season subnetwork **(iii)** was comprised of well-connected *Alphaproteobacteria* to *Bacteroidetes* and *Actinobacteria*, and was largest in the switchgrass 2016 time series. The smallest subnetwork **(ii)** included mid-season "bloomers", including *Alphaproteobacteria* and few taxa belonging to *Beta*- and *Gamma-proteobacteria, Cytophagia* or *Actinobacteria*; they were generally positively connected with taxa in the late-season subnetwork. Our data suggested a compensatory relationship between members within the Proteobactera, where *Gammaproteobacteria* and *Alphaproteobacteria* replace one another over time (**Figure 6**). Such community transitions have been observed on the phyllosphere of crops such as sugarcane (Hamonts et al. 2018), common beans, soybeans, and canola (Copeland et al. 2015). Interestingly, a study of endophytic bacteria of prairie grasses, including switchgrass, showed the same trend in abundance of *Gamma*- and *Alphaproteobacteria* (Ding and Melcher 2016) suggesting that these phyllosphere taxa are facultative endophytes or are similarly affected by the plant development. The benefits plants gain from these taxa are well characterized (see review from (Bringel and Couée 2015)), however it remains unknown what drives the exclusion of *Pseudomonas* and gives rise to *Alphaproteobacteria* (predominantly *Methylobacteria*) in the phyllopshere and endopshere. One possible explanation would be nutrient availability regulated by the plant development which would selectively influence the abundances of these taxa. Delmotte and colleagues (Delmotte et al. 2009) hypothesized that *Pseudomonas* are probably specialized to monosaccharide, disaccharide and amino acid

utilization whereas *Sphingomonas* and *Methylobacteria* are scavenging various substrates present at low amounts.

Despite similarity in the membership and dynamics of the core microbiota on both crop plants, there were nuances in relative abundances of the same taxa across plant hosts, suggesting microbiome selectivity for or by the host plant (**Figure 4, Figure 5**). For example, the core *Sphingomonas* taxa OTU2 and OTU430 showed nuances not only by crop by also over the growing season. OTU2 had maximum relative abundance in the early stage of miscanthus growth, but in switchgrass it reached maximum abundance late in the season, a trend observed in both 2016 and 2017. *Sphingomonas* OTU430 showed very different patterns, with consistently high abundance in the earliest time points and low abundance in the late season, across crops and years. As another example, *Polaromonas* sp. (OTU41) was highly abundant in the early stages of the switchgrass growth and with constant decrease in relative abundance and prevalence afterwards. However, this same taxon was maintained throughout the 2016 season on the miscanthus leaves, peaking in abundance at the end of the season.

Drivers of phyllosphere assembly: contributions of abiotic variables, space, time, and host

The observed influence of the host plant on the phyllosphere microbiota agrees with previous research on perennial plants such as wild mustard (Wagner et al. 2016), sugar cane (Hamonts et al. 2018), and tree species like birch, maple, and pine (Laforest-Lapointe, Messier, and Kembel 2016). However, for annual crops such as common beans, canola and soybean, the phyllosphere community was also influenced by plant development and sampling location (Copeland et al. 2015). We used variance partitioning to determine the shared contributions of

host plant (crop), space, time, and abiotic variables to the assembly of the core phyllosphere community. We focused our analysis on the 29 core members of the 2016 season so that influence of the host plant could be assessed. Abiotic factors included soil chemistry, plant chemistry and weather variables that were not colinear with time. While spatial distance between the plots had no explanatory value (Mantel statistic r: 0.013, p = 0.256), measured abiotic factors, time, and their interaction explained the largest proportion of variance (**Figure S5**, collectively 45.9%). The host plant explained 5.3% variation alone. However, 46.2% of the variance in community structure of the core community could not be explained by any of these three measured factors or their interactions. We posit that the unexplained variance is attributed to unmeasured abiotic factors of importance for the phyllosphere.

To conclude, we investigated the assembly and seasonal dynamics of the phyllosphere and soil microbes of two perennial grasses, switchgrass and miscanthus, and found very repeatable community trajectories and memberships across growing seasons, suggesting that their key players are highly predictable. We considered the sources of the phyllosphere communities and found that the associated soil is likely the primary reservoir for these taxa. The majority of phyllosphere taxa, including the abundant core members, were found in very low abundance in the associated soil suggesting the specificity for life on the leaf surface. We found 15 core phyllosphere taxa that were predominantly classified as *Proteobacteria*. However, even within this core, nuances in their relative abundances were detected during the growth season and across plants, including replacements indicating compensatory dynamics. These nuanced dynamics suggested either selection by or functionally specificity for the host.

238 The characteristics of the core leaf microbiome described here could be an general attribute of
239 perennial plants or of grasses.
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243 Methods
244 *Site description & sampling scheme*
245 Our study system is located within the Great Lakes Bioenergy Research Center (GLBRC)
246 Biofuel Cropping System Experiment (BCSE) in Hickory Corners, Michigan (42°23'41.6"N,
247 85°22'23.1"W). We collected samples from two biofuel crops within the BCSE, switchgrass
248 (*Panicum virgatum* L. cultivar "Cave- in-rock") and miscanthus (*Miscanthus x giganteus)*. Both
249 crops had been continuously grown since 2008, in replicate 30 x 40 m plots arrayed in a
250 randomized complete block design. Within each plot, nitrogen-free (no fertilizer) subplots were
251 maintained in the western-most 3 m of each plot. We sampled replicate plots 1-4 in both the
252 main and the nitrogen free subplots. We collected leaf and bulk soil samples every three weeks
253 across the 2016 growing season, including bare soil in April (pre-emergence) through
254 senescence in October and November (for switchgrass and miscanthus, respectively). In total,
255 we collected 152 soil samples (72 switchgrass and 80 miscanthus) and 136 leaf samples (64
256 switchgrass and 72 miscanthus). At each sampling time, leaves were collected and pooled at
257 three flags along a standardized path within each plot. Leaves were removed from the plant
258 stem using ethanol sterilized gloves, then stored in sterile whirl-pak bags until processing. Bulk
259 soil cores (2 x 10 cm) were collected at the same three locations within a plot, sieved through 4

mm mesh, then pooled and stored in whirl-pak bags. All samples were kept on wet ice for transport, then stored at -80 °C long term.

Contextual data

Soil physico-chemical characteristics (pH, lime, P, K, Ca, Mg, organic matter, NO_3-N, NH_4-N, and percent moisture) were measured by the Michigan State University (MSU) Soil and Plant Nutrient Lab (East Lansing, MI, USA, http://www.spnl.msu.edu/) according to their standard protocols. From each plot, 10 switchgrass leaves or 5 miscanthus leaves were processed for leaf dry matter content according to (Cornelissen et al. 2003). Dried leaves were ground to a fine powder using a Sampletek 200 vial rotator and iron roll bars (Mavco Industries, Lincoln, NE, USA), then carbon and nitrogen were measured on an elemental analyzer (Costech ECS 4010; Costech Analytical Technologies Inc, Valencia, CA, USA). Weather data was collected from the MSU Weather Station Network, for the Kellogg Biological Station location (https://mawn.geo.msu.edu) for each sampling day, and plant height and soil temperature were measured on a per-plot basis.

Nucleic acid extraction & sequencing

Soil microbial DNA was extracted using a powersoil microbial DNA kit (MOBio Inc. Carlsbad, California, USA) according to manufacturer's instructions. Phyllosphere epiphytic DNA was extracted from intact leaves using a benzyl chloride liquid:liquid extraction, followed by an isopropanol precipitation as described in (Suda et al. 2008), using approximately 5 g of leaves (5-10 switchgrass leaves, or a minimum of 2 miscanthus leaves). Metagenomic DNA from both

soil and phyllosphere was quantified using a qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA), and DNA concentrations were normalized between all samples prior to sequencing. Paired-end amplicon sequencing was completed by the Department of Energy's Joint Genome Institute using an Illumina MiSeq sequencer, and using the 16S-V4 (515f-804r) primer set (Caporaso et al. 2011).

Sequence quality control and operational taxonomic units

BBDuk (v 37.96) was used to remove contaminants and trim adaptor sequences from reads. Reads containing 1 or more 'N' bases, having an average quality score of less than ten or less than 51 bases were removed. Reads were mapped to the genomes of human, cat, dog, mouse and common microbial contaminants with BBMap (v 37.96) and were removed from analysis.

Primers were removed from reads using cutadapt (v1.17). Reads were merged, dereplicated, clustered into 97% identity with usearch (v10.0.240), and classified against version 123 of the Silva Database (Quast et al. 2013) using sintax (Edgar 2016). Our full sequence analysis workflow can be found on Github (https://github.com/ShadeLab/PAPER_GradySorensenStopnisek_InPrep)

Statistical analyses

Before analyses, we filtered all the sequences classified as Mitochondria or Cyanobacteria and subsampled (rarefied) to 146 reads per sample. We removed the low read count samples (May 2016 samples, n = 23) from the dataset and conducted a Mantel statistic

304 on the Bray-Curtis distance matrices of the two datasets (rarified to 146 or 500 reads). The
305 Mantel test showed no effect of the subsampling on overarching changes in community
306 structure (Mantel statistic r: 0.929, significance: 0.001). We used 146 reads for all of the
307 analysis, unless specified differently. For alpha diversity indices, richness as total number of
308 OTUs, Shannon and Pielou indexes were calculated. We used ANOVA to look for differences in
309 alpha diversity over time. We used the protest function in R to test the significance of
310 ordination results from the subsetted data to crop and year. For calculation of the beta
311 dispersion, we used the betadisper function in R, which is a multivariate analogue of Levene's
312 test for homogeneity of variances. PERMANOVA was used to test hypothesis of beta diversity
313 using adonis function in R. Variance partitioning was performed on the 2016 core taxa (n = 29)
314 using the varpart function in the vegan package. Each taxon's abundance was relativized to the
315 total number of reads comprising the core taxa in each site. Abiotic variables were tested for
316 correlations with time, and those with significant correlations were removed from analysis.
317 To infer the core phyllosphere taxa, we used occupancy-abundance plots (e.g. (Shade et
318 al. 2018)). For each OTU we calculated prevalence and mean relative abundance across the
319 dataset. For plotting we used the log10 mean relative abundance. We selection the core taxa
320 based on few criteria i) present in at least 1 out of 4 replicates, ii) found in at least 2 consequent
321 sampling time points, and iii) high relative abundance. With that criteria we selected the
322 threshold to be at occupancy greater than 0.4 and log10(mean relative abundance) greater
323 than -2.5.
324
325 *Temporal analyses and network construction*

We identified significant temporal correlations in the relative abundances of individual taxa derived from soils that were treated to remove relic DNA using extended Local Similarity Analysis (eLSA). To satisfy the eLSA requirement of equal number of replicates per time point we eliminated number of samples from each time point to the number of replicates lowest for each dataset (3 for switchgrass 2017 and 6 for switchgrass and miscanthus 2016). We included also a delay parameter which was set to 1 (-d 1) and permutation based on 1000 replications (-p 'perm' -b 1000). We defined significant temporal associations as those with a local similarity (LS) score lower than -0.34 and greater than 0.34 and a p value ≤ 0.05. Pairs of significantly correlated taxa were visualized in Cytoscape (version 3.6.1). Network modules were guided by the hierarchical cluster analysis (hclust function in R) on the dissimilatory distance (Bray-Curtis) matrix including z-scores. Node IDs (individual taxa) were color coded by their membership based on class level and node sizes were based on the connectivity.

Availability of data, workflows, and material

The datasets generated and/or analyzed during the current study are available in the Joint Genomes Institute, Integrated Microbial Genomes repository with JGI Projects designated by year and sample type (Project ID 1139694, 1139696 for 2016 season phyllosphere and soil, and 1191516 and 1191517 for 2017 season phyllosphere and soil sequences, respectively). Computing workflows are available at https://github.com/ShadeLab/PAPER_GradySorensenStopnisek_InPrep

Competing interests

348 The authors declare that they have no competing interests.

349

350 Authors' contributions

351 AS designed the study. AS, KLG, JS, and NS conducted field work. KLG executed lab work. JS,

352 NS, and AS analyzed the data. All authors wrote the manuscript.

353

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363

364

365 Figure Legends

366 **Figure 1. Seasonal patterns in the structures of bacterial and archaeal communities**

367 **inhabiting the phyllosphere and associated soils of the biofuel feedstocks switchgrass and**

368 **miscanthus.** (A) Principal coordinates analysis (PCoA) of switchgrass and miscanthus

369 phyllosphere communities (Bray-Curtis dissimilarity), error bars show 1 deviation around the

370 centroid. Subsampling depth was 146 sequences per sample and environmental vectors are

371 fitted when $r^2 > 0.4$ and $p < 0.05$ (B) PCoA of the associated soil communities, subsampled to
372 19,967 sequences per sample. (C) PCoA of the phyllosphere communities relative to the soil,
373 subsampled to 146 sequences per sample. NF is not fertilized and F is fertilized and fitted
374 environmental vectors are $R^2 > 0.40$, $p < 0.05$.
375
376 **Figure 2. Seasonal patterns in the number of observed phyllosphere taxa (richness).**
377 Operational taxonomic units (OTUs) were defined at 97% amplicon sequence identity. (**A**)
378 Switchgrass phyllosphere richness in 2016 (senescence in November). (**B**) Miscanthus
379 phyllosphere richness in 2016 (senescence in October). (**C**) Switchgrass phyllosphere richness in
380 2017. (**D**) Seasonal taxa accumulation of switchgrass in 2016 (Sw '16), 2017 (Sw '17) and
381 miscanthus in 2016 (Mi '16).
382
383 **Figure 3. Comparative detection of core members of the phyllosphere microbiome across**
384 **leaves and soil. (A)** Occupancy **(B)** relative abundance of leaf taxa detected in the phyllosphere
385 and soil during the 2016 field season.
386
387 **Figure 4. Occurrence patterns of core phyllosphere members.** Colors show standardized
388 temporal dynamics (z-score), and circle size shows the proportion of plot replicates in which the
389 taxon was detected at each sampling time (e.g, 1 = 100% or detected in 4/4 plots). Each panel
390 is a core leaf OTU that has been identified to the most-resolved taxonomic level possible, where
391 *p*: is phylum, *c:* is class, *o:* is order, *f:* is family, and *g:* is genus.
392

393 **Figure 5. Co-occurrence networks of the core phyllosphere microbiome members for (A)**
394 **miscanthus 2016, (B) switchgrass 2016 and (C) switchgrass 2017.** Networks are calculated by
395 local similarity analysis for each crop's replicated time series (n = 3-4 plots per time point, per
396 crop) and included a lag time of t-1. Node colors are phylum-level taxonomy, node size is the
397 connectivity of each hub, and black edges are positive relationships while gray are negative.
398
399 **Figure 6.** Compensatory patterns of Protobacteria classes over crops and season in the
400 phyllosphere of switchgrass and miscanthus.
401
402 Supporting Figures
403 **Figure S1. Rarefaction curves for switchgrass and miscanthus phyllosphere and soils.**
404 Operational taxonomic units (OTUs) were defined at 97% sequence identity of 16S rRNA gene
405 amplicons. Complete sequencing is shown in **(A)**, and **(B)** is an inset of the first 400 sequences.
406
407 **Figure S2. Phyllosphere communities become more stable over time**. Variability in
408 phyllosphere microbiome structure over time for (**A)** miscanthus 2016; **(B)** switchgrass 2016
409 and **(C)** switchgrass 2017 field seasons. Distance to median was calculated by analysis of beta-
410 dispersion.
411
412 **Figure S3. Taxa shared across the switchgrass and miscanthus phyllosphere,** in 2016 and
413 2017.
414

Figure S4. **Seasonal patterns in the evenness of phyllosphere taxa** for **(A)** miscanthus 2016; **(B)** switchgrass 2016 and **(C)** switchgrass 2017 field seasons. Evenness was calculated using Pielou's metric.

Figure S5. Variance partitioning of abiotic factors, host, and time for 2016 phyllosphere communities. Communities were subset to the 29 core taxa from 2016 and normalized for variance partitioning. Only abiotic factors not correlated with time were used (precipitation, maximum and minimum air temperature, air pressure, absolute humidity, soil temp at 10 cm, pH, calcium, and nitrate).

Figure S6. Abundance-occupancy of leaf taxa for **(A)** switchgrass 2016, **(B)** switchgrass 2017, and **(C)** miscanthus 2016. Members shared across crops are indicated by open circles, while crop-specific taxa are indicated by filled circles. The most persistent and abundant taxa were determined using cut-offs of 0.4 occupancy and log mean -2.5 relative abundance (dashed lines).

Supporting Tables

Table S1. Fitted environmental variables that explain changes in microbiome community structure. All are $p < 0.05$ unless designated as not significant (NS). Values in bold (EnvFit $R^2 >$ 0.40) were plotted as vectors in Figure 1.

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