

1 Title

2 Assembly and seasonality of core phyllosphere microbiota on perennial biofuel crops

3

4 Authors

5 Keara L Grady^{1,2,a}, Jackson W. Sorensen^{1,2,a}, Nejc Stopnisek^{2,3,a}, and Ashley Shade^{1,2,3,4*}

6

7 1. Department of Microbiology and Molecular Genetics, Michigan State University, East

8 Lansing MI 48840 USA

9 2. The DOE Great Lakes Bioenergy Research Center, Michigan State University, East

10 Lansing, MI 48840

11 3. The Plant Resilience Institute, Michigan State University, East Lansing MI 48840

12 4. Department of Plant, Soil and Microbial Sciences; and Program in Ecology, Evolutionary

13 Biology and Behavior, Michigan State University, East Lansing MI 48840

14

15 ^aContributed equally

16 *correspondence, shadeash@msu.edu

17

18 Keywords

19 Miscanthus, switchgrass, temporal dynamics, microbiome, agroecosystems, bioenergy,

20 sustainability, plant-microbe interactions, leaf, phytobiome

21

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

37 The phyllosphere represents the largest environmental surface area of microbial
38 habitation on the planet (Peñuelas and Terradas 2014; S.E. Lindow and Brandl 2003; Vorholt
39 2012), and much of that surface area is cultivated agriculture. Eleven percent of the world's
40 land is dedicated to arable and permanent agricultural crops (Bruinsma et al. 2002), including
41 an estimated 1.5×10^7 km² of cropland (Foley et al. 2011). Biofuel crops like miscanthus and
42 switchgrass are selected to have extended growing seasons, to produce ample phyllosphere
43 biomass, and to maintain high productivity when grown on marginal lands that are not optimal

44 for food agriculture (Heaton, Dohleman, and Long 2008; Tornqvist et al. 2017; Robertson et al.
45 2017; Stoof et al. 2015). In the field, these crops provide the microbial equivalent of mansions
46 to their phyllosphere microbiota that are, upon senescence, harvested for conversion to
47 biofuels and related bioproducts. Improved understanding of the phyllosphere microbiome is
48 expected to advance goals to predict or manage changes in biomass quality in response to
49 abiotic stress like drought (Ong et al. 2016; Emerson et al. 2014) or biotic stress like foliar
50 pathogens (Cox et al. 2013; Alexander et al. 2017; Sattler and Funnell-Harris 2013).

51 Phyllosphere microorganisms may provide numerous benefits to plants, including
52 increased stress tolerance (Hamilton et al. 2012; Redman et al. 2002; Steven E Lindow and
53 Leveau 2002), promotion of growth and reproduction (Canto and Herrera 2012; Doty et al.
54 2009; Taghavi et al. 2009), protection from foliar pathogens (Lee et al. 2014), and, with soil
55 microbes, control of flowering phenology (Wagner et al. 2014). Phyllosphere microorganisms
56 are also thought to play important roles in Earth's biogeochemical cycles by moderating
57 methanol emissions from plants (Iguchi, Yamanaka, and Budhiono 2000; Galbally and Kirstine
58 2002) and contributing to global nitrogen fixation (Fürnkranz et al. 2008). Despite this
59 importance, knowledge of phyllosphere microbiomes remains relatively modest, especially for
60 agricultural crops (Weyens et al. 2009; Hacquard and Schadt 2015; Kinkel 1997; Vorholt 2012).
61 However, to leverage plant microbiomes to support productivity and resilience both above and
62 below ground (Lebeis 2014; Vandenkoornhuys et al. 2015; Turner, James, and Poole 2013),
63 there is a need to advance foundational knowledge of phyllosphere microbiome diversity and
64 dynamics.

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

70

71 Results and Discussion

72 *Seasonal microbiome dynamics*

73 With our sequencing efforts, we exhaustively sampled the phyllosphere communities,
74 and also approached richness asymptotes with soils (**Figure S1A**). There were clear seasonal
75 changes in the structures of switchgrass and miscanthus phyllosphere bacterial and archaeal
76 communities (**Figure 1A**, PERMANOVA pseudoF for time = 40.79, $p < 0.001$), and these could be
77 attributed to changes in both soil and leaf properties, as well as to weather (**Table S1**). Over the
78 2016 season, miscanthus and switchgrass phyllosphere communities were synchronous
79 (changed at the same pace and to the same extent, Procrustes Sum of Squares = 0.398,
80 Correlation = 0.776, $p = 0.015$), and community structure became less variable as the growing
81 season progressed (**Figure S2**). Switchgrass 2016 and 2017 leaf communities were highly
82 synchronous, suggesting a predictable, interannual assembly (Procrustes Sum of Squares:
83 0.1187, Procrustes rotation: 0.9388, Significance: 0.001). The switchgrass community structures
84 were overall equivalent between 2016 and 2017, with the exception of the final time points
85 that were collected post-senescence.

86 Phyllosphere communities were relatively simple in their within-sample (alpha)
87 diversity, with 1,115 total taxa observed across both crops (**Figure S3**), observed at an
88 exhaustive sequencing depth that was rarefied to 146 reads per sample (**Figure S1B**). At first,
89 we were surprised that 146 reads could well-describe the leaf diversity (we had performed
90 much deeper sequencing), but inclusion of additional reads did not alter analysis outcomes and
91 rarefaction suggests that coverage is more than sufficient. Indeed, richness ranged from 30 to
92 77 average taxa per sampling time (range is 7 to 105 taxa per plot, $n = 171$, **Figure 2A-C**). In
93 2016, switchgrass and miscanthus phyllosphere communities were consistent in their richness,
94 with the exception of one time point in early June that had a transient increase in richness
95 (**Figure 2A**). This increase may be attributed to fertilizer application 17 days prior, though no
96 overall impact of fertilizer was seen on community structure or richness (PERMANOVA PseudoF
97 = 0.490, $p = 0.926$, t-test $p = 0.714$). Evenness had similar seasonal trends to richness (**Figure**
98 **S4**). Also, cumulative richness increased most between the two earliest time points, and then
99 tapered gradually upward until senescence (**Figure 2D**), showing that the contributions of new
100 taxa was low but consistent over time. Together, these data suggest that these phyllosphere
101 communities are not stochastically assembled, nor are they a linear accumulation over seasonal
102 leaf exposure to whatever taxa are dispersed. The communities instead follow a directional
103 assembly over the growing season, and the assembly was highly consistent over two years in
104 the switchgrass.

105

106 *Contribution of soil microorganisms to phyllosphere assembly*

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

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

123 Next, we used the full soil dataset (not rarified) to explore whether any phyllosphere
124 members were detected even among very rare soil members, and this also allowed us to
125 characterize the seasonal nuances of soil contributions to the leaf. Surprisingly, 87.2% and
126 77.7% of taxa detected in the phyllosphere were also detected in soil in 2016 and 2017,
127 respectively. A closer look at the relationships between abundance and occupancy (persistence
128 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

173 hypothesized to have substantial overlap in their functional repertoire (Martiny et al. 2015).
174 The early-season subnetworks (i) included several *Gammaproteobacteria* with positive
175 relationships to *Betaproteobacteria*, and these then negatively connected to the mid- and late-
176 subnetworks. The late-season subnetwork (iii) was comprised of well-connected
177 *Alphaproteobacteria* to *Bacteroidetes* and *Actinobacteria*, and was largest in the switchgrass
178 2016 time series. The smallest subnetwork (ii) included mid-season “bloomers”, including
179 *Alphaproteobacteria* and few taxa belonging to *Beta-* and *Gamma-proteobacteria*, *Cytophagia*
180 or *Actinobacteria*; they were generally positively connected with taxa in the late-season
181 subnetwork. Our data suggested a compensatory relationship between members within the
182 Proteobacteria, where *Gammaproteobacteria* and *Alphaproteobacteria* replace one another
183 over time (Figure 6). Such community transitions have been observed on the phyllosphere of
184 crops such as sugarcane (Hamonts et al. 2018), common beans, soybeans, and canola
185 (Copeland et al. 2015). Interestingly, a study of endophytic bacteria of prairie grasses, including
186 switchgrass, showed the same trend in abundance of *Gamma-* and *Alphaproteobacteria* (Ding
187 and Melcher 2016) suggesting that these phyllosphere taxa are facultative endophytes or are
188 similarly affected by the plant development. The benefits plants gain from these taxa are well
189 characterized (see review from (Bringel and Couée 2015)), however it remains unknown what
190 drives the exclusion of *Pseudomonas* and gives rise to *Alphaproteobacteria* (predominantly
191 *Methylobacteria*) in the phyllosphere and endosphere. One possible explanation would be
192 nutrient availability regulated by the plant development which would selectively influence the
193 abundances of these taxa. Delmotte and colleagues (Delmotte et al. 2009) hypothesized that
194 *Pseudomonas* are probably specialized to monosaccharide, disaccharide and amino acid

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

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

209

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

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

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

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

240

241

242

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

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

262

263 *Contextual data*

264 Soil physico-chemical characteristics (pH, lime, P, K, Ca, Mg, organic matter, NO₃-N, NH₄-
265 N, and percent moisture) were measured by the Michigan State University (MSU) Soil and Plant
266 Nutrient Lab (East Lansing, MI, USA, <http://www.spnl.msu.edu/>) according to their standard
267 protocols. From each plot, 10 switchgrass leaves or 5 miscanthus leaves were processed for
268 leaf dry matter content according to (Cornelissen et al. 2003). Dried leaves were ground to a
269 fine powder using a Sampletek 200 vial rotator and iron roll bars (Mavco Industries, Lincoln, NE,
270 USA), then carbon and nitrogen were measured on an elemental analyzer (Costech ECS 4010;
271 Costech Analytical Technologies Inc, Valencia, CA, USA). Weather data was collected from the
272 MSU Weather Station Network, for the Kellogg Biological Station location
273 (<https://mawn.geo.msu.edu>) for each sampling day, and plant height and soil temperature
274 were measured on a per-plot basis.

275

276 *Nucleic acid extraction & sequencing*

277 Soil microbial DNA was extracted using a powersoil microbial DNA kit (MOBio Inc.
278 Carlsbad, California, USA) according to manufacturer's instructions. Phyllosphere epiphytic DNA
279 was extracted from intact leaves using a benzyl chloride liquid:liquid extraction, followed by an
280 isopropanol precipitation as described in (Suda et al. 2008), using approximately 5 g of leaves
281 (5-10 switchgrass leaves, or a minimum of 2 miscanthus leaves). Metagenomic DNA from both

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

287

288 *Sequence quality control and operational taxonomic units*

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

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

299

300 *Statistical analyses*

301 Before analyses, we filtered all the sequences classified as Mitochondria or
302 Cyanobacteria and subsampled (rarefied) to 146 reads per sample. We removed the low read
303 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*

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

338

339 *Availability of data, workflows, and material*

340 The datasets generated and/or analyzed during the current study are available in the
341 Joint Genomes Institute, Integrated Microbial Genomes repository with JGI Projects designated
342 by year and sample type (Project ID 1139694, 1139696 for 2016 season phyllosphere and soil,
343 and 1191516 and 1191517 for 2017 season phyllosphere and soil sequences, respectively).

344 Computing workflows are available at

345 https://github.com/ShadeLab/PAPER_GradySorensenStopnisek_InPrep

346

347 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

354 Acknowledgements

355 We thank SH Lee, M Sleda, S Wu and M Nunez for technical assistance in the field and
356 laboratory. This material is based upon work supported by the Great Lakes Bioenergy Research
357 Center, U.S. Department of Energy, Office of Science, Office of Biological and Environmental
358 Research under Award Numbers DE-SC0018409 and DE-FC02-07ER64494. The work conducted
359 by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility,
360 is supported under Contract No. DE-AC02-05CH11231. This work was supported in part by
361 Michigan State University through computational resources provided by the Institute for Cyber-
362 Enabled Research. NS acknowledges support from the Michigan State Plant Resilience Institute.

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

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

418
419 **Figure S5. Variance partitioning of abiotic factors, host, and time for 2016 phyllosphere**
420 **communities.** Communities were subset to the 29 core taxa from 2016 and normalized for
421 variance partitioning. Only abiotic factors not correlated with time were used (precipitation,
422 maximum and minimum air temperature, air pressure, absolute humidity, soil temp at 10 cm,
423 pH, calcium, and nitrate).

424
425 **Figure S6.** Abundance-occupancy of leaf taxa for (A) switchgrass 2016, (B) switchgrass 2017,
426 and (C) miscanthus 2016. Members shared across crops are indicated by open circles, while
427 crop-specific taxa are indicated by filled circles. The most persistent and abundant taxa were
428 determined using cut-offs of 0.4 occupancy and log mean -2.5 relative abundance (dashed
429 lines).

430
431 Supporting Tables

432 **Table S1.** Fitted environmental variables that explain changes in microbiome community
433 structure. All are $p < 0.05$ unless designated as not significant (NS). Values in bold (EnvFit $R^2 >$
434 0.40) were plotted as vectors in Figure 1.

435
436 **References**

- 437 Alexander, Helen M., Emily Bruns, Hayley Schebor, and Carolyn M. Malmstrom. 2017. "Crop-
438 Associated Virus Infection in a Native Perennial Grass: Reduction in Plant Fitness and
439 Dynamic Patterns of Virus Detection." *Journal of Ecology* 105 (4):1021–31.
440 <https://doi.org/10.1111/1365-2745.12723>.
- 441 Barret, Matthieu, Martial Briand, Sophie Bonneau, Anne Préveaux, Sophie Valière, Olivier
442 Bouchez, Gilles Hunault, Philippe Simoneau, and Marie-Agnès Jacquesa. 2015. "Emergence
443 Shapes the Structure of the Seed Microbiota." *Applied and Environmental Microbiology* 81
444 (4):1257–66. <https://doi.org/10.1128/AEM.03722-14>.
- 445 Bodenhausen, Natacha, Matthew W. Horton, and Joy Bergelson. 2013. "Bacterial Communities
446 Associated with the Leaves and the Roots of *Arabidopsis Thaliana*." *PLoS ONE* 8 (2).
447 <https://doi.org/10.1371/journal.pone.0056329>.
- 448 Bringel, Françoise, and Ivan Couée. 2015. "Pivotal Roles of Phyllosphere Microorganisms at the
449 Interface between Plant Functioning and Atmospheric Trace Gas Dynamics." *Frontiers in*
450 *Microbiology* 6 (MAY). <https://doi.org/10.3389/fmicb.2015.00486>.
- 451 Bruinsma, Jell, Günther Fischer, Freddy Nachtergaele, Jean-Marc Faurès, Jippe Hoogeveen, Jan
452 Poulisse, Dat Tran, et al. 2002. "Crop Production and Natural Resource Use." In *World*
453 *Agriculture: Towards 2015 / 2030*. Food and Agriculture Organization (FAO) of the United
454 Nations. [https://doi.org/10.1016/S0264-8377\(03\)00047-4](https://doi.org/10.1016/S0264-8377(03)00047-4).
- 455 Canto, A., and C.M. Herrera. 2012. "Micro-Organisms behind the Pollination Scenes: Microbial
456 Imprint on Floral Nectar Sugar Variation in a Tropical Plant Community." *Annals of Botany*
457 110 (6):1173–83. <https://doi.org/10.1093/aob/mcs183>.
- 458 Caporaso, J. Gregory, Christian L. Lauber, Elizabeth K. Costello, Donna Berg-Lyons, Antonio

- 459 Gonzalez, Jesse Stombaugh, Dan Knights, et al. 2011. "Moving Pictures of the Human
460 Microbiome." *Genome Biology* 12 (5). <https://doi.org/10.1186/gb-2011-12-5-r50>.
- 461 Copeland, Julia K, Lijie Yuan, Mehdi Layeghifard, Pauline W Wang, and David S Guttman. 2015.
462 "Seasonal Community Succession of the Phyllosphere Microbiome." *Mol Plant-Microbe*
463 *Interact* 28 (3):274–85. <https://doi.org/10.1094/MPMI-10-14-0331-FI>.
- 464 Cornelissen, J.H.C., S. Lavorel, E. Garnier, S. Díaz, N. Buchmann, D.E. Gurvich, P.B. Reich, et al.
465 2003. "A Handbook of Protocols for Standardised and Easy Measurement of Plant
466 Functional Traits Worldwide." *Australian Journal of Botany* 51 (4):335–80.
467 <https://doi.org/10.1071/BT02124>.
- 468 Cox, C.M., W.W. Bockus, R.D. Holt, L.Fang, and K.A. Garrett. 2013. "Spatial Connectedness of
469 Plant Species: Potential Links for Apparent Competition via Plant Diseases." *Plant*
470 *Pathology* 62 (6):1195–1204. <https://doi.org/10.1111/ppa.12045>.
- 471 Delmotte, Nathanaël, Claudia Knief, Samuel Chaffron, Gerd Innerebner, Bernd Roschitzki, Ralph
472 Schlapbach, Christian von Mering, and Julia a Vorholt. 2009. "Community Proteogenomics
473 Reveals Insights into the Physiology of Phyllosphere Bacteria." *Proceedings of the National*
474 *Academy of Sciences of the United States of America* 106 (38):16428–33.
475 <https://doi.org/10.1073/pnas.0905240106>.
- 476 Ding, Tao, and Ulrich Melcher. 2016. "Influences of Plant Species, Season and Location on Leaf
477 Endophytic Bacterial Communities of Non-Cultivated Plants." *PLoS ONE* 11 (3):1–13.
478 <https://doi.org/10.1371/journal.pone.0150895>.
- 479 Doty, Sharon L., Brian Oakley, Gang Xin, Jun Won Kang, Glenda Singleton, Zareen Khan, Azra
480 Vajzovic, and James T. Staley. 2009. "Diazotrophic Endophytes of Native Black Cottonwood

- 481 and Willow.” *Symbiosis* 47 (1):23–33. <https://doi.org/10.1007/BF03179967>.
- 482 Edgar, Robert. 2016. “SINTAX: A Simple Non-Bayesian Taxonomy Classifier for 16S and ITS
483 Sequences.” *BioRxiv*. <https://doi.org/10.1101/074161>.
- 484 Emerson, Rachel, Amber Hoover, Allison Ray, Jeffrey Lacey, Marnie Cortez, Courtney Payne,
485 Douglas Karlen, et al. 2014. “Drought Effects on Composition and Yield for Corn Stover,
486 Mixed Grasses, and Miscanthus as Bioenergy Feedstocks.” *Biofuels* 5 (3):275–91.
487 <https://doi.org/10.1080/17597269.2014.913904>.
- 488 Foley, Jonathan A., Navin Ramankutty, Kate A. Brauman, Emily S. Cassidy, James S. Gerber, Matt
489 Johnston, Nathaniel D. Mueller, et al. 2011. “Solutions for a Cultivated Planet.” *Nature* 478
490 (7369):337–42. <https://doi.org/10.1038/nature10452>.
- 491 Fürnkranz, Michael, Wolfgang Wanek, Andreas Richter, Guy Abell, Frank Rasche, and Angela
492 Sessitsch. 2008. “Nitrogen Fixation by Phyllosphere Bacteria Associated with Higher Plants
493 and Their Colonizing Epiphytes of a Tropical Lowland Rainforest of Costa Rica.” *The ISME*
494 *Journal* 2 (5):561–70. <https://doi.org/10.1038/ismej.2008.14>.
- 495 Galbally, I. E., and W. Kirstine. 2002. “The Production of Methanol by Flowering Plants and the
496 Global Cycle of Methanol.” *Journal of Atmospheric Chemistry* 43 (3):195–229.
497 <https://doi.org/10.1023/A:1020684815474>.
- 498 Hacquard, Stéphane, and Christopher W. Schadt. 2015. “Towards a Holistic Understanding of
499 the Beneficial Interactions across the *Populus* Microbiome.” *New Phytologist* 205 (4):1424–
500 30. <https://doi.org/10.1111/nph.13133>.
- 501 Hamilton, Cyd E., P. E. Gundel, M. Helander, and K. Saikkonen. 2012. “Endophytic Mediation of
502 Reactive Oxygen Species and Antioxidant Activity in Plants: A Review.” *Fungal Diversity* 54

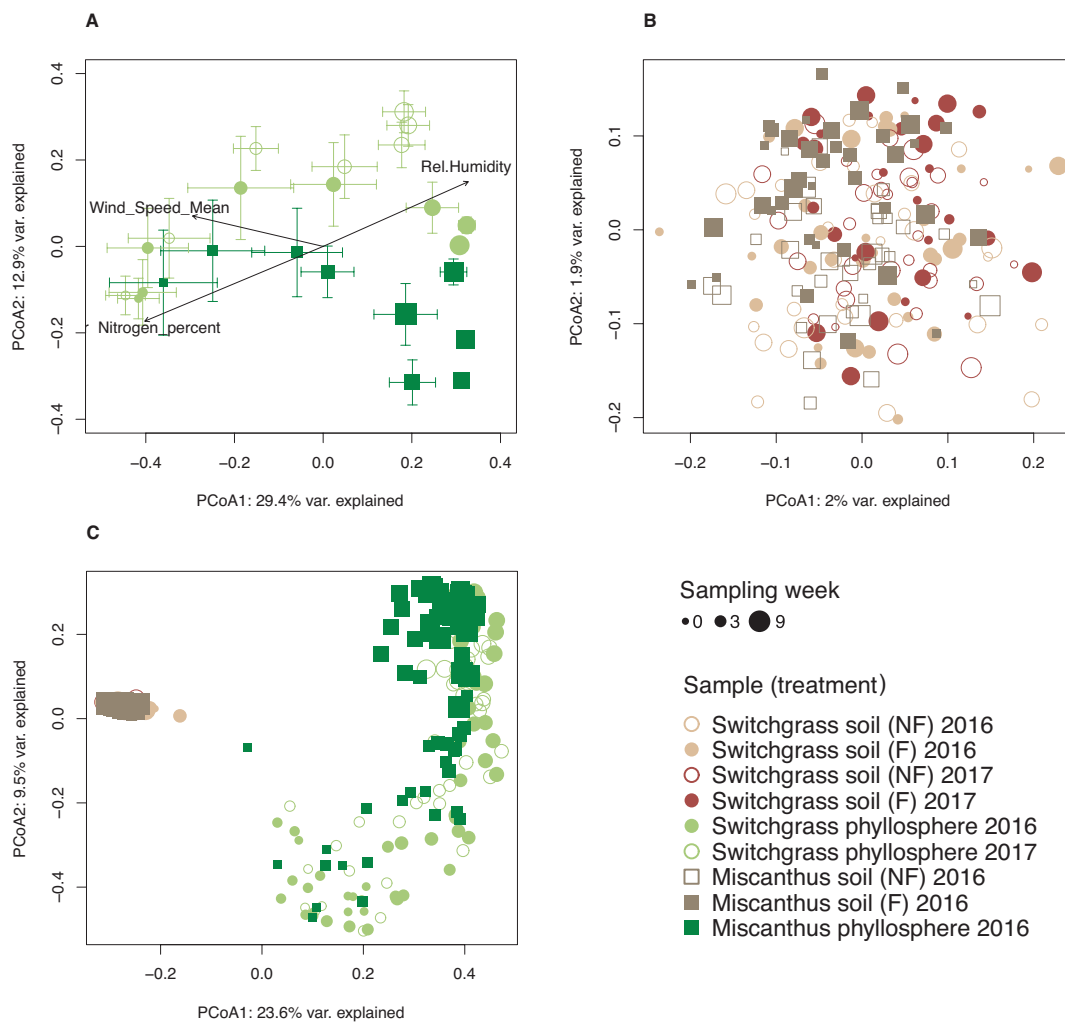
- 503 (1):1–10. <https://doi.org/10.1007/s13225-012-0158-9>.
- 504 Hamonts, Kelly, Pankaj Trivedi, Anshu Garg, Caroline Janitz, Jasmine Grinyer, Paul Holford,
505 Frederik C. Botha, Ian C. Anderson, and Brajesh K. Singh. 2018. “Field Study Reveals Core
506 Plant Microbiota and Relative Importance of Their Drivers.” *Environmental Microbiology*
507 20 (1). Wiley/Blackwell (10.1111):124–40. <https://doi.org/10.1111/1462-2920.14031>.
- 508 Heaton, Emily A., Frank G. Dohleman, and Stephen P. Long. 2008. “Meeting US Biofuel Goals
509 with Less Land: The Potential of Miscanthus.” *Global Change Biology* 14 (9):2000–2014.
510 <https://doi.org/10.1111/j.1365-2486.2008.01662.x>.
- 511 Iguchi, M, S Yamanaka, and A Budhiono. 2000. “Bacterial Cellulose—a Masterpiece of Nature’s
512 Arts.” *Journal of Materials Science* 35 (2):261–70.
- 513 Kinkel, L L. 1997. “Microbial Population Dynamics on Leaves.” *Annual Review of Phytopathology*
514 35 (January):327–47. <https://doi.org/10.1146/annurev.phyto.35.1.327>.
- 515 Knief, Claudia, Alban Ramette, Lisa Frances, Carlos Alonso-Blanco, and Julia A Vorholt. 2010.
516 “Site and Plant Species Are Important Determinants of the Methylobacterium Community
517 Composition in the Plant Phyllosphere.” *ISME J* 4:719–28.
518 <https://doi.org/10.1038/ismej.2010.9>.
- 519 Laforest-Lapointe, Isabelle, Christian Messier, and Steven W. Kembel. 2016. “Tree Phyllosphere
520 Bacterial Communities: Exploring the Magnitude of Intra- and Inter-Individual Variation
521 among Host Species.” *PeerJ* 4:e2367. <https://doi.org/10.7717/peerj.2367>.
- 522 Lebeis, Sarah L. 2014. “The Potential for Give and Take in Plant–microbiome Relationships.”
523 *Frontiers in Plant Science* 5 (June):1–6. <https://doi.org/10.3389/fpls.2014.00287>.
- 524 Lee, Dong Wan, Jin-Sung Hong, Sun-Hyung Kim, Jin Won Kim, and Beom Seok Kim. 2014. “First

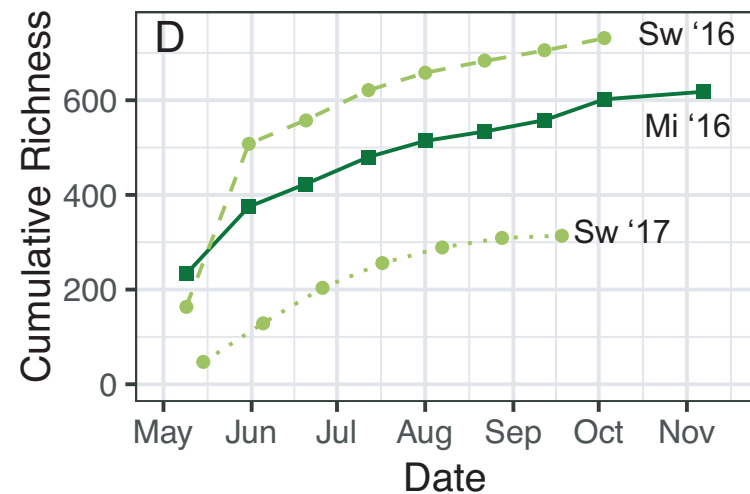
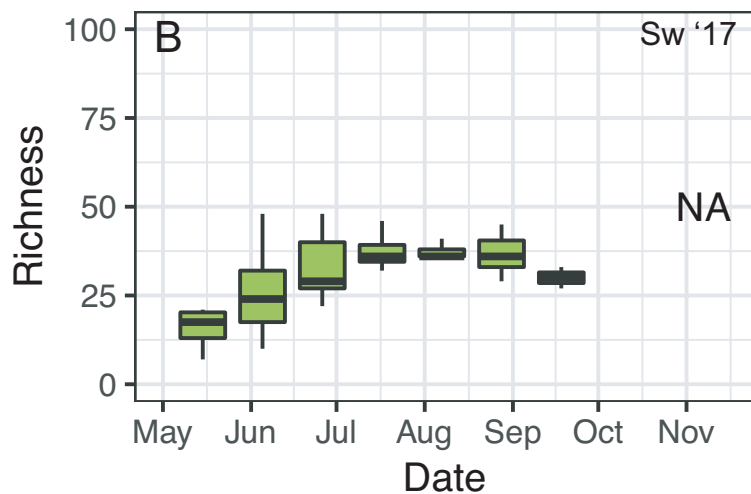
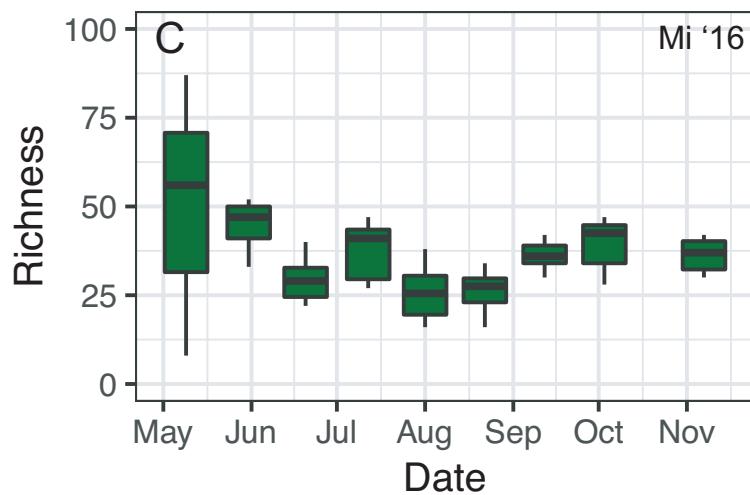
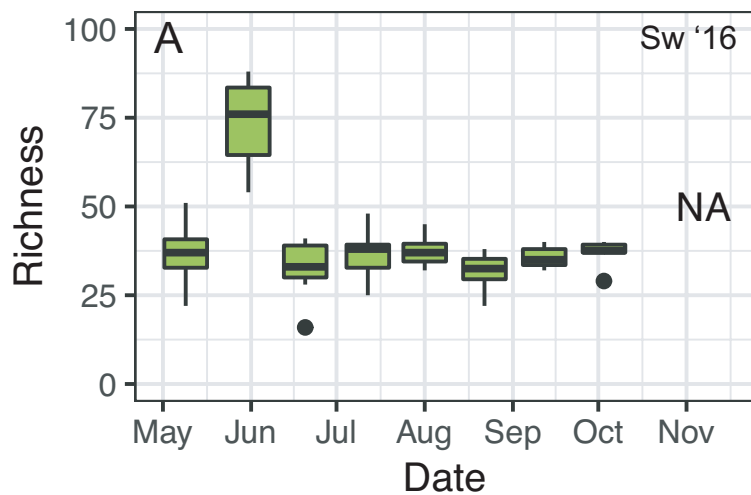
- 525 Report of *Pseudomonas Lurida* Causing Bacterial Leaf Spot on *Miscanthus Sinensis*.”
- 526 *Journal of Phytopathology* 162 (3):195–200. <https://doi.org/10.1111/jph.12176>.
- 527 Lindow, S.E., and M.T. Brandl. 2003. “Microbiology of the Phyllosphere.” *Applied and*
- 528 *Environmental Microbiology* 69 (4). Am Soc Microbiol:1875–83.
- 529 <https://doi.org/10.1128/AEM.69.4.1875>.
- 530 Lindow, Steven E, and Johan H J Leveau. 2002. “Phyllosphere Microbiology.” *Current Opinion in*
- 531 *Biotechnology* 13 (3). Elsevier:238–43.
- 532 Maignien, Loïs, Emelia A. DeForce, Meghan E. Chafee, A. Murat Eren, and Sheri L. Simmons.
- 533 2014. “Ecological Succession and Stochastic Variation in the Assembly of Arabidopsis
- 534 Thaliana Phyllosphere Communities.” *MBio*. <https://doi.org/10.1128/mBio.00682-13>.
- 535 Martiny, Jennifer B H, Stuart E. Jones, Jay T. Lennon, and Adam C. Martiny. 2015. “Microbiomes
- 536 in Light of Traits: A Phylogenetic Perspective.” *Science (New York, N.Y.)* 350
- 537 (6261):aac9323. <https://doi.org/10.1126/science.aac9323>.
- 538 Ong, Rebecca Garlock, Alan Higbee, Scott Bottoms, Quinn Dickinson, Dan Xie, Scott A. Smith,
- 539 Jose Serate, et al. 2016. “Inhibition of Microbial Biofuel Production in Drought-Stressed
- 540 Switchgrass Hydrolysate.” *Biotechnology for Biofuels* 9 (1). BioMed Central:1–14.
- 541 <https://doi.org/10.1186/s13068-016-0657-0>.
- 542 Ottesen, Andrea R., Sasha Gorham, Elizabeth Reed, Michael J. Newell, Padmini Ramachandran,
- 543 Travis Canida, Marc Allard, Peter Evans, Eric Brown, and James Robert White. 2016. “Using
- 544 a Control to Better Understand Phyllosphere Microbiota.” Edited by Marie-Joelle Virolle.
- 545 *PLOS ONE* 11 (9):e0163482. <https://doi.org/10.1371/journal.pone.0163482>.
- 546 Peñuelas, Josep, and Jaume Terradas. 2014. “The Foliar Microbiome.” *Trends in Plant Science*

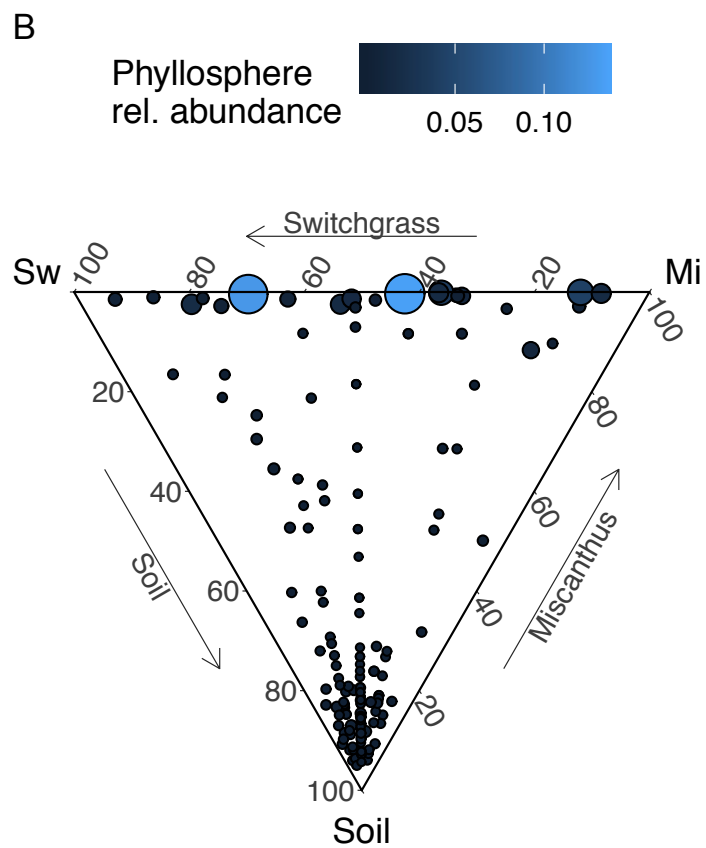
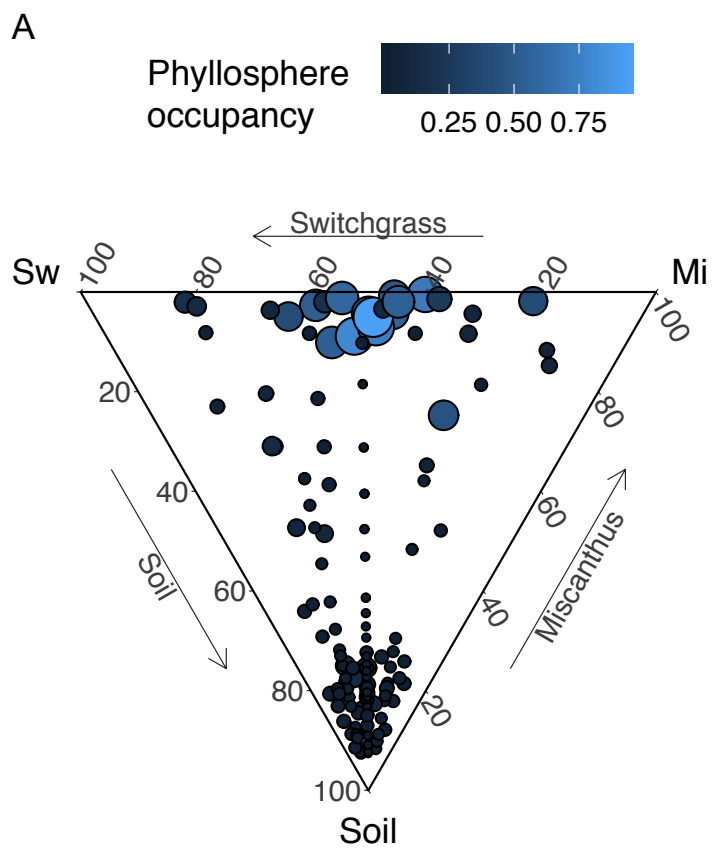
- 547 19 (5):278–80. <https://doi.org/10.1016/j.tplants.2013.12.007>.
- 548 Quast, Christian, Elmar Pruesse, Pelin Yilmaz, Jan Gerken, Timmy Schweer, Pablo Yarza, Jörg
549 Peplies, and Frank Oliver Glockner. 2013. “The SILVA Ribosomal RNA Gene Database
550 Project: Improved Data Processing and Web-Based Tools.” *Nucleic Acids Research* 41.
551 <https://doi.org/10.1093/nar/gks1219>.
- 552 Rastogi, Gurdeep, Gitta L. Coaker, and Johan H J Leveau. 2013. “New Insights into the Structure
553 and Function of Phyllosphere Microbiota through High-Throughput Molecular
554 Approaches.” *FEMS Microbiology Letters* 348 (1):1–10. [https://doi.org/10.1111/1574-
555 6968.12225](https://doi.org/10.1111/1574-6968.12225).
- 556 Redman, Regina S, Kathy B Sheehan, Richard G Stout, Russell J Rodriguez, and Joan M Henson.
557 2002. “Thermotolerance Generated by Plant/Fungal Symbiosis.” *Science (New York, N.Y.)*
558 298 (5598):1581. <https://doi.org/10.1126/science.1072191>.
- 559 Robertson, G. Philip, Stephen K. Hamilton, Bradford L. Barham, Bruce E. Dale, R. Cesar
560 Izaurralde, Randall D. Jackson, Douglas A. Landis, Scott M. Swinton, Kurt D. Thelen, and
561 James M. Tiedje. 2017. “Cellulosic Biofuel Contributions to a Sustainable Energy Future:
562 Choices and Outcomes.” *Science* 356 (6345). <https://doi.org/10.1126/science.aal2324>.
- 563 Sattler, Scott E., and Deanna L. Funnell-Harris. 2013. “Modifying Lignin to Improve Bioenergy
564 Feedstocks: Strengthening the Barrier against Pathogens?” *Frontiers in Plant Science* 4
565 (April):1–8. <https://doi.org/10.3389/fpls.2013.00070>.
- 566 Shade, A, RR Dunn, SA Blowes, P Keil, BJ Bohannon, M Herrmann, K Küsel, et al. 2018.
567 “Macroecology to Unite All Life, Large and Small.” *Trends in Ecology & Evolution*.
568 <https://doi.org/https://doi.org/10.1016/j.tree.2018.08.005>.

- 569 Stoof, Cathelijne R., Brian K. Richards, Peter B. Woodbury, Eric S. Fabio, Alice R. Brumbach, Jerry
570 Cherney, Srabani Das, et al. 2015. "Untapped Potential: Opportunities and Challenges for
571 Sustainable Bioenergy Production from Marginal Lands in the Northeast USA." *BioEnergy
572 Research* 8 (2):482–501. <https://doi.org/10.1007/s12155-014-9515-8>.
- 573 Suda, Wataru, Michiei Oto, Seigo Amachi, Hirofumi Shinoyama, and Masahiro Shishido. 2008.
574 "A Direct Method to Isolate DNA from Phyllosphere Microbial Communities without
575 Disrupting Leaf Tissues." *Microbes and Environments / JSME* 23 (3):248–52.
576 <https://doi.org/10.1264/jsme2.23.248>.
- 577 Taghavi, Safiyh, Craig Garafola, Sébastien Monchy, Lee Newman, Adam Hoffman, Nele Weyens,
578 Tanja Barac, Jaco Vangronsveld, and D. Daniel Van Der Lelie. 2009. "Genome Survey and
579 Characterization of Endophytic Bacteria Exhibiting a Beneficial Effect on Growth and
580 Development of Poplar Trees." *Applied and Environmental Microbiology* 75 (3):748–57.
581 <https://doi.org/10.1128/AEM.02239-08>.
- 582 Tornqvist, Carl Erik, Brieanne Vaillancourt, Jeongwoon Kim, C. Robin Buell, Shawn M. Kaeppler,
583 and Michael D. Casler. 2017. "Transcriptional Analysis of Flowering Time in Switchgrass."
584 *Bioenergy Research* 10 (3). *BioEnergy Research*:700–713. [https://doi.org/10.1007/s12155-
585 017-9832-9](https://doi.org/10.1007/s12155-017-9832-9).
- 586 Turner, Thomas R, Euan K James, and Philip S Poole. 2013. "The Plant Microbiome." *Genome
587 Biology* 14 (209):1–10. <https://doi.org/10.1016/B978-0-12-417163-3.00011-1>.
- 588 Vandenkoornhuysse, Philippe, Achim Quaiser, Marie Duhamel, Amandine Le Van, and Alexis
589 Dufresne. 2015. "The Importance of the Microbiome of the Plant Holobiont." *New
590 Phytologist* 206 (4):1196–1206. <https://doi.org/10.1111/nph.13312>.

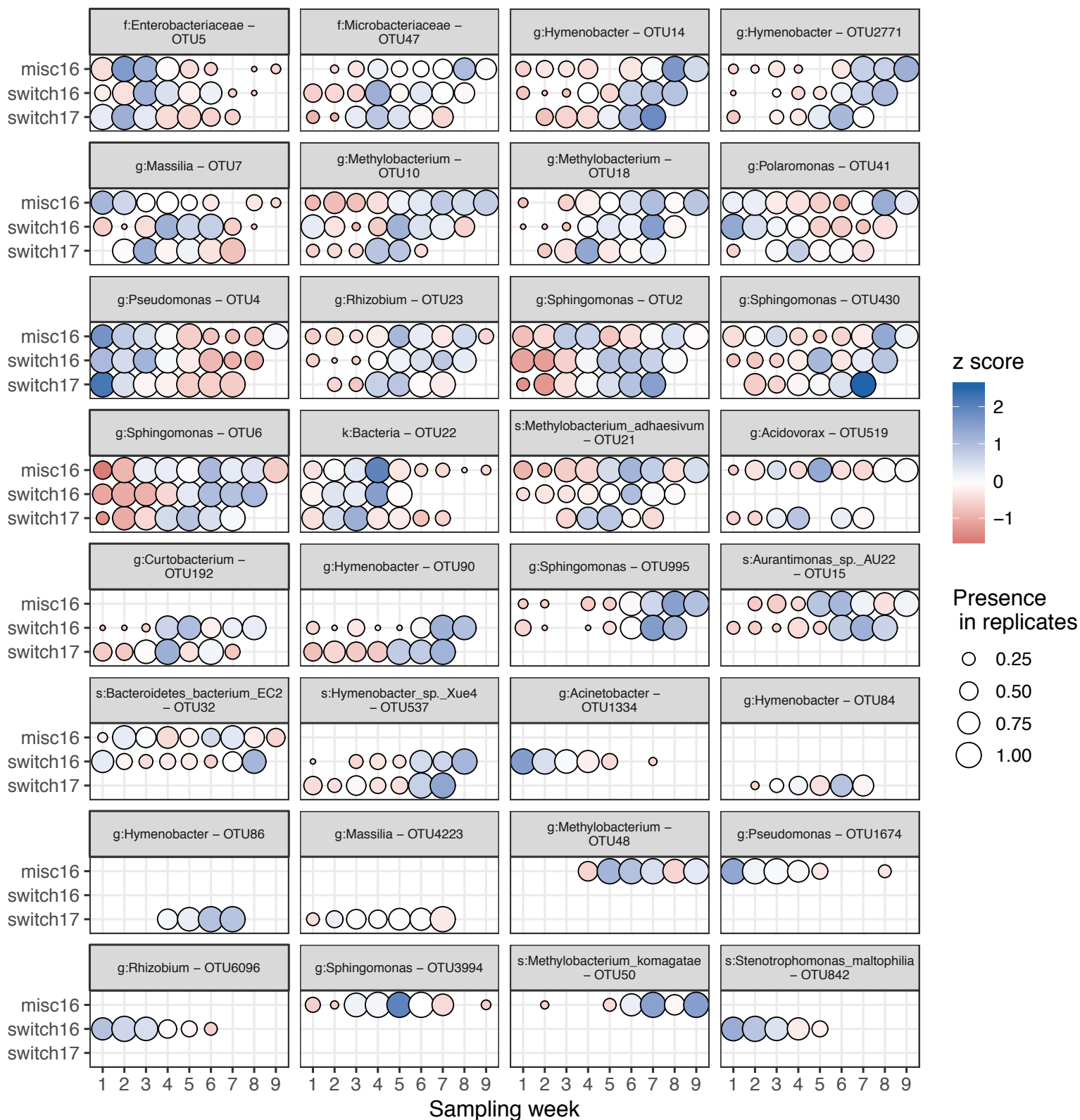
- 591 Vokou, Despoina, Katerina Vareli, Ekaterini Zarali, Katerina Karamanoli, Helen-Isis A.
592 Constantinidou, Nikolaos Monokrousos, John M. Halley, and Ioannis Sainis. 2012.
593 “Exploring Biodiversity in the Bacterial Community of the Mediterranean Phyllosphere and
594 Its Relationship with Airborne Bacteria.” *Microbial Ecology* 64 (3). Springer-Verlag:714–24.
595 <https://doi.org/10.1007/s00248-012-0053-7>.
- 596 Vorholt, Julia A. 2012. “Microbial Life in the Phyllosphere.” *Nature Reviews Microbiology* 10
597 (12):828–40. <https://doi.org/10.1038/nrmicro2910>.
- 598 Wagner, Maggie R., Derek S. Lundberg, Devin Coleman-Derr, Susannah G. Tringe, Jeffery L.
599 Dangl, and Thomas Mitchell-Olds. 2014. “Natural Soil Microbes Alter Flowering Phenology
600 and the Intensity of Selection on Flowering Time in a Wild Arabidopsis Relative.” *Ecology*
601 *Letters* 17 (6):717–26. <https://doi.org/10.1111/ele.12276>.
- 602 Wagner, Maggie R., Derek S Lundberg, Tijana G. Del Rio, Susannah G. Tringe, Jeffery L. Dangl,
603 and Thomas Mitchell-Olds. 2016. “Host Genotype and Age Shape the Leaf and Root
604 Microbiomes of a Wild Perennial Plant.” *Nature Communications* 7 (July):12151.
605 <https://doi.org/10.1038/ncomms12151>.
- 606 Weyens, Nele, Daniel van der Lelie, Safiyh Taghavi, Lee Newman, and Jaco Vangronsveld. 2009.
607 “Exploiting Plant-Microbe Partnerships to Improve Biomass Production and Remediation.”
608 *Trends in Biotechnology*. <https://doi.org/10.1016/j.tibtech.2009.07.006>.
- 609 Xia, L C, J A Steele, J A Cram, Z G Cardon, S L Simmons, J J Vallino, J A Fuhrman, and F Sun. 2011.
610 “Extended Local Similarity Analysis (ELSA) of Microbial Community and Other Time Series
611 Data with Replicates.” *BMC Systems Biology* 5 (Suppl 2):S15.
612



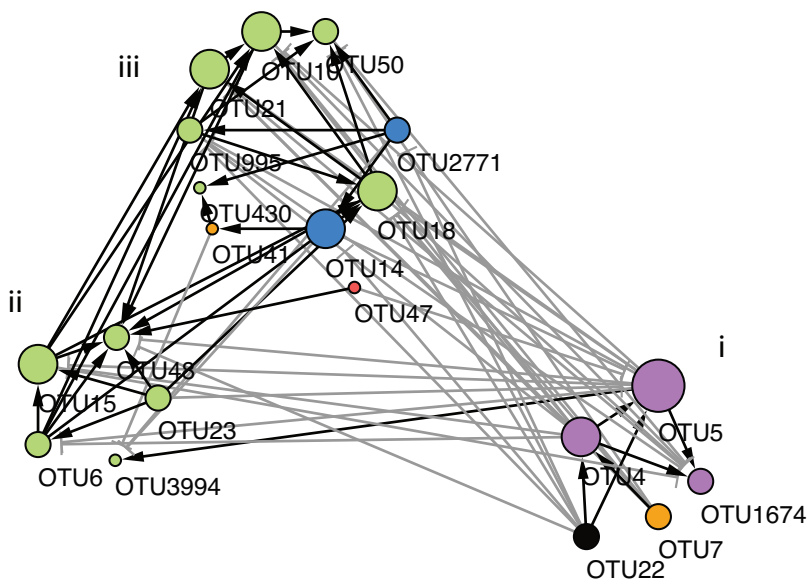




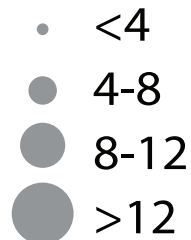
Core



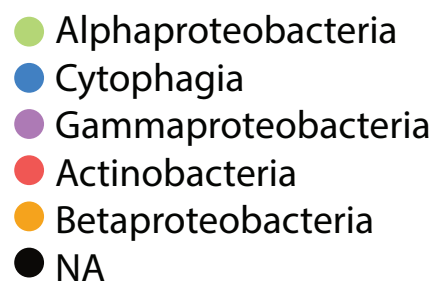
A



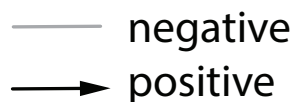
Connections



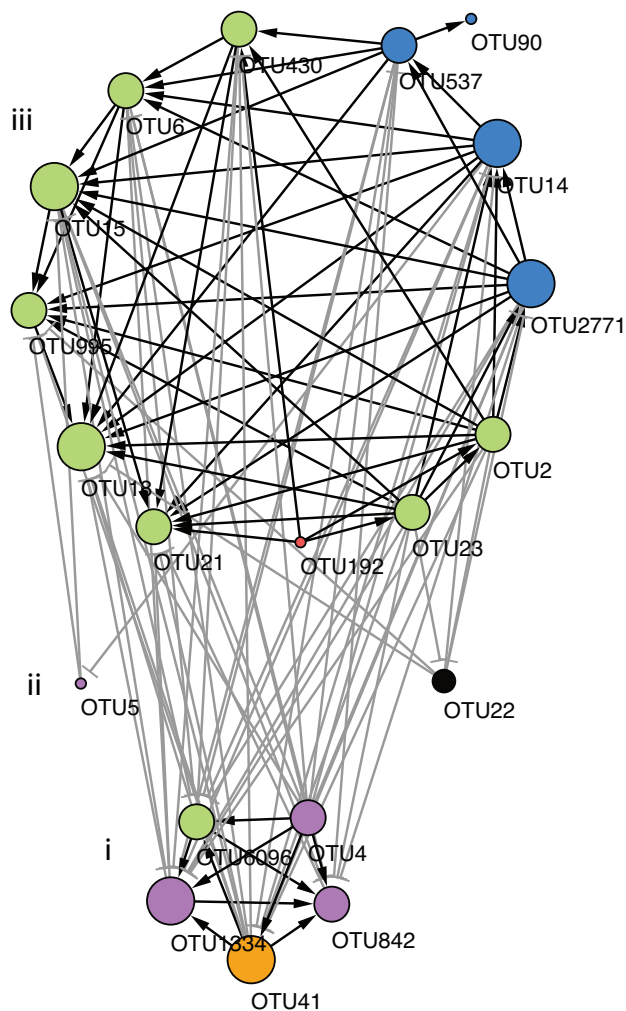
Phylogeny



Correlation



B



C

