MULTI-GENERATION SELECTION OF RHIZOSPHERE ECOSYSTEM ASSOCIATED WITH PLANT GENOTYPE AND BIOMASS IN *ARABIDOPSIS THALIANA*

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Abstract

The role of the microbiome in shaping the host phenotype has emerged as a critical area of investigation, with implications in ecology, evolution, and host health. The complex and dynamic interactions involving plants and their diverse rhizosphere microbial communities are influenced by a multitude of factors, including but not limited to soil type, environment, and plant genotype. Understanding the impact of these factors on microbial community assembly is key to yielding host-specific and robust benefits for plants, yet remains challenging. Here we ran an artificial ecosystem selection experiment, over eight generations, in *Arabidopsis thaliana* L*er* and Cvi to select soil microbiomes associated with higher or lower biomass of the host. This resulted in divergent microbial communities, shaped by a complex interplay between random environmental variations, plant genotypes, and biomass selection pressures. In the initial phases of the experiment, the genotype and the biomass selection treatment have modest but significant impacts. Over time, the plant genotype and biomass treatments gain more influence, explaining ~40% of the variation in the microbial community composition. Furthermore, a genotype-specific association of plant growth-promoting rhizobacterial taxa, *Labraceae* with L*er* and *Rhizobiaceae* with Cvi, is observed under selection for high biomass. This study enhances our understanding of

both microbial community dynamics and plant genetics and can be applied to agricultural settings to improve plant growth and soil restoration.

Introduction

The conventional understanding of the host phenotype involves genetics and environment shaping observable traits. Yet, the last two decades have underscored the microbiome's significance in shaping the host phenotype, driven by extensive research on its role in ecology, evolution, and host health. The plant microbiome represents a rich source of functional diversity that is not encoded within the host genome. The interactions between the plant host and its microbiome are dynamic and reciprocal, with the plant shaping its immediate environment by exuding specific metabolites, thereby promoting the growth of specific microbial taxa, while the microbiome in turn influences plant health and growth (1). A multitude of biotic and abiotic factors influence the dynamic nature of the microbiome, including but not limited to plant root exudates (2–4), soil type (5–9), environment (10), and various aspects of the plant, including species (11), genotype (4,12–15) and developmental stage (16,17).

Previous studies in *Arabidopsis thaliana* have shown that the microbial diversity of the soil reduced with proximity to the endophytic compartment, and microbes found in the bulk soil were not enriched in the endophytic compartment and vice-versa (18–20). Furthermore, the microbiome of greenhouse-grown plants was similar to that of the field-grown plants, in the different plant compartments – rhizosphere, woody stem, and endophytic compartment, indicating an active role of the plant host in creating and maintaining an environment where certain microbes have improved fitness (21). The soil environment was a stronger predictor of microbiome structure, though plant genotype had a weak influence in some studies (20,22,23). A follow-up study

47 confirmed that variation in microbiome communities depends more on the environment than on
48 different *Arabidopsis* varieties and sister species. Though there were some differences between
49 different host species, the core microbiome interacting with the plant host at the root endophytic
50 zone was largely consistent and reproducible (24).

51 Unfortunately, most prior studies were conducted in a single plant growth cycle, precluding
52 an analysis of the temporal stability of the plant-microbiome association. Observations from plant-
53 soil feedback studies highlight that plant-microbiome interactions can be altered by successive
54 growth cycles of a given plant in the same soil (25,26). Prior studies have employed plant-mediated
55 selection on the soil ecosystem over multiple generations, and resulted in consistent effects on
56 plant characteristics such as biomass, flowering time, and germination (27,28). By selecting for
57 soils where hosts show the desired phenotype, such as high biomass or altered flowering time, it
58 is possible to enrich microbes that modulate host traits. Additionally, studies showed the plant's
59 response to abiotic stress, such as drought and salt stress, could be influenced in two ways. First,
60 through generational selection, where plants with desirable stress responses were chosen over
61 multiple generations (29–31). Second, by introducing beneficial microbes that were associated
62 with plants and had previously lived in similar drought or salt conditions (32). Mueller et al., (29)
63 used generational selection to create microbial communities that could improve plant seed
64 production by up to 205%. Soil inoculum obtained from drought-exposed soils improved wheat
65 biomass under drought conditions (32). Older studies, such as Swenson et al. (28), also have shown
66 similar results under optimal growing conditions without necessarily exploring the microbiome. A
67 recent study conducted on the rhizosphere microbiome of wild and domesticated tomato plants
68 over multiple generations demonstrated an escalating influence of host genotype on the

69 microbiome community (33). A separate study on the phyllosphere microbiome of various tomato
70 genotypes indicated a declining impact of host genotype across successive generations (34).
71 Despite several earlier efforts, the development of a robust and host-specific microbial
72 community with long-lasting beneficial effects on the plant host remains a challenge.
73 Using an artificial ecosystem selection experiment, we sought to advance our understanding of
74 how plant genotype, environment, and biomass selection treatment (henceforth referred to as
75 biomass treatment) impact the assembly of host microbial communities. Treatments selecting for
76 the microbial communities of high and low biomass plants did affect the growth of plants in
77 subsequent generations, though unknown environmental factors had substantial effects throughout
78 the experiment. In the first few generations of the experiment, the genotype and biomass treatments
79 played a modest role in shaping the microbial community. Over time, the plant genotype and
80 biomass treatments had increasing influence, explaining ~40% of the difference in the microbial
81 community composition. Moreover, we observe an enrichment of known plant growth-promoting
82 rhizobacteria (PGPR) in the high biomass treatment, indicating potentially beneficial host-specific
83 interactions.

84 **Material and Methods**

85 **Multi-generation selection of soil ecosystem**

86 *Arabidopsis thaliana* Cvi and L*er* accessions were cultivated using custom-made
87 "rhizotubes," (Stuart Morey, Univ. of Massachusetts, Boston, unpublished). The tubes were
88 equipped with a black polyethylene sleeve, which effectively blocked light penetration. This
89 design also facilitated the convenient removal of the plant from the pot, granting full access to
90 the root system. (Supplementary Figure S2.1).
91 The potting soil used was PRO-MIX PGX, a commercial mixture comprising 80-90%
92 sphagnum peat moss and small quantities of perlite. It was autoclaved twice for 40 minutes with

93 a 48-hour interval between each sterilization. The potting soil was then sifted through a 3mm
94 sieve and combined with field soil in a 6:1 ratio respectively. The field soil obtained from the
95 Center for Agricultural Research in Waltham, Massachusetts, was composed of 44% sand, 49%
96 silt, and 7% clay representative of agricultural and grassland ecosystems.

97 The mixture was homogenized using a custom-made cement mixer and attached with
98 sterile bins to avoid cross-contamination. Distilled water was gradually added to achieve a final
99 ratio of 1:2 (water to soil). The soil was incubated at room temperature for two days following
100 inoculation before planting the seeds. All seeds used in the experiment were obtained from a
101 single parent plant. Before planting, the seeds were treated with a solution of 50% bleach vol/vol
102 and a drop of tween 20 for 10 minutes and rinsed ten times with sterile distilled water. We placed
103 3-4 seeds in the center of each pot, and kept only one seedling per pot after emergence. All plants
104 were grown at 22ºC day/ 18ºC night with a 12/12 hour day night cycle in a controlled growth
105 chamber. Relative humidity ranged from 35-60 and the light intensity 96uE. No fertilization was
106 applied to plants.

107 In the first generation of the experiment, 100 plants of each accession (Cvi and L*er*) were
108 grown separately in individual pots. The plants were arranged in a randomized block design to
109 avoid any batch effects. All plants were harvested 35 days after germination. The above-ground
110 portion of the plant, the rhizosphere, and the bulk soil from each rhizotube were separated,
111 ensuring there was no cross-contamination (see Supplementary Figure S2.1). The above-ground
112 part of the plant, stem, and leaves were dried at 70ºC for 4 days. All the plants were weighed
113 individually on a closed weighing scale accurate to 1 mg. The root-soil complex (comprising the
114 rhizosphere and the endosphere, henceforth referred to as the rhizosphere) was obtained by
115 shaking the excess soil off the root and placing the root and remaining attached soil in a sterile

116 5 ml tube. Bulk soil samples were moved to a sterile Ziplock bag. Tubes and bags were
117 immediately transferred to dry ice and then stored at -80ºC for DNA analysis.
118 The inoculation process for the next generation of soil involved using the bulk soil of the
119 top five and bottom five plants by weight, resulting in two different biomass treatments for each
120 genotype: high biomass L*er*, low biomass L*er*, high biomass Cvi, and low biomass Cvi. Starting
121 from the second generation of the experiment, 50 plants are grown on each of the four soil
122 inoculants to maintain a manageable experiment size. This process is repeated for eight
123 generations. There was substantial stochastic variation in above-ground plant biomass and during
124 generation 6 all plants died for unknown reasons. That experiment was therefore restarted using
125 soil from generation 5. Plants in uninoculated sterile potting soil control were grown from
126 generation 3 onwards to act as a reference for random environmental variation (REV).
127
128 **DNA extraction and 16S rRNA amplicon library prep**
129 Microbial DNA was isolated from the frozen rhizosphere samples using the Machery-
130 Nagel Nucleospin Soil DNA extraction kit (MACHEREY-NAGEL Inc., Allentown, PA, USA).
131 Approximately 0.1g of rhizosphere soil sample was used for DNA isolation. All samples were
132 diluted to 5 ng ul-1 with PCR-grade water. 16S rRNA gene was amplified from the isolated
133 DNA samples in triplicate in 96-well PCR plates. The PCR primers used were for the 16S rRNA
134 V4 region, 515F (5'-GTGYCAGCMGCCGCGGTAA-3') (ref. 35) and 806R (5'-
135 GGACTACNVGGGTWTCTAAT-3') (ref. 36) for downstream paired-end Illumina (Illumina,
136 Inc., San Diego, CA, USA) barcoded sequencing (37). The PCR cycling conditions were as
137 follows: 94ºC for 3 min; 25 cycles of 94ºC for 45 s, 50ºC for 60 s, 72ºC for 90 s, and final
138 elongation at 72ºC for 10 min. The triplicate amplified samples were pooled and then purified

139 and normalized using the SequalPrep™ Normalization Plate Kit (Invitrogen Corporation,
140 Carlsbad, Canada). Finally, multiplexed paired-end sequencing was carried out in the Illumina
141 MiSeq (SY-410-1003) platform using earth microbiome primers (38).
142
143 **Sequence Data Analysis & Statistics**
144 The paired-end sequences obtained from the Illumina MiSeq were demultiplexed with
145 QIIME 2 and converted into individual sequence fastq files for each sample (37,39). The rest of
146 the sequence processing was carried out in R (Version 4.2.2) using the DADA2 package
147 (Version 1.24.0) (ref. 40). The reads were processed in R using the following command in
148 DADA2; `filterAndTrim(fnFs, filtFs, fnRs, filtRs, truncLen=c(145,145), minLen=50, maxN=0,
149 maxEE=c(2,2), truncQ=2, rm.phix=TRUE, compress=TRUE, multithread=TRUE). `
150 *De novo* OTU (Operational Taxonomic Units) picking was performed using DADA2 (37) which
151 resolves amplicon sequence variants (ASVs) to single nucleotide differences. Chimeras were
152 removed. The taxonomy was assigned using a 100% cut-off for species-level identification with
153 the (2022) GTDB 16S rRNA reference database (41). The phylogenetic tree was constructed
154 using IQTree (42), with 1000 ultrafast bootstraps and Modelfinder to identify the best model. In
155 this study, the best-fit model was SYM+R10 chosen according to BIC. The data comprising the
156 OTU table, phylogenetic tree, taxonomy table, and sample meta-data were then parsed through R
157 studio using the phyloseq package (43). Here, it was processed to remove: OTUs identified as
158 mitochondrial or chloroplast, which had less than five reads across all samples, and samples that
159 had fewer than 8000 reads. The packages phytools, phyloseq, microbiome, and ggplot2 were
160 used for further data analysis.
161

Diversity Analyses

Alpha diversity was computed using four metrics, Observed, Shannon, Chao1, and Simpson diversity indices. Beta diversity was calculated using weighted and unweighted UniFrac, and subsequently Principle Coordinate Analysis (PCoA) plots were constructed using the first two axes that explain the most variance. Linear mixed effect models using, *lmerTest* package in R were used to determine changes in alpha and beta diversity over time (lmer (DiveristyMetric, Genotype*Generation*Biomass Treatment, (1|Generation)), with generation as a fixed and random effect. Pairwise comparisons were determined with *emmeans* and adjusted p-value obtained with Tukey.

Adonis

PERMANOVA was carried out for each generation using the adonis2 test. Both weighted and unweighted UniFrac distance matrices were used to account for the abundance, presence/absence, and phylogeny of the microbiome. The following model was used `adonis2(distance.matrix~Biomass Treatment+Genotype, data = meta, permutations=999)`.

Neutral Model

To determine the impact of neutral processes like drift and dispersal or by deterministic selective forces such as plant genotype and biomass treatments on microbial community assembly, we carried out the analysis as described in Burns et al. (44), which fits the data to the neutral model for Prokaryotes from Sloan et al. (45). The following design was used, sncm.fit (spp=generation(n)_Biomass Treatment, pool = generation(n), stats=T), where n is the nth generation and ‘Biomass Treatment’ is high or low biomass samples.

185

186 *Differential Abundance*

187 There are several challenges in estimating differentially abundant (DA) taxa in
188 microbiome data, these include high variability in abundance, zero-inflated data, and the
189 compositional nature of the data. Nearing et al. (46) compared several methods across 38
190 datasets and found that different methods often identified different sets of DA taxa. The DA taxa
191 are estimated using ANCOMBC2 (47), a conservative and robust approach. ANCOMBC2
192 incorporates bias correction, effectively addressing sampling-specific and sequencing biases
193 present in the data. This feature ensures that the analysis is not skewed by any systematic errors
194 introduced during the sampling or sequencing process. It also conducts a sensitivity analysis for
195 the pseudo-count addition to assess the impact of different pseudo-count values on zero counts
196 for each taxon.The analysis was run on L*er* and Cvi samples, from OTU to Family taxonomic
197 levels with (fixed effect = (Biomass Treatment + Genotype + Generation), group = Biomass
198 Treatment).

199

200 **Results**

201

202 **Plant Biomass**

203 The ecosystem selection experiment was carried out for the trait plant biomass on two
204 different accessions of *Arabidopsis thaliana,* L*er* and Cvi for eight generations. The two selected
205 treatments were high and low above-ground biomass. The absolute values for the mean biomass
206 of plants (n=50) from each genotype and treatment changed substantially through the course of
207 eight generations of the experiment. Despite the stochastic fluctuations in biomass caused by

208 REV, the high biomass selected lines were always the same or greater than those of the low
209 selected biomass for both genotypes (Figures 2.1a and 2.1b). Due to the significant drop in
210 biomass between generations 1 and 2, a sterile potting soil uninoculated control was planted in
211 all subsequent generations to serve as a reference for REV. The uninoculated control does show
212 that the drastic drop in biomass after generations 1 and 2, was not a result of the inoculated soil
213 but other largely unknown factors and growing conditions. Above-ground biomass in terms of
214 deviations from the mean removes some of these REVs and gives a clearer representation of the
215 difference in phenotype seen in every generation (Figures 2.2a and 2.2b). Significant differences
216 between the low and high biomass treatments become apparent from generation 4 onwards.
217
218 **Microbial community Composition**
219 After preprocessing in DADA 2, a total of 1,897,732 reads, with an average of 12822
220 reads per sample were obtained. Singletons and chimeras were removed during pre-processing.
221 The most well-represented phyla were *Proteobacteria* (36.2%), *Bacteroidetes* (10.3%),
222 *Planctomycetes* (7.8%), and *Actinobacteria* (7.6%) (Figure 3). The most well-represented
223 Classes identified in the dataset were, *Alphaproteobacteria* (24.7%), *Gammaproteobacteria*
224 (11.4%), *Bacteroidia (10.1%)*, and *Verrucomicrobiae* (6.2%) (Figure 4). The relative abundance
225 is typical of microbiome data, heavily weighted to a few abundant groups with a long tail of rare
226 taxa.
227
228 **Alpha Diversity**
229 We used four metrics to assess alpha diversity (Figure 5). Observed ($F_{7,160}$ =333.054,
230 $P<0.001$) and Chao1 ($F_{7,160}$ =195.54, $P<0.001$) estimate species richness, and both showed a

231 statistically significant decrease in OTUs (1477 to ~580) and richness across generations. The
232 Shannon diversity index ($F_{7,160}$ =82.23, P<0.001), which is more sensitive to the difference in
233 abundance, also showed this steady decrease across generations. The Simpson diversity ($F_{7,160}$
234 =14.46, P<0.001), which is more sensitive to evenness, exhibits fluctuations across successive
235 generations, albeit without any distinct trend. In addition, a linear mixed-effect model shows
236 significant interactions between genotype:biomass treatment, genotype:generation and
237 genotype:biomass treatment:generation, for both Shannon and Simpson diversity indices
238 (Supplementary Tables 2.7 and 2.8). No significant interaction terms were found for Observed
239 and Chao1, which do not consider the abundance of OTUs in their calculations.
240
241 **Beta Diversity**
242 Principal coordinate analyses (PCoA) of the unweighted UniFrac distances were plotted
243 with the first two axes that captured the most variance in the data, from 13.4% in generation 1 to
244 greater than 52.3% in generation 8 (Figure 6). UniFrac distance considers the evolutionary
245 relationships between taxa, which makes it more biologically meaningful than other distance
246 metrics that do not consider the evolutionary history. Unweighted UniFrac considers
247 presence/absence of taxa. The microbiomes associated with the treatments and genotypes are
248 very similar in generation 1, but begin to diverge by generation 2. By generation 3, the
249 microbiomes of the L*er* and Cvi genotypes have diverged and by generation 5 the high and low
250 biomass treatments appear to have further distinguished the microbial communities. The
251 divergence of the microbial community in generations 4 and 5 caused by the high/low biomass
252 treatments also aligns with the onset of notable differences in the above-ground plant biomass
253 within these generations.

254 The complex interplay between genotype and biomass treatments was modeled using a
255 PERMANOVA test with both weighted and unweighted UniFrac distances to account for
256 differences in abundance and presence-absence of OTUs respectively. The resulting R^2 values
257 for genotype and biomass treatment and residuals as a proxy for REVs were plotted (Figure 7a
258 and 7b). For both weighted and unweighted UniFrac, the variance in microbial community
259 explained by both genotype and biomass treatment increased significantly over the eight
260 generations. Despite observing a decrease in the variance accounted for by the residuals, which
261 could potentially signify a decline in the influence of REVs, they accounted for ~50 percent of
262 the variability within the microbial community in generation 8. For all metrics, weighted
263 UniFrac exhibits more fluctuations compared to the unweighted UniFrac. Weighted UniFrac
264 analyses found that genotype ($F_{1,39}$=1.55, P<0.05) and biomass treatment ($F_{1,39}$=1.33, P<0.001)
265 explained 5.7% and 13.7%, respectively, of the dissimilarity between microbiomes in generation
266 1. By generation 8, genotype ($F_{1,14}$=5.14, P<0.05) and biomass treatment ($F_{1,14}$=5.48, P<0.001)
267 explain 24% and 22%, respectively, of the dissimilarity between microbiomes (Supplementary
268 Tables 2.9 to 2.10). For unweighted UniFrac, the influence of genotype on the microbial
269 community increased from 3.3% in generation 1 ($F_{1,39}$=1.33, P<0.01) to 26% in generation 8
270 ($F_{1,39}$=5.13, P<0.001), and for biomass increased from 3.8% in generation 1 ($F_{1,14}$=1.55 ,P<.001)
271 to 13% in generation 8 ($F_{1,14}$=2.58, P<0.05) (Supplementary Tables 2.11 and 2.12).
272
273 **Sloan Neutral Model**
274 Many forces can alter microbial community structure and dynamics and in our study
275 these can be divided into selective forces such as the plant genotype being colonized and the
276 biomass selection process, or neutral, stochastic forces innate in any experiment and

277 environment. The Sloan neutral model was fit to the data to assess the importance of selective
278 versus neutral drivers of change in the experiment. If neutral processes were the driving force in
279 the microbial community assembly, the null hypothesis would be that all generations of the
280 experiment would fit the model equally well. The model fit to neutrality represented by the R^2
281 value decreases from 0.795 in generation 1 to 0.58 in generation 8 demonstrating the increasing
282 importance of selective drivers through the course of the experiment (Figure 8).
283
284 **Differential Abundance**
285 Differentially abundant (DA) taxa that distinguished between the high and low biomass
286 treatments were determined using ANCOMBC2 at multiple taxonomic levels, ranging from the
287 OTU to the Class. Taxa lacking classification at the Family level were denoted by the subsequent
288 identifiable taxonomic tier. The OTUs that were present in higher (red) and lower (blue)
289 abundance in the high biomass treatment are identified (Figure 9). Among the DA taxa in this
290 study, several are known to benefit plants. The Class *Bacilli* promote plant growth (48). The
291 Class *Gemmatimonadetes* are positively associated with vegetation restoration, plant richness,
292 and soil nutrients (49). *Cytophagacea* are chemoorganotrophs and important for remineralizing
293 organic materials into micronutrients. They could support both mycelial growth and plant
294 nutrition (50).
295 *Enterobacteriaceae*, *Paenibacillaceae*, and *JACDCH01* were all present in higher
296 abundance in the high biomass treatments of both L*er* and Cvi. Most *Paenibacillaceae* members
297 predominantly inhabited soil, frequently in close association with plant roots (51). These
298 rhizobacteria were known to play a significant role in enhancing plant growth and possessing

potential applications in agriculture and *Enterobacteriaceae* had been reported to enhance plant growth (52–54).

Additionally, *Order_NS11-12g*, *BJHT01*, *JACDCH01*, and *UBA6156* were more abundant in Cvi under high biomass conditions and less abundant in L*er* under high biomass conditions. The majority of these were uncultivated or candidate taxa. Two alternative families in the Order *Rhizobiales* appeared differentially associated with the two plant genotypes: *Labraceae* with Ler and *Rhizobiaceae* with Cvi (55). These could indicate genotype-specific interactions with different members of the microbial community.

309 **Discussion**

310 We applied artificial ecosystem selection for eight generations, in *Arabidopsis thaliana*
311 L*er* and Cvi to select soil microbiomes associated with higher or lower biomass of the host. In
312 contrast to some previous studies (27,33), we did not fertilize plants thus maintaining nutrient
313 limitation, and promoting the plant's interaction with the microbiome (56). Over the course of
314 eight generations, a response to the selection, most apparent after generations 4 and 5, was
315 observed both in the microbiome characteristics that shifted gradually and in the plant biomass.
316 Microbiome selection noticeably influenced plant biomass despite large phenotypic variation
317 from one generation to the next. Stochasticity in growth through generations may be attributed to
318 random environmental variations (REVs) as indicated by Swenson et al. (28). This was observed
319 in the uninoculated sterile potting soil reference that showed similar patterns of variability as the
320 inoculated treatments across generations (Figure 1). Ecological variability among generations
321 even under controlled growth chamber conditions is a common characteristic of studies (57) and
322 may be due to minute fluctuations in growth chamber conditions or batch and age of potting soil.

323 We observed a gradual decline in microbial species richness during the initial generations
324 of the experiment. This was evident from the observed OTU counts, as well as the Chao1 and
325 Shannon diversity indices. This pattern is consistent with findings from other studies (32–34),
326 highlighting the impact of selection pressures as the microbial communities adapted to the host
327 plant environment. However, in our study which continued for about twice the number of
328 generations of these earlier studies, we observed a stabilization of richness and alpha diversity in
329 the later generations.

330 To further understand the complex interplay between the forces of directed selection (plant
331 genotype and biomass treatment), we did a PCoA. Over successive generations, the results

332 demonstrate a strengthening effect of plant genotypes and biomass treatment on the microbial
333 community (Figure 6). This result was modeled using PERMANOVA , for both weighted and
334 unweighted UniFrac distances. Both weighted and unweighted UniFrac show marked increases in
335 the proportion of variance explained by genotype and biomass treatment, with unweighted UniFrac
336 exhibiting larger increases from generation to generation (Figure 7). Both metrics consider
337 phylogenetic relatedness. Weighted UniFrac considers the absolute abundance of OTUs and is
338 generally used to study changes in microbial community structure. Unweighted UniFrac considers
339 only the presence/absence of OTUs and is generally used to study changes in microbial community
340 composition. The results suggest that variance in microbiome composition as explained by
341 genotype consistently increases, whereas variance in microbiome structure as explained by
342 genotype, fluctuates more with changes in abundant taxa.

343 Here we would like to highlight that pronounced shifts in biomass between the high/low
344 biomass treatments, alpha diversity, and beta diversity all occur around generations 4 and 5 (Figure
345 1). This marks the point at which the changes in alpha diversity stabilize (Figure 5) and the
346 divergence between microbial communities by high/low biomass treatments in the PCoA plots
347 becomes more pronounced (Figure 6). It is possible that over the first four to five generations, the
348 starting microbial community underwent a period of restructuring before it stabilized and formed
349 four distinct communities under selection by high/low biomass treatments and by plant genotype.
350 We propose a complex interaction between plants and their associated microbiome ensued, where
351 differences in root exudation patterns between the two genotypes presumably established
352 associations with microbes in the early generations. Simultaneously, during this period, the
353 biomass treatment likely promoted host-specific microbe-mediated interactions that modulate
354 plant biomass. This restructuring of the microbial community is driven by both structural

355 (abundance of OTUs) and compositional (presence/absence of OTUs) effects in the microbiome
356 (Figure 7). Differences in the abundance of taxa driven by the biomass and genotype selection
357 pressures, was a larger contributor to the generation to generation variation during the experiment.
358 Changes in the assembly of microbial communities can arise from either selective pressure,
359 like the plant genotype or biomass treatment observed in this experiment, or stochastic processes,
360 like minute changes in growth chamber humidity or microenvironment. To gain deeper insights
361 into the influence of selection on microbial community assembly, a neutral model was fit to the
362 data (44,45). Interestingly, this revealed a progressive decline in fit to neutrality, indicated by
363 decreasing R^2 values and increasing AIC values (Figure 8). This suggests an increasing influence
364 of selective forces such as biomass treatment and genotype over multiple generations of ecosystem
365 selection. This was also previously observed by Morella et al. (34).
366 Previous studies in *Arabidopsis thaliana* have often detected only a weak genotypic effect
367 on the rhizosphere microbiome (23,24,58). However, a majority of these studies are focused on a
368 single growth cycle. Insights from plant-soil feedback research emphasize that the interactions
369 between plants and their microbiomes can be modified by consecutive growth cycles of the same
370 plant species in its respective soil (25,26). Recent research has presented conflicting findings on
371 the influence of host genotype (33,34,59). The different experimental designs, communities being
372 assessed and the length of these experiments, alongside high stochastic environmental variation
373 that is clearly associated with these microbial studies likely account for the conflicting findings.
374 In our investigation, we find that during the initial stages, genotype and biomass treatments have
375 modest but significant impacts. Over time, the plant genotype and biomass treatments have an
376 increasing influence. Together these selective forces explained ~40% of the variation in the
377 microbial community composition in these later generations.

378 A key aim of microbiome engineering has been to develop host-specific microbial
379 communities that impart lasting beneficial effects on the plant host (27,29,32,60). Here we show
380 the enrichment of some common taxa in high biomass treatments but also some genotype specific
381 changes. Despite starting with the same soil, only three common families were enriched in the
382 high biomass treatment samples of both the L*er* and Cvi genotypes. In addition, well-known plant
383 growth promoting rhizobacteria were enriched in both L*er* and Cvi high biomass treatment
384 samples, however possible genotype-specific interactions are involved. In the Order *Rhizobiales*,
385 Labraceae was enriched in L*er,* while *Rhizobiaceae* was enriched in Cvi (55). Moreover, in high
386 biomass conditions, Cvi had increased levels of the taxa *Order_NS11-12g*, *BJHT01*, *JACDCH01*,
387 and *UBA6156*, while L*er* had decreased levels of these. These data suggest genotype specific plant
388 genetic control. Multiple studies have shown variations in the structure of the rhizosphere
389 microbiome, even within closely related plant genotypes, highlighting the importance of genotype-
390 specific root exudates in forming associations with the corresponding microbiome (61–65).
391 Further exploration must prioritize highlighting the mechanisms that uphold these interactions
392 through the utilization of comparative metagenome and metatranscriptome analyses. This will play
393 a part in decoding chemical crosstalk, which could eventually foster interactions between plants
394 and microbes, leading to an overall enhancement in plant fitness.

395 The relationship between plants and microorganisms in the rhizosphere involves complex
396 and diverse interactions, which influence crucial ecological and physiological processes. These
397 interactions can be mutualistic, competitive, or antagonistic in nature. Many biotic and abiotic
398 factors act in concert to influence the dynamic rhizosphere microbiome. Gaining insights into how
399 these factors influence the assembly of microbial communities is crucial for obtaining targeted and
400 long-lasting advantages for plants. Nevertheless, establishing a strong and host-specific microbial

401 community that consistently provides beneficial effects continues to be a persistent challenge. We
402 have shown that, despite stochastic fluctuation due to REVs, it is possible to select for microbial
403 communities that impact biomass in a host-specific manner within four generations, addressing
404 this key challenge in microbial community engineering (32,60). The rhizosphere microbiome that
405 evolves under plant-mediated selection offers better chances of survivability and efficacy when
406 applied as inoculum to the plant (66,67). This study enhances our understanding of the temporal
407 dynamics involved in microbial assembly in the rhizosphere and has implications for sustainable
408 agriculture, evolution, and ecology.
409

FIGURES

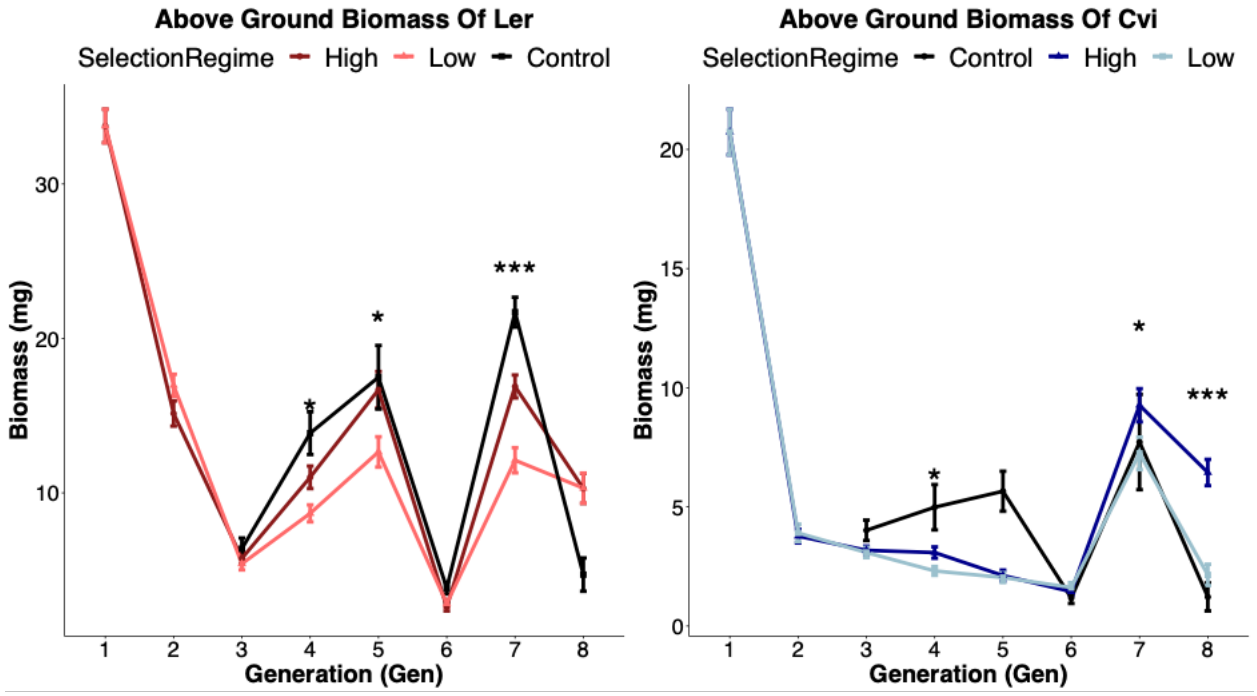

Figure 1. Results of the ecosystem selection on above-ground plant biomass for genotypes L*er* (a) and Cvi (b) of Arabidopsis thaliana. Dark lines represent the high biomass treatment, and light lines represent the low biomass treatment. Each point represents the mean of n=50 microcosms. The mean biomass of the selection lines differed for several generations of both genotypes (t.test p-value * < 0.05; **<0.01;*** <0.001).

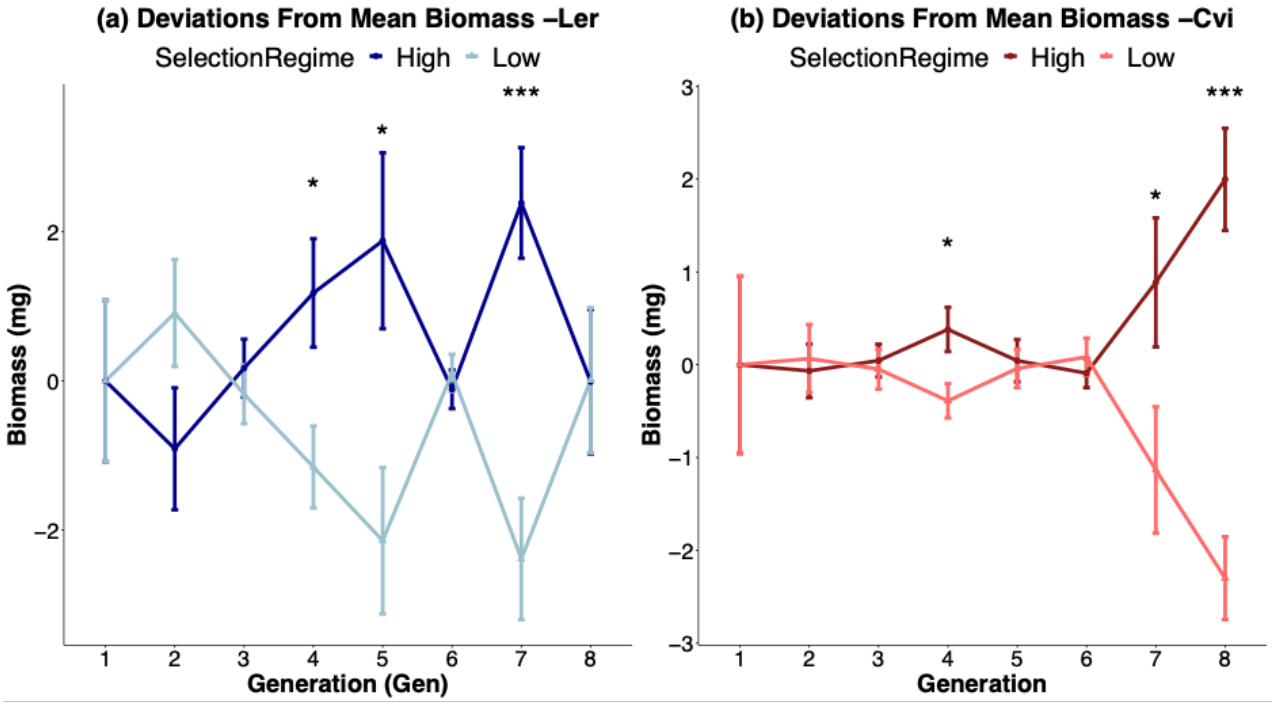

Figure 2 By plotting deviations from the mean, the influence of REVs on above-ground biomass is mitigated for genotypes L*er* (a) and Cvi (b). The dark lines denote high biomass treatments, while the light lines represent low biomass treatments. Each data point is the average of n=50 microcosms. (t.test p-value * < 0.05;**<0.01; *** <0.001).

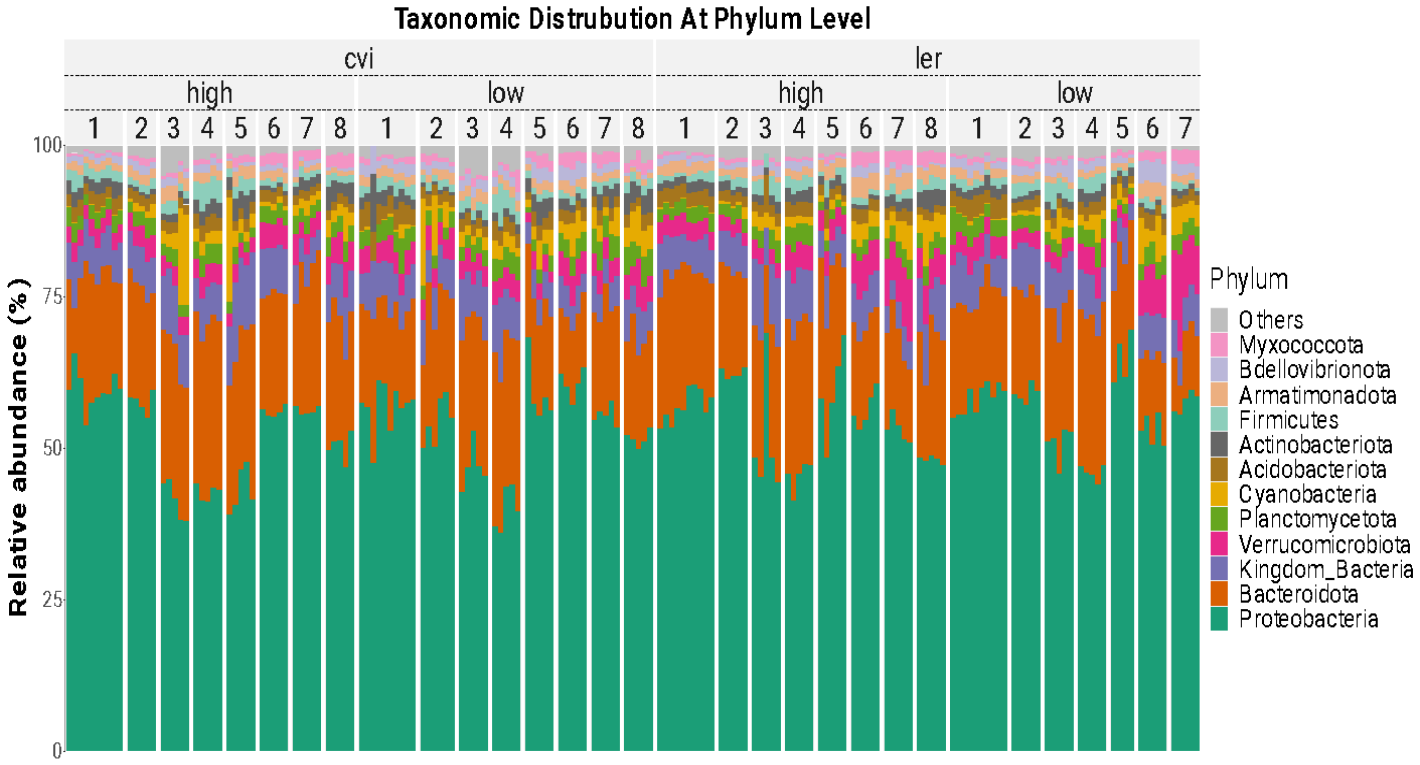

Figure 3. Taxonomic distribution of the microbial community represented in terms of the relative abundance at the Phylum level. The plot shows eight generations (1-8) for the high and low biomass treatments of both the Cvi (left) and L*er* (right) genotypes of *Arabidopsis thaliana*. The microbial community is dominated by *Proteobacteria*, *Bacteroidetes*, *Verrucomicrobia, and Planctomycetes* which together comprise nearly 73% of the bacterial community.

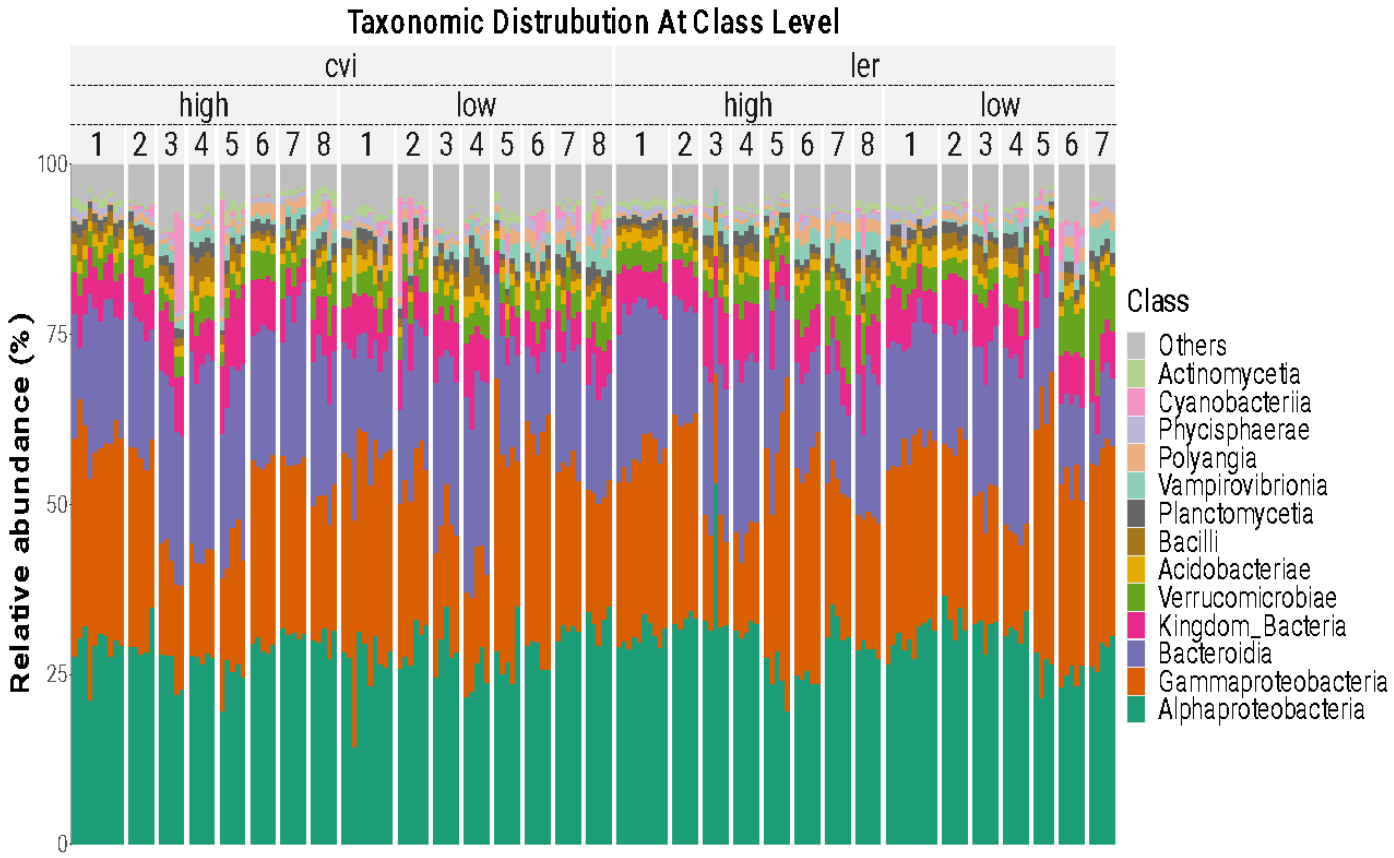

Figure 4. Taxonomic distribution of the microbial community is represented in terms of the relative abundance at the Class level. The plot shows eight generations (1-8) for the high and low biomass treatments of both the Cvi (left) and L*er* (right) genotypes of *Arabidopsis thaliana*. Ten samples were assayed for generation 1 experiments while five were done for all subsequent generations. The most represented classes across all samples were *Alphaproteobacteria* (24.7%), *Gammaproteobacteria* (11.4%), *Bacteroidia* (10.1%), and *Verrucomicrobiae* (6.2%).

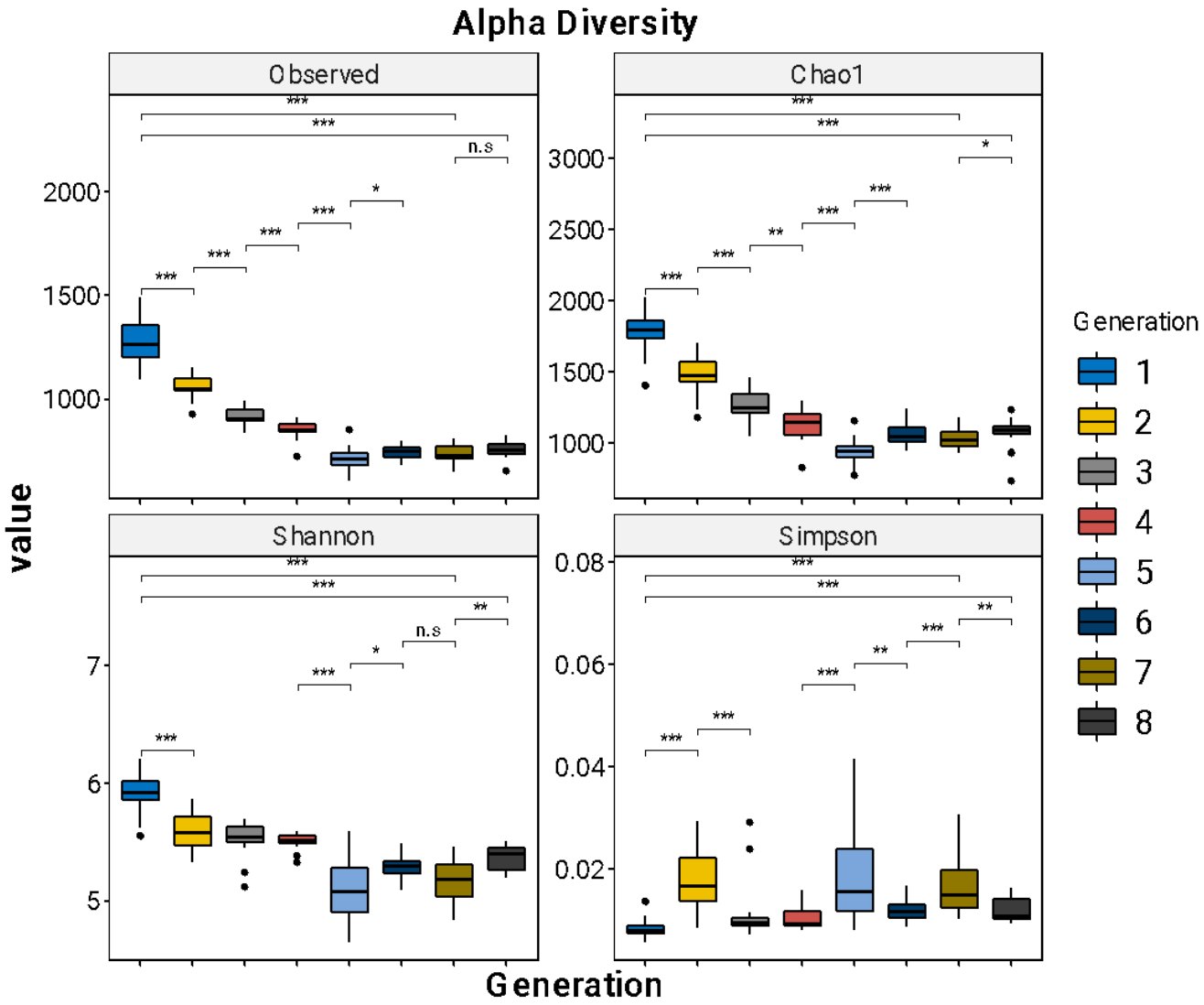

Figure 5. Over the course of eight generations, four Alpha diversity indices were computed, including Observed, Chao1 estimate, Shannon, and Simpson indices. Results indicate a significant decline in both Observed and Chao1 estimate species richness. Additionally, the Shannon index was more responsive to species richness and exhibited a noteworthy decrease from generation 1 to 8. In contrast, the Simpson index was more sensitive to evenness and demonstrated fluctuations across successive generations without any distinct trend. (wilcox test *<0.05, **<0.01, *** <0.001).

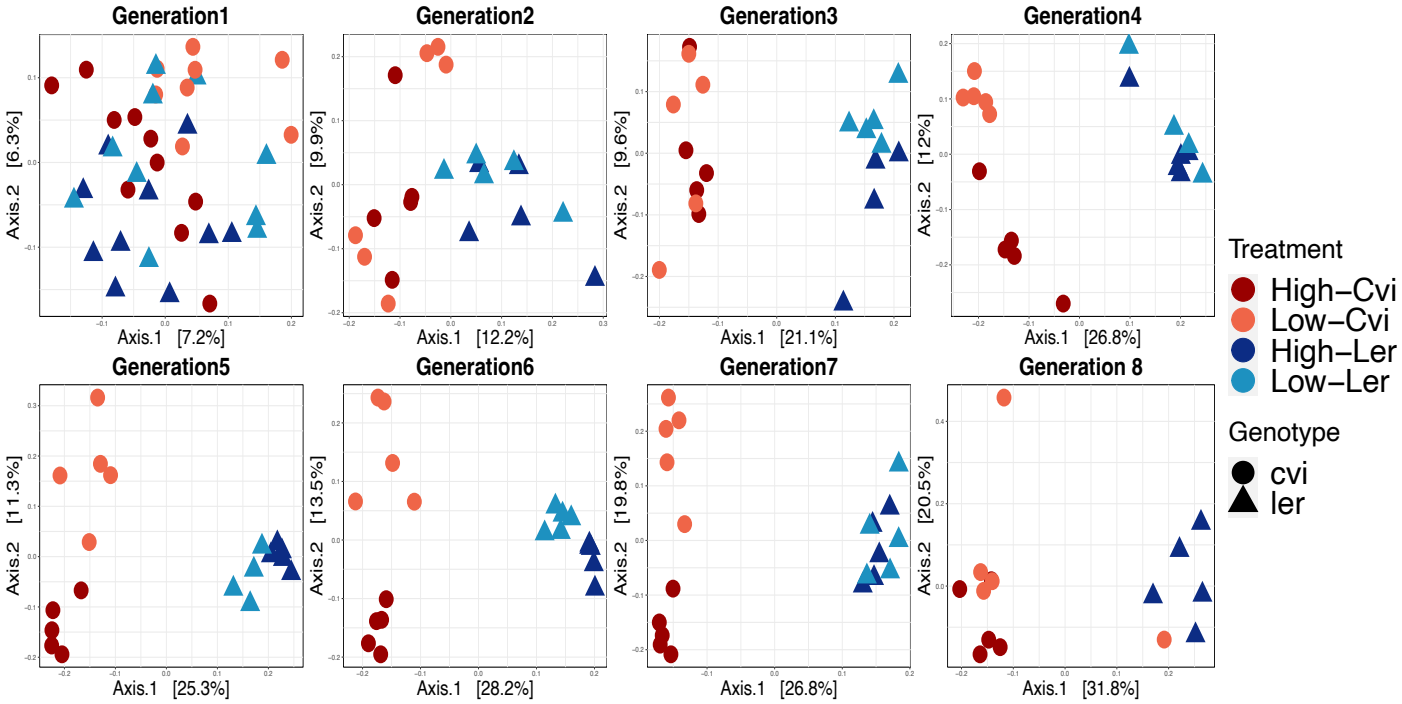

Figure 6. Principle Coordinate Analysis (PCoA) using unweighted UniFrac for generations 1 through 8. Each point represents an individual sample with microbial communities defined of Cvi represented by red/orange circles and L*er* by dark/light blue triangles. The first two coordinate axes plotted accounted highest variation in the data, ranging from 13% in generation 1 to approximately 52% in generation 8. While there was no discernible clustering in generation 1, both genetic lines and high/low biomass treatments exhibited greater clustering over the course of the experiment.

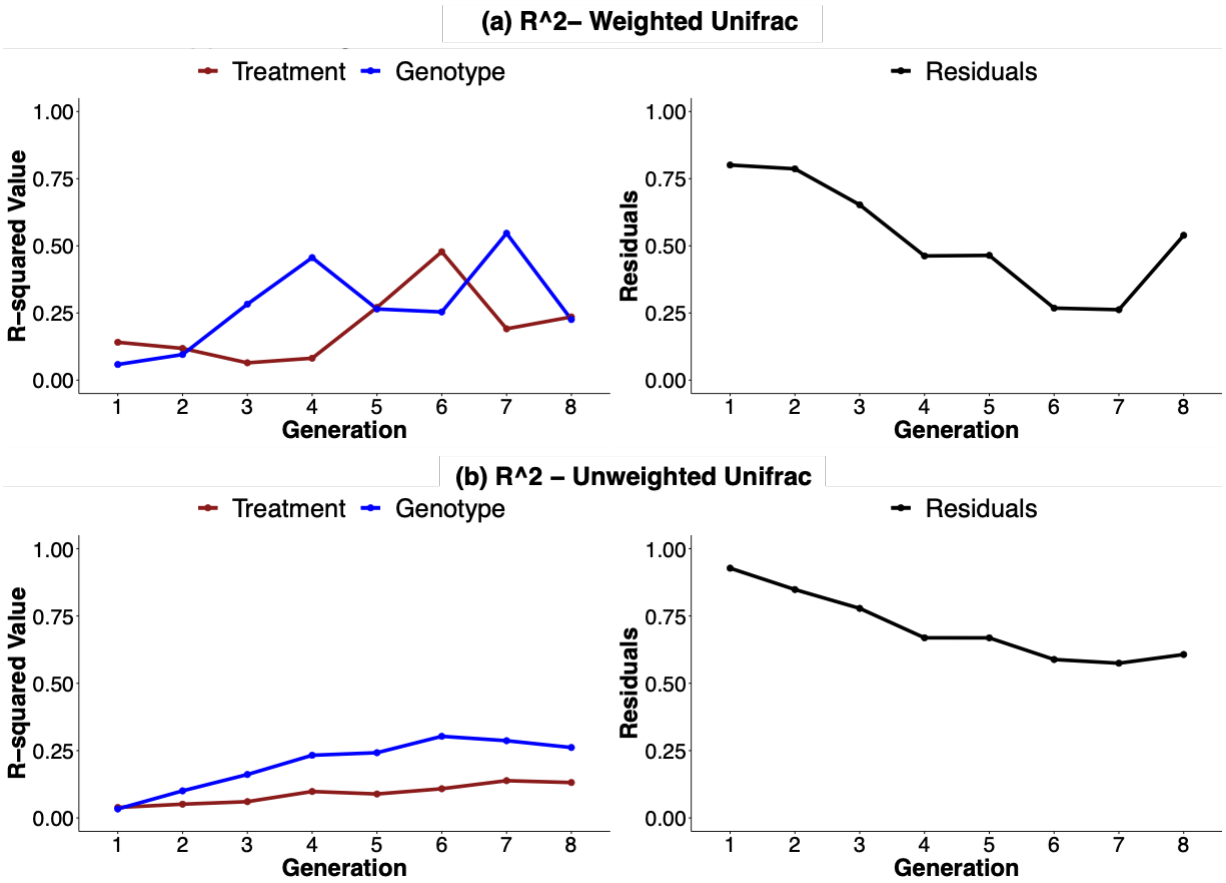

Figure 7. The R^2 value obtained from PERMANOVA using adonis2 test on generations 1 through 8 for (a) weighted and (b) unweighted-UniFrac distances. The resulting R^2 and variance explained by residuals values for the biomass treatment (brown), genotype (blue) and residuals (black) are plotted. Residuals are plotted as a proxy for random environmental variations (REVs). There is a marked increase in the influence of both genotype and biomass treatment on differences in the microbial community from generations 1 through 8. Despite observing a decrease in the variance accounted for by the residuals, which could potentially signify a decline in the influence of REVs, the model elucidates more than 50 percent of the variability within the microbial community in the 8th generation. These differences are more pronounced in the weighted vs unweighted UniFrac. Model (distance.matrix~biomass treatment +genotype).

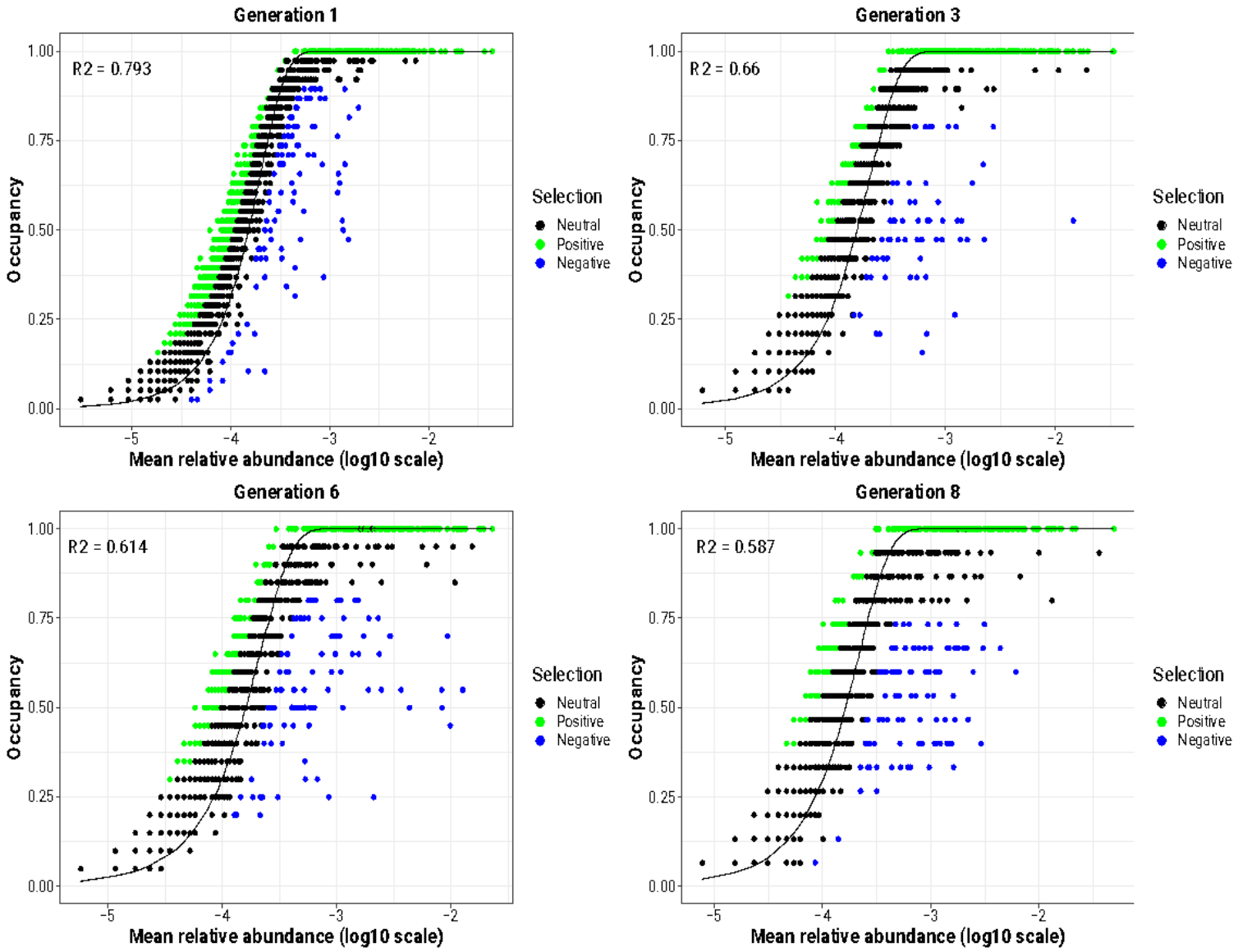

Figure 8. The occupancy (prevalence of OTU in samples) plotted against log(10) abundance is depicted using the Burns model. OTUs that are neutral, i.e., not selected for or against, are indicated in black. Green and blue-colored OTUs signify positive and negative selection, respectively. This plot highlights the presence of numerous microbes that undergo selection throughout the experiment.

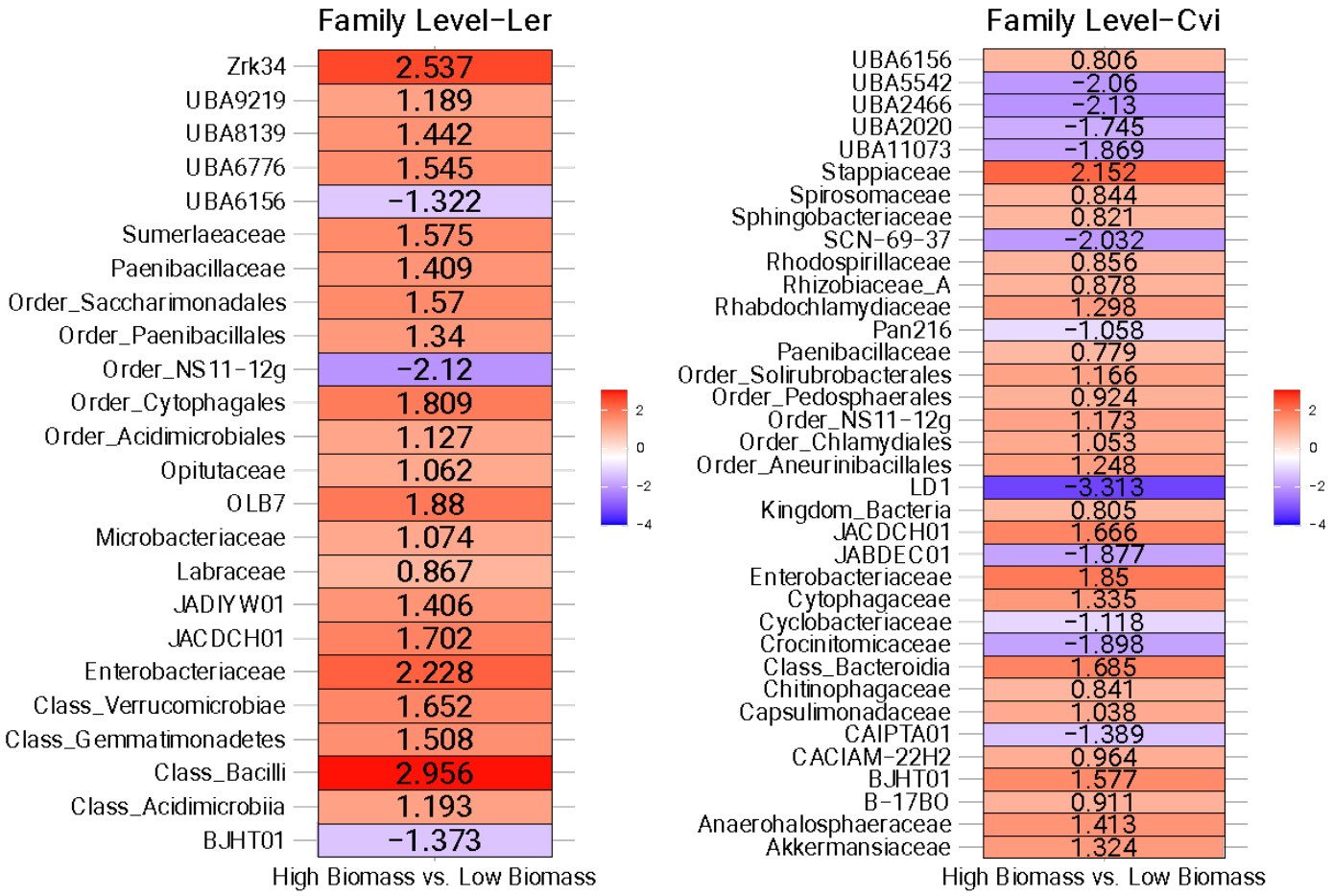

Figure 9. ANCOMBC2 results are illustrated as log fold changes at the family level for high biomass vs low biomass samples. Each accession listed in the table represents a single OTU in either the L*er* or Cvi microbial community identified to the family level if possible. The scale indicates log fold change, red and blue for positive and negative fold change in the high biomass samples respectively. Well-known plant growth-promoting bacteria, such as *Paenibacillaceae, Bacilli, Labraceae, Rhizobiaceae* and *Bdellovibrio* are present in higher abundance. A vast majority of the other taxa identified are uncultivated and have little to no information on them.

References

1. Gouda S, Kerry RG, Das G, Paramithiotis S, Shin HS, Patra JK. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol Res. 2018;206.

2. Jones DL, Nguyen C, Finlay RD. Carbon flow in the rhizosphere: Carbon trading at the soil-root interface. Plant Soil. 2009;321(1–2):5–33.

3. Dennis PG, Miller AJ, Hirsch PR. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol Ecol. 2010;72(3):313–27.

4. Hartmann A, Schmid M, van Tuinen D, Berg G. Plant-driven selection of microbes. Plant Soil. 2009;321(1–2):235–57.

5. Chaparro JM, Sheflin AM, Manter DK, Vivanco JM. Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils. 2012;48(5):489–99.

6. Marschner P, Marschner P, Crowley D, Crowley D, Yang CH, Yang CH, et al. Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. Plant Soil. 2004;261(1–2):199–208.

7. Garbeva P, van Veen J a, van Elsas JD. Microbial diversity in soil: selection microbial populations by plant and soil type and implications for disease suppressiveness. Annu Rev Phytopathol. 2004;42(29):243–70.

8. Tkacz A, Cheema J, Chandra G, Grant A, Poole PS. Stability and succession of the rhizosphere microbiota depends upon plant type and soil composition. ISME J. 2015;1–11.

9. Berg G, Smalla K. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol. 2009;68(1):1–13.

10. Jin H, Yang X, Liu R, Yan Z, Li X, Li X, et al. Bacterial community structure associated with the rhizosphere soils and roots of Stellera chamaejasme L. along a Tibetan elevation gradient. Ann Microbiol. 2018 May 1;68(5):273–86.

11. Barbosa Lima A, Cannavan FS, Navarrete AA, Teixeira WG, Kuramae EE, Tsai SM. Amazonian Dark Earth and Plant Species from the Amazon Region Contribute to Shape Rhizosphere Bacterial Communities. Microb Ecol. 2015 May 1;69(4):855–66.

12. Marques JM, da Silva TF, Vollu RE, Blank AF, Ding GC, Seldin L, et al. Plant age and genotype affect the bacterial community composition in the tuber rhizosphere of field-grown sweet potato plants. FEMS Microbiol Ecol. 2014;88(2):424–35.

13. Bodenhausen N, Bortfeld-Miller M, Ackermann M, Vorholt JA. A Synthetic Community Approach Reveals Plant Genotypes Affecting the Phyllosphere Microbiota. PLoS Genet. 2014;10(4).

14. Zancarini A, Mougel C, Voisin AS, Prudent M, Salon C, Munier-Jolain N. Soil Nitrogen Availability and Plant Genotype Modify the Nutrition Strategies of M. truncatula and the Associated Rhizosphere Microbial Communities. PLoS ONE. 2012;7(10).

15. Wei S, Liu B, Ni K, Ma L, Shi Y, Leng Y, et al. Rhizosphere Microbial Community Shows a Greater Response Than Soil Properties to Tea (Camellia sinensis L.) Cultivars. Agronomy. 2023 Jan 1;13(1).

16. Yuan J, Chaparro JM, Manter DK, Zhang R, Vivanco JM, Shen Q. Roots from distinct plant developmental stages are capable of rapidly selecting their own microbiome without the influence of environmental and soil edaphic factors. Soil Biol Biochem. 2015;89(AUGUST):206–9.

17. Houlden A, Timms-Wilson TM, Day MJ, Bailey MJ. Influence of plant developmental stage on microbial community structure and activity in the rhizosphere of three field crops. FEMS Microbiol Ecol. 2008;65(2):193–201.

18. Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, et al. Defining the core Arabidopsis thaliana root microbiome. Nature. 2013 Aug 1;488(7409):86–90.

19. Bulgarelli D, Rott M, Schlaeppi K, Ver E, Themaat LV, Ahmadinejad N, et al. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature. 2012;488(7409):91–5.

20. Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, et al. Plant compartment and biogeography affect microbiome composition in cultivated and native *Agave* species. New Phytol. 2016 Jan;209(2):798–811.

21. Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, et al. Structure, variation, and assembly of the root-associated microbiomes of rice. Proc Natl Acad Sci U S A. 2015 Feb 24;112(8):E911–20.

22. Wagner MR, Lundberg DS, Del Rio TG, Tringe SG, Dangl JL, Mitchell-Olds T. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. Nat Commun. 2016 Jul 12;7.

23. Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, et al. Defining the core Arabidopsis thaliana root microbiome. Nature. 2012;488(7409):86–90.

24. Schlaeppi K, Dombrowski N, Oter RG, Ver Loren Van Themaat E, Schulze-Lefert P. Quantitative divergence of the bacterial root microbiota in Arabidopsis thaliana relatives. Proc Natl Acad Sci U S A. 2014;111(2):585–92.

25. Hu L, Robert CAM, Cadot S, Zhang X, Ye M, Li B, et al. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nat Commun. 2018 Dec 1;9(1).

26. Van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, et al. Plant-soil feedbacks: The past, the present and future challenges. J Ecol. 2013 Mar;101(2):265–76.

27. Panke-buisse K, Poole AC, Goodrich JK, Ley RE, Kao-kniffin J. Selection on soil microbiomes reveals reproducible impacts on plant function. ISME J. 2014;1–10.

28. Swenson W, Wilson DS, Elias R. Artificial ecosystem selection. Proc Natl Acad Sci U S A. 2000 Aug 1;97(16):9110–4.

29. Mueller UG, Juenger TE, Kardish MR, Carlson AL, Burns KM, Edwards JA, et al. Artificial Selection on Microbiomes To Breed Microbiomes That Confer Salt Tolerance to Plants METHODS AND PROTOCOLS [Internet]. 2021. Available from: https://journals.asm.org/journal/msystems

30. Lau JA, Lennon JT. Rapid responses of soil microorganisms improve plant fitness in novel environments. Proc Natl Acad Sci. 2012;109(35):14058–62.

31. terHorst CP, Lennon JT, Lau JA. The relative importance of rapid evolution for plant-microbe interactions depends on ecological context. Proc R Soc B Biol Sci. 2014 Jun 22;281(1785):20140028.

32. Jochum MD, McWilliams KL, Pierson EA, Jo YK. Host-mediated microbiome engineering (HMME) of drought tolerance in the wheat rhizosphere. PLoS ONE. 2019 Dec 1;14(12).

33. Cordovez V, Rotoni C, Dini-Andreote F, Oyserman B, Carrión VJ, Raaijmakers JM. Successive plant growth amplifies genotype-specific assembly of the tomato rhizosphere microbiome. Sci Total Environ. 2021 Jun 10;772.

34. Norma M. Morella, Francis Cheng-Hsuan Weng, Pierre M. Joubert, C. Jessica E. Metcalf, Steven Lindow, Britt Koskella. Successive passaging of a plant-associated microbiome reveals robust habitat and host genotype-dependent selection. PNAS. 2020 Jan 14;117(2).

35. Parada AE, Needham DM, Fuhrman JA. Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol. 2016 May 1;18(5):1403–14.

36. Apprill A, Mcnally S, Parsons R, Weber L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol. 2015 Jun 4;75(2):129–37.

37. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012 Aug;6(8):1621–4.

38. Thompson LR, Sanders JG, McDonald D, Amir A, Ladau J, Locey KJ, et al. A communal catalogue reveals Earth's multiscale microbial diversity. Nature. 2017 Nov 1;551(7681):457.

39. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019 Aug 1;37(8):852–7.

40. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016 Jun 29;13(7):581–3.

41. Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk v2: memory friendly classification with the genome taxonomy database. Bioinforma Oxf Engl. 2022 Nov 30;38(23):5315–6.

42. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, et al. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Mol Biol Evol. 2020 May 1;37(5):1530–4.

43. Bunn AG. A dendrochronology program library in R (dplR). Dendrochronologia. 2008 Oct 1;26(2):115–24.

44. Burns AR, Stephens WZ, Stagaman K, Wong S, Rawls JF, Guillemin K, et al. Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. ISME J. 2016;10(3):655–64.

45. Sloan WT, Woodcock S, Lunn M, Head IM, Curtis TP. Modeling taxa-abundance distributions in microbial communities using environmental sequence data. In: Microbial Ecology. 2007. p. 443–55.

46. Nearing JT, Douglas GM, Hayes MG, MacDonald J, Desai DK, Allward N, et al. Microbiome differential abundance methods produce different results across 38 datasets. Nat Commun. 2022 Dec 1;13(1).

47. Lin H, Peddada SD. Analysis of compositions of microbiomes with bias correction. Nat Commun. 2020 Dec 1;11(1).

48. Radhakrishnan R, Hashem A, Abd Allah EF. Bacillus: A biological tool for crop improvement through bio-molecular changes in adverse environments. Front Physiol. 2017 Sep 6;8(SEP).

49. Mujakić I, Piwosz K, Koblížek M. Phylum Gemmatimonadota and Its Role in the Environment. Microorganisms. 2022 Jan 1;10(1).

50. Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E. The Prokaryotes: A Handbook on the Biology of Bacteria: Archaea. Bacteria: Firmicutes, Actinomycetes. The Prokaryotes: A Handbook on the Biology of Bacteria. 2006.

51. Grady EN, MacDonald J, Liu L, Richman A, Yuan ZC. Current knowledge and perspectives of Paenibacillus: A review. Microb Cell Factories. 2016 Dec 1;15(1).

52. Ramesh A, Sharma SK, Sharma MP, Yadav N, Joshi OP. Plant Growth-Promoting Traits in Enterobacter cloacae subsp. dissolvens MDSR9 Isolated from Soybean Rhizosphere and its Impact on Growth and Nutrition of Soybean and Wheat Upon Inoculation. Agric Res. 2014;3(1):53–66.

53. Kämpfer P, Ruppel S, Remus R. Enterobacter radicincitans sp. nov., a plant growth promoting species of the family Enterobacteriaceae. Syst Appl Microbiol. 2005 Apr 22;28(3):213–21.

54. Khalifa AYZ, Alsyeeh AM, Almalki MA, Saleh FA. Characterization of the plant growth promoting bacterium, Enterobacter cloacae MSR1, isolated from roots of non-nodulating Medicago sativa. Saudi J Biol Sci. 2016;23(1):79–86.

55. Harkes P, van Steenbrugge JJM, van den Elsen SJJ, Suleiman AKA, de Haan JJ, Holterman MHM, et al. Shifts in the Active Rhizobiome Paralleling Low Meloidogyne chitwoodi Densities in Fields Under Prolonged Organic Soil Management. Front Plant Sci. 2020 Jan 10;10.

56. Meier MA, Xu G, Lopez-Guerrero MG, Li G, Smith C, Sigmon B, et al. Association analyses of host genetics, root-colonizing microbes, and plant phenotypes under different nitrogen conditions in maize. eLife. 2022;11.

57. Porter AS, Evans-Fitz.Gerald C, McElwain JC, Yiotis C, Elliott-Kingston C. How well do you know your growth chambers? Testing for chamber effect using plant traits. Plant Methods. 2015;11(1).

58. Bulgarelli D, Garrido-Oter R, Münch PC, Weiman A, Dröge J, Pan Y, et al. Structure and function of the bacterial root microbiota in wild and domesticated barley. Cell Host Microbe. 2015;17(3):392–403.

59. Brown SP, Grillo MA, Podowski JC, Heath KD. Soil origin and plant genotype structure distinct microbiome compartments in the model legume Medicago truncatula. Microbiome. 2020 Sep 28;8(1).

60. Basu A, Prasad P, Das SN, Kalam S, Sayyed RZ, Reddy MS, et al. Plant Growth Promoting Rhizobacteria (PGPR) as Green Bioinoculants: Recent Developments, Constraints, and Prospects. Sustainability. 2021 Jan 22;13(3):1140.

61. Micallef S, Colón-Carmona A. Genetic and Developmental Control of Rhizosphere Bacterial Communities. In: de Bruijn FJ, editor. Molecular Microbial Ecology of the Rhizosphere [Internet]. 1st ed. Wiley; 2013 [cited 2023 Aug 20]. p. 257–63. Available from: https://onlinelibrary.wiley.com/doi/10.1002/9781118297674.ch24

62. Micallef SA, Channer S, Shiaris MP, Colón-Carmona A. Plant age and genotype impact the progression of bacterial community succession in the Arabidopsis rhizosphere. Plant Signal Behav. 2009 Aug;4(8):777–80.

696 63. Lee S, I. Sergeeva L, Vreugdenhil D. Natural variation of hormone levels in *Arabidopsis*
697 roots and correlations with complex root architecture: Natural variation of phytohormones in
698 *Arabidopsis* roots. J Integr Plant Biol. 2018 Apr;60(4):292–309.

699 64. Matsushima C, Shenton M, Kitahara A, Wasaki J, Oikawa A, Cheng W, et al. Multiple
700 analysis of root exudates and microbiome in rice (Oryza sativa) under low P conditions.
701 Arch Microbiol. 2021 Nov 1;203(9):5599–611.

702 65. Huang XF, Chaparro JM, Reardon KF, Zhang R, Shen Q, Vivanco JM. Rhizosphere
703 interactions: Root exudates, microbes, and microbial communities1. Botany.
704 2014;92(4):267–75.

705 66. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. Plant–microbiome interactions: from
706 community assembly to plant health. Nat Rev Microbiol. 2020;18(11).

707 67. Gu S, Wei Z, Shao Z, Friman VP, Cao K, Yang T, et al. Competition for iron drives
708 phytopathogen control by natural rhizosphere microbiomes. Nat Microbiol. 2020 Aug
709 1;5(8):1002–10.

710