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1 Stable, multigenerational transmission of the bean seed microbiome despite
2 abiotic stress

3

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15

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17

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19 *Phaseolus vulgaris*, nutrients, endophyte

20

21

22 **Abstract**

23 Seed microbiomes initiate plant microbiome assembly, but the consequences of environmental
24 conditions of the parent plant for seed microbiome assembly and transmission are unknown.

25 We tracked endophytic seed bacterial communities of common bean lines exposed to drought
26 or excess nutrients, and discovered stable transmission of 22 bacterial members regardless of
27 parental plant treatment. This study provides insights into the maintenance of plant
28 microbiomes across generations, even under challenging environmental stress.

29

30 **Main Text**

31 The seed microbiome plays a role in both pathogen transmission and in shaping a plant's
32 beneficial microbiome^{1,2}. Understanding the inheritance of microbiome members over plant
33 generations, especially as mediated through seed transmission, is of interest for plant
34 microbiome management and conservation³⁻⁷. Plant microbiomes can be assembled via both
35 horizontal transmission from the environment and vertical transmission from the parent plant
36 through the seed microbiome⁸⁻¹⁰. For seed transmission, microbial cells can either migrate
37 through the vascular tissue or floral compartments and become packaged within the internal
38 seed tissues or colonize seed surfaces¹⁰⁻¹⁴. During germination, seed microbiome members that
39 first colonize the plant can determine priority effects and drive the trajectory of microbiome
40 assembly¹⁵⁻¹⁷. Additionally, these "inherited" microbiome members enriched by the parent
41 plant may provide important benefits for the offspring¹⁸⁻²¹.

42 A remaining unknown is how the environmental conditions of the parent plant influence
43 seed microbiome assembly and whether stress can drive long-term alterations in the

44 microbiome across generations^{22–24}. A few studies have found an influence of parental plant
45 line on the resulting seed microbiome of the next generation^{5,25}. Other studies have suggested
46 an impact of parental stress on the seed microbiome after one generation, particularly drought
47 in beans and wheat^{26,27}, excess nutrients in beans²⁶, and salt stress in rice²⁸. A legacy effect of
48 environmental stress on the seed microbiome could have consequences for the healthy
49 assembly of the microbiome in the next plant generation²⁹.

50 We conducted a multigenerational experiment in which we exposed common bean
51 plants (*Phaseolus vulgaris* L.) to drought or high nutrients during their early vegetative growth
52 (**Fig. S1**). Plants were grown in agricultural soil in environmental chambers to allow for the
53 natural assembly of their native microbiome while also controlling environmental conditions.
54 We were motivated to study *Phaseolus vulgaris* L. because it is a critical legume for global food
55 security, supporting the health and livelihood of millions of people worldwide^{30,31}. We chose
56 these two abiotic treatments, drought, and nutrient excess, because of their relevance to bean
57 production in a changing climate. Bean production is threatened by drought associated with
58 warming and changes in precipitation patterns^{32–34}. Furthermore, beans grown in areas of high
59 production are often managed with excess mineral fertilizer, which can impact the selection
60 and stability of the plant microbiome³⁵.

61 We hypothesized that abiotic treatment of the parent plant characteristically alters the
62 seed endophyte microbiome. Furthermore, we expected that each abiotic treatment would
63 have specific consequences for the composition and stability of taxa transmitted across
64 generations. We tested our hypothesis by planting a starting set of G0 bean seeds through two
65 generations, exposed to either control growth conditions, drought, or excess nutrient

66 treatments while tracking each plant's parental line within a fully factorial design (**Fig. S1**). We
67 applied standard 16S V4 rRNA gene amplicon sequencing of the seed bacterial endophyte
68 communities for each of the three generations to assess the impact of the treatments on the
69 seed microbiome and the potential for transmission of the bacterial taxa.

70 The drought and nutrient treatments did not influence microbiome alpha or beta
71 diversity in either generation (**Fig. S2, Fig. S3, Table S1**). Furthermore, we detected no legacy
72 influence of the G1 treatments on the G2 seed microbiomes and no overarching differences in
73 the microbiomes across the G1 and G2 generations (**Fig. S3, Table S1**). However, there was an
74 appreciable influence of parental line for those G2 plants that originated from the G1 drought
75 treatment (**Fig. S4B**, PERMANOVA *post-hoc* $r^2=0.40311$ $F=1.4637$ $p=0.0021$), but not for the
76 control or nutrient parental lines.

77 Instead, we found evidence of stable transmission of 128 of 658 detected Amplicon
78 Sequence Variants (ASVs) across all three generations, regardless of the experimental
79 treatment (**Fig. 1A**). The 128 ASVs detected in all three generations were in higher relative
80 abundance than the ASVs that were only seen in one or two generations (Kruskal-Wallis test:
81 test-statistic=363.59, $df=2$, $p<0.0001$; Post-hoc Dunn's test with Benjamini-Hochberg
82 correction: 1 v. 3 generations: test-statistic=17.78, adjusted $p<0.0001$, 2 v. 3 generations: test-
83 statistic=5.879, adjusted $p<0.0001$) (**Fig. 1B**). Furthermore, the Genus-level taxonomic profiles
84 of G0, G1, and G2 microbiomes were highly comparable (**Fig. S5**), suggesting a consistent
85 taxonomic signature of the ASVs detected across seed generations.

86 We identified ASVs overlapping between a G1 parent plant and at least one of its
87 offspring in G2, which we call "overlapping ASVs." There were 99 overlapping ASVs discovered

88 among the 36 lines, 70 of which were found in at least two parent lines and 43 of which were
89 common to all treatments. The proportion of overlapping ASVs in each line ranged from 17% to
90 45% of the total ASVs detected (**Fig. 1C, 1D**).

91 Nine overlapping ASVs were present in all 36 parental lines, and an additional 13
92 ASVs were found in at least half of all parental lines. These 22 ASVs generally had 100%
93 transmission to all three G2 offspring per parent line (**Fig. 2**). At the same time, we noticed that
94 less prevalent ASVs were not consistently transmitted in all offspring within lines. The G1
95 treatment did not impact the average transmission of the ASVs in the G2 offspring, and there
96 was no significant difference in average transmission between parental lines (Pearson's Chi-
97 squared Test. G1 Treatment: $X^2=0.67413$, $df=4$, $p=0.9545$. Line: $X^2=63.48$, $df=70$, $p=0.6958$) (**Fig.**
98 **2**).

99 Most overlapping ASVs that were found in at least two parental lines were also detected
100 in the G0 microbiomes (61 out of 70 ASVs) (**Fig. 2**). These ASVs were taxonomically diverse and
101 included 39 Families and 26 Orders. Many overlapping ASVs were also highly abundant (**Fig. 2**).
102 Their Genus-level taxonomic compositions in G2 were very similar within and across parent
103 lines (**Fig. S7**).

104 To seek generalities with other relevant studies, we compared the 70 overlapping ASVs
105 detected in this study to the six “core” common bean seed microbiome ASVs identified by
106 Simonin *et al.* 2022³⁶ and to the 48 “core” common bean rhizosphere OTUs identified by
107 Stopnisek and Shade 2021³⁷. These previously reported core taxa were identified across
108 multiple studies and are hypothesized to be important for health in common beans. Three core
109 common bean seed ASVs³⁶ were found in the overlapping G1-G2 dataset, specifically from the

110 *Pseudomonas*, *Bacillus*, and *Pantoea* genera (**Table 1**, Fig. 2, **Fig. S6**). Two ASVs, the
111 *Pseudomonas* and *Bacillus* core members, were detected in all 36 parental lines, while the
112 *Pantoea* core seed microbiome member was found in only three parental lines (**Table 1**). There
113 were nine ASVs aligned at >96% identity to the previously identified core rhizosphere taxa³⁷
114 (**Table 1**, **Table S3**, **Fig. 2**). These ASVs were classified in the families Comamonadaceae,
115 Devosiaceae (genus *Devosia*), Methyloligellaceae, Oxalobacteraceae (genus *Massilia*),
116 Rhizobiaceae (genus *Ochrobactrum*), Sphingomonadaceae (genus *Sphingomonas*),
117 Streptomycetaceae (genus *Streptomyces*), and Micrococcaceae in the genus *Arthrobacter*, of
118 which our seed ASV aligned at 98.8% identity to the most abundant core OTU in the
119 rhizosphere study (**Table 1**, **Table S3**). These genera are commonly associated with plant
120 microbiomes^{38–42}.

121 Our multigenerational study indicates that numerous seed microbiome members were
122 consistently packaged in the seed endophytes of the G0, G1, and G2 plants, regardless of the
123 growth conditions (field or environmental chamber), the starting soil or abiotic treatment
124 applied to the plants. This suggests a consistent and stable seed microbiome transmission for
125 common beans. This is an unexpected result because, for many plant species, the seed
126 microbiome has been reported to have relatively low diversity (tens to hundreds of taxa), low
127 biomass/small community size (dozens of cells), and notably high compositional variability^{4,36,43},
128 suggesting an influence of stochasticity in the assembly. Our results indicate that against a
129 background of high variability, a handful of members of the seed endophyte microbiome
130 exhibit stable transmission. These transmitted taxa could not have been identified before,
131 because previous studies have focused on only one generation, and, perhaps more importantly,

132 on seeds that were pooled across multiple parent plants. These stable seed microbiome taxa
133 may establish mutualistic or commensal associations and persist through the plant life cycle
134 until packaged within the seed for the next generation. It also suggests that a parental effect on
135 the seed microbiome has the potential to outweigh abiotic effects.

136 Our results cannot address the exact mode of transmission of the seed endophyte
137 microbiome, which is a limitation of the work. It remains unclear whether the seed endophytes
138 are passed directly *via* vertical transmission (e.g., from seed to plant to seed via the vascular
139 tissue as previously reported as the primary pathway for common bean^{11,44}) or re-acquisition
140 (e.g., parent plants selectively recruit the same taxa from the environment). Regardless of the
141 transmission mode, the seed microbiome members' stability despite abiotic treatment, their
142 prevalence and high relative abundance, and their consistent detection across several bean
143 microbiome investigations suggest a strong selection of the plant seed environment for these
144 taxa. Thus, while our initial hypothesis about the importance of abiotic treatment in driving
145 seed endophyte microbiome variation was not supported, there was clear evidence that a
146 stable seed microbiome was transmitted in all conditions in our study.

147 As the need for sustainable solutions to maintain or improve agricultural productivity
148 increases, plant microbiome management, microbiome engineering, and breeding plants for
149 improved microbiomes will be critical strategies^{45–47}. Applying beneficial plant microbiome
150 members via seed treatments or soil inoculation has shown promise in improving plant growth
151 or health^{48,49}. The stably transmitted bean seed microbiome members identified here provide
152 targets for future research to understand how to shape legume microbiomes to improve crop
153 yield, health, and resilience^{50,51}.

154

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160 la Recherche Scientifique (CNRS), France.

161 **Online Methods**

162 **Bean cultivar**

163 *Phaseolus vulgaris* L. var. Red Hawk⁵², developed by the Michigan State University Bean
164 Breeding program, was selected as a representative dry bean crop. Red Hawk seeds were
165 obtained from the Michigan State University Bean Breeding Program from their 2019 harvest
166 and stored at 4°C until ready for use in experiments. These seeds were obtained as “Generation
167 0” and used to plant the first generation of the experiment.

168

169 **Soil preparation**

170 Agricultural field soil was collected for each planting group in September 2019,
171 December 2020, May 2021, and September 2021 from a Michigan State University Agronomy
172 Farm field that was growing common beans in 2019 (42°42'57.4"N, 84°27'58.9"W, East Lansing,
173 MI, USA). The soil was a sandy loam with an average pH of 7.2. When collecting the soil, we
174 avoided the dry top layer of soil and plant debris. Field soil was covered with air-tight lids and
175 stored at 4°C until the experiment began. The soil was passed through a 4mm sieve to remove
176 rocks and plant debris and then mixed with autoclaved coarse vermiculite at a 50% v/v ratio.

177

178 **Surface sterilization and seed germination**

179 Red Hawk seeds were surface sterilized prior with a solution of 10% bleach and 0.1%
180 Tween20. Seeds were randomly selected from the bulk G0 seed supply or the harvested
181 Generation 1 (G1) seed supply, but we avoided seeds that were visibly cracked or moldy.
182 Approximately 20 seeds were placed in a sterile 50 mL conical tube, 20-30 mL of bleach solution

183 was added, and then the seeds were soaked for 10 minutes, with agitation at 5 minutes. After
184 soaking, the seeds were rinsed 5 times with sterile DI H₂O. On the final rinse, 100 µL of rinse
185 water was spread onto Tryptic Soy Agar (TSA) and Potato Dextrose Agar (PDA) plates to assess
186 the efficacy of the seed surface sterilization. TSA plates were incubated overnight at 28°C and
187 PDA plates at room temperature for 48 hours. Seeds corresponding to plates that had microbial
188 growth were discarded from the experiment and replaced with surface-sterile ones. For
189 germination, seeds were placed in a Petri dish lined with sterile filter paper and supplemented
190 with 1-2 mL sterile DI H₂O. Petri dishes were stored in the dark at room temperature for 3-4
191 days, with an additional 2 mL sterile DI H₂O added after two days. Once seeds had sprouted
192 radicle roots, they were transferred to soil.

193

194 **Growth conditions**

195 For G1, three germinated seeds were planted into 3.78-liter pots filled with the soil-
196 vermiculite mixture and placed in a high-light BioChambers FLEX™ LED growth chamber with a
197 16-hour day/8-hour night cycle at 26°C and 22°C, respectively, and 50% relative humidity. Once
198 seedlings emerged and reached the VC growth stage (vegetative growth with two cotyledons
199 and primary leaves expanded), they were thinned to one seedling per pot. Plants were watered
200 every other day with 300 mL 0.05% 15-10-30 water-soluble fertilizer solution (control
201 condition) (Masterblend International, Morris, IL, USA). At the V3 stage (vegetative growth with
202 third trifoliolate leaves expanded), treatments began for the drought- and nutrient-treated
203 plants. Drought plants received 100 mL of 0.15% 15-10-30 fertilizer solution every other day
204 (66% less water than control with the same concentration of nutrients), and nutrient plants

205 received 300 mL of 0.15% 15-10-30 fertilizer solution every other day (3X concentrated
206 nutrients with the same volume of water as control). After approximately 14 days of the
207 treatment period, when plants reached the R1 stage (reproductive stage, first open flowers),
208 they were returned to the water regime for the control plants (every other day) until
209 senescence. As plants began to dry, they were watered less frequently as needed. Mature
210 seeds were collected from 12 plants per treatment for seed microbiome assessment and for
211 germination for the next generation.

212 For Generation 2 (G2), seeds from the 36 G1 parental lines that received either control,
213 drought, or nutrient conditions were planted in a full factorial design and grown under one of
214 the three treatment conditions in G2. There were nine cross-generational treatment
215 combinations total (G1_G2, n=12 plants per treatment): Control_Control, Control_Drought, and
216 Control_Nutrient; Drought_Control, Drought_Drought, and Drought_Nutrient;
217 Nutrient_Control, Nutrient_Drought, and Nutrient_Nutrient. Six seeds from each parental line
218 were surface sterilized and germinated as described above, then planted in the field soil-
219 vermiculite mixture in seedling trays in the growth chamber under the conditions stated above.
220 G2 plants were grown in three randomized planting groups, with each planting group
221 containing parental lines from all three treatments. Once plants reached the VC stage, three
222 healthy seedlings per parent line were transferred to 3.78-liter pots. Each G1 parental line
223 provided one offspring per G2 treatment for a total of 108 plants in G2. Plants were watered
224 according to the conditions and treatment timeline in G1.

225

226 **Seed harvest**

227 Once the plants had senesced and pods were dried, seeds were harvested for planting
228 or microbiome analysis. Seed pods were removed from each plant and stored in sterile
229 Whirlpak bags. Pods and seeds per plant were counted, and then seeds were removed from the
230 pods and pooled by the parent plant in 50 mL conical tubes and stored at 4°C for use in
231 planting. Five seeds per plant were selected for microbiome analysis and stored in a 15 mL
232 conical tube at -80°C until DNA extraction was performed.

233

234 **DNA extractions**

235 DNA extractions were performed on sets of five randomly selected seeds per plant,
236 which is our unit of microbiome sampling. For the G0 bulk seed, twenty sets of five randomly
237 selected seeds from each plant were analyzed (these five seeds did not necessarily come from
238 the same parent plant). Seeds were analyzed from 36 parent plants for G1 and from 108
239 offspring for G2. Seeds were thawed and surface sterilized according to the method above, and
240 then microbial DNA was extracted from the endophytic compartment using a protocol adapted
241 from Barret *et al.* 2015 and Bintarti *et al.* 2021^{8,43}. Following surface sterilization, the seeds
242 were sliced in half lengthwise along the natural division of the cotyledons with a sterile razor
243 blade. Sliced seeds were placed in a 50 mL conical tube, and 20-30 mL of sterile Phosphate-
244 buffered Saline (PBS) with 0.05% Tween 20 was added. Seeds were soaked overnight at 4°C
245 with constant agitation on a surface shaker at 160 rpm. After soaking, tubes were centrifuged
246 at 4500xg, 4°C, for one hour. Seed tissue and supernatant were removed, and the remaining
247 pellet was transferred to a 1.5 mL microcentrifuge tube. Pellets were stored at -80°C until
248 extraction with the E.Z.N.A. Bacterial DNA kit (Omega Bio-tek, Inc., Norcross, GA, USA) following

249 the manufacturer's protocol with the following modifications. The seed material pellet was
250 resuspended in 100 μ L TE Buffer, 10 μ L kit-provided Lysozyme was added, and the samples
251 were vortexed thoroughly and incubated at 37°C for 1 hour. The glass bead step from the
252 E.Z.N.A. kit was utilized with 25-30 mg glass beads provided, and samples were vortexed at
253 maximum speed for 10 minutes in a 24-tube vortex adapter. After adding the Proteinase K, the
254 samples were incubated in a shaking heat block at 55°C for 2 hours. In the final step, DNA was
255 eluted in 60 μ L Elution Buffer and incubated at 65°C for 10 minutes before centrifuging into the
256 final tube.

257 DNA extractions were performed in randomized batches within each generation (**Table**
258 **S2**). For each batch, negative and positive controls were included. The negative control was 3
259 mL sterile PBS+Tween buffer, and the positive control was an aliquot of a mixture of cells from
260 a custom-made mock bacterial community in 3 mL buffer⁵³. These controls were soaked
261 overnight alongside the seed samples and then processed and sequenced as described for the
262 seeds, and then ultimately used to perform batch-informed bioinformatic sequence
263 decontamination⁵⁴.

264

265 **Amplicon Sequencing**

266 Sequencing of the V4 region of the 16S rRNA gene (515F-806R)^{55,56} was performed at
267 the Environmental Sample Preparation and Sequencing Facility (ESPSF) at Argonne National
268 Laboratory (Lemont, IL, USA). The DNA was PCR amplified with region-specific primers that
269 include sequencer adapter sequences used in the Illumina Nextseq2K flowcell;
270 FWD:GTGYCAGCMGCCGCGGTAA; REV:GGACTACNVGGGTWTCTAAT⁵⁵⁻⁵⁹. Each 25 μ L PCR

271 reaction contained 9.5 μ L of MO BIO PCR Water (Certified DNA-Free), 12.5 μ L of QuantaBio's
272 AccuStart II PCR ToughMix (2x concentration, 1x final), 1 μ L Golay barcode tagged Forward
273 Primer (5 μ M concentration, 200 pM final), 1 μ L Reverse Primer (5 μ M concentration, 200 pM
274 final), and 1 μ L of template DNA. The conditions for PCR were as follows: 94 $^{\circ}$ C for 3 minutes to
275 denature the DNA, with 35 cycles at 94 $^{\circ}$ C for 45 s, 50 $^{\circ}$ C for 60 s, and 72 $^{\circ}$ C for 90 s, with a final
276 extension of 10 min at 72 $^{\circ}$ C to ensure complete amplification. Amplicons were then quantified
277 using PicoGreen (Invitrogen) and a plate reader (Infinite[®] 200 PRO, Tecan). Once quantified, the
278 volumes of each of the products were pooled into a single tube in equimolar amounts. This pool
279 was then cleaned up using AMPure XP Beads (Beckman Coulter) and then quantified using a
280 fluorometer (Qubit, Invitrogen). After quantification, the pool was diluted to 2 nM, denatured,
281 and then diluted to a final concentration of 6.75 pM with a 10% PhiX spike for sequencing.
282 Amplicons were sequenced on a 251bp x 12bp x 251bp NextSeq2000.

283

284 **Sequence data processing**

285 Fastq files were processed in QIIME2 after primer removal by the sequencing center
286 (QIIME2 version: 2022.8.0)⁶⁰. Sample fastq files were imported to QIIME2 format, and samples
287 were denoised, truncated, and merged using DADA2 with a forward truncation length of 191
288 and reverse truncation length of 84⁶¹. Amplicon sequence variants (ASVs) were defined at 100%
289 sequence identity, and 16S taxonomy was assigned with the Silva database release 138 at the
290 default confidence value of 0.7, and taxonomy and ASV tables were exported for further
291 analysis in R⁶².

292 Data analyses were performed in R version 4.3.1 and R Studio version 2023.06.1+524⁶³.
293 There were 126.8 million merged DNA reads prior to host removal and decontamination. ASV,
294 taxonomy, metadata tables, and phylogenetic tree files were imported into the phyloseq
295 package, and host reads classified as chloroplast and mitochondria were removed using the
296 `subset_taxa()` command in the phyloseq package version 1.44.0⁶⁴. 90% of the total DNA reads,
297 and 13% of the ASVs were removed as host reads, leaving 12.3 million total bacterial DNA
298 reads. Datasets were decontaminated with the decontam package version 1.20.0 at the 0.1
299 threshold using the negative and positive controls from each extraction group⁶⁵. After
300 decontamination, there were 422,719 total DNA reads with a range of 456-5788 reads per
301 sample in the full dataset (**Fig. S8**). Rarefaction curves were created using the `rarecurve()`
302 command in the vegan package version 2.6-4⁶⁶. Datasets were then subset for further analysis
303 using the `ps_filter()` command in the microViz package version 0.10.10⁶⁷. Since seed
304 microbiomes typically have low bacterial diversity containing tens to hundreds of taxa, and
305 vertical transmission of specific ASVs was a primary area of investigation in this study, the full
306 dataset was preserved to ensure full observation ASVs⁴³.

307

308 **Ecological Analysis**

309 Alpha diversity was assessed in R using `estimate_richness()` in phyloseq with an ANOVA,
310 and figures were created using the `plot_richness()` command from phyloseq with the ggplot2
311 package version 3.4.2⁶⁸. Faith's Phylogenetic Diversity was calculated with `calculatePD()` from
312 the biomeUtils package version 0.022⁶⁹. ANOVAs were performed with the base R stats
313 command `aov()`. Weighted UniFrac distances were calculated with `distance()` in phyloseq and

314 used for all analyses of beta diversity, and PERMANOVA statistical tests were performed with
315 `adonis2()` from the `vegan` package. We used Weighted UniFrac distance because it explained
316 the most microbiome variation relative to other resemblances we also considered (e.g., Bray-
317 Curtis, Jaccard, etc). Post-hoc analysis on the PERMANOVA results was performed with
318 `pairwise.adonis2()` from `pairwise Adonis` version 0.4.1⁷⁰. Beta dispersion was assessed with the
319 `betadisper()` and `permutest()` commands from the `vegan` package. **Figure 1A** was created with
320 the `UpSetR` package version 1.4.0⁷¹ and statistical analyses were performed with `leveneTest()`
321 from the `car` package⁷² and `kruskal_test()` and `dunn_test()` from the `rstatix` package⁷³. Beta
322 diversity ordinations were created with `ordinate()` from `phyloseq` with `ggplot2`. Additional data
323 analysis was performed in the `tidyverse` package version 2.0.0⁷⁴ and `dplyr` package version
324 1.1.2⁷⁵. Amplicon sequence variant (ASV) transmission was analyzed as count data and
325 Pearson's Chi-squared Tests were performed with `chisq.test()` from the base R stats package.
326 Seed core microbiota identified in Simonin *et al.* 2022³⁶ were compared to transmitted ASVs,
327 and Venn Diagrams were produced with the `VennDiagram` package version 1.7.3⁷⁶. To compare
328 the prevalent ASVs in this study to the 48 core bean rhizosphere microbiome taxa identified by
329 Stopnisek and Shade 2021³⁷, the fasta sequences for each core OTU were used as a query set in
330 a two sequence nucleotide BLAST on the National Center for Biotechnology Information (NCBI)
331 database website, and the fasta sequences from the seed ASVs were compared to the 48 core
332 taxa at >96% identity⁷⁷.

333

334 **Data Availability**

335 Data analysis code can be found at
336 (https://github.com/ShadeLab/Seed_transmission_Common_Bean). Raw sequences can be
337 found on the NCBI Sequence Read Archive under BioProject number PRJNA1058980.

338

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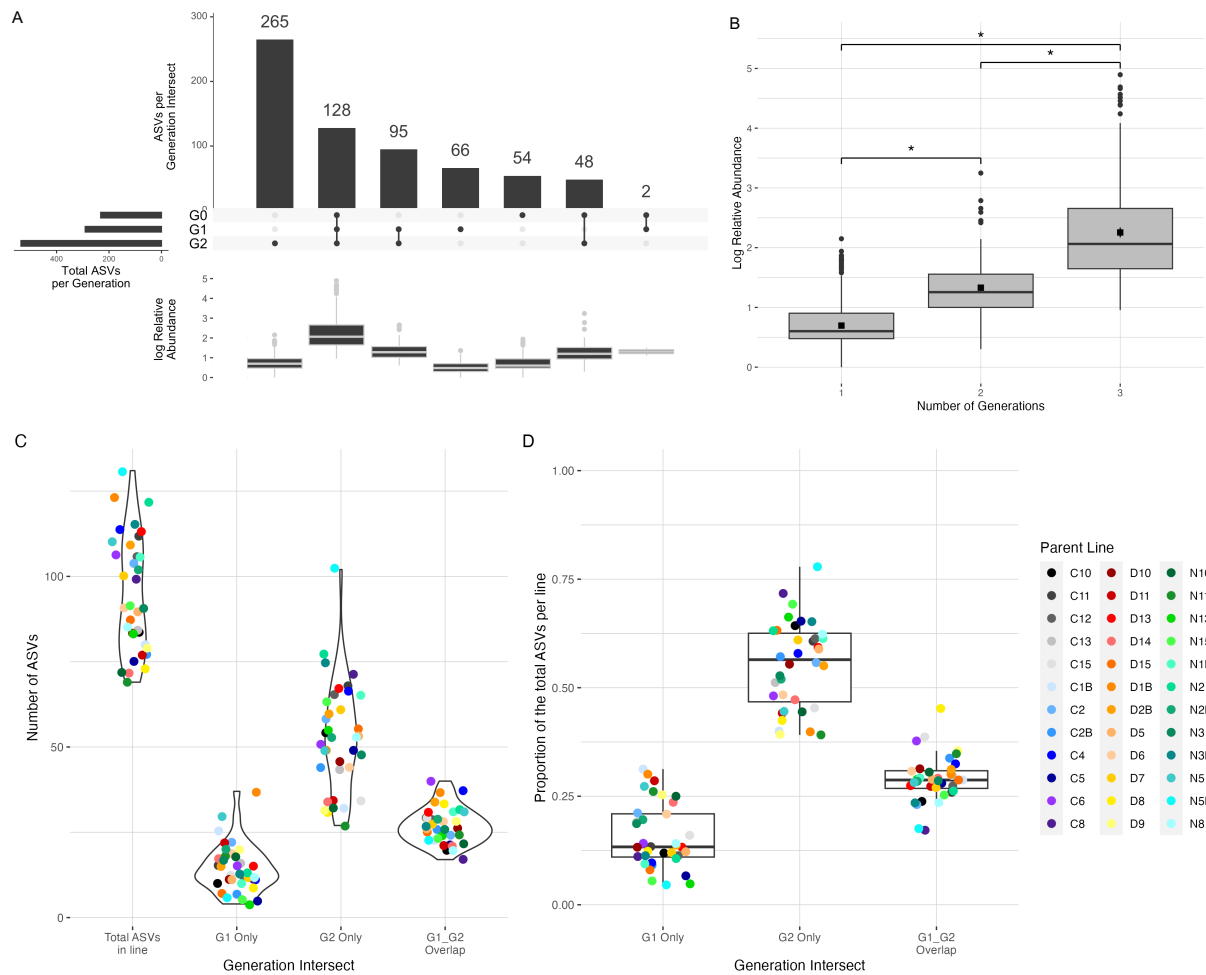
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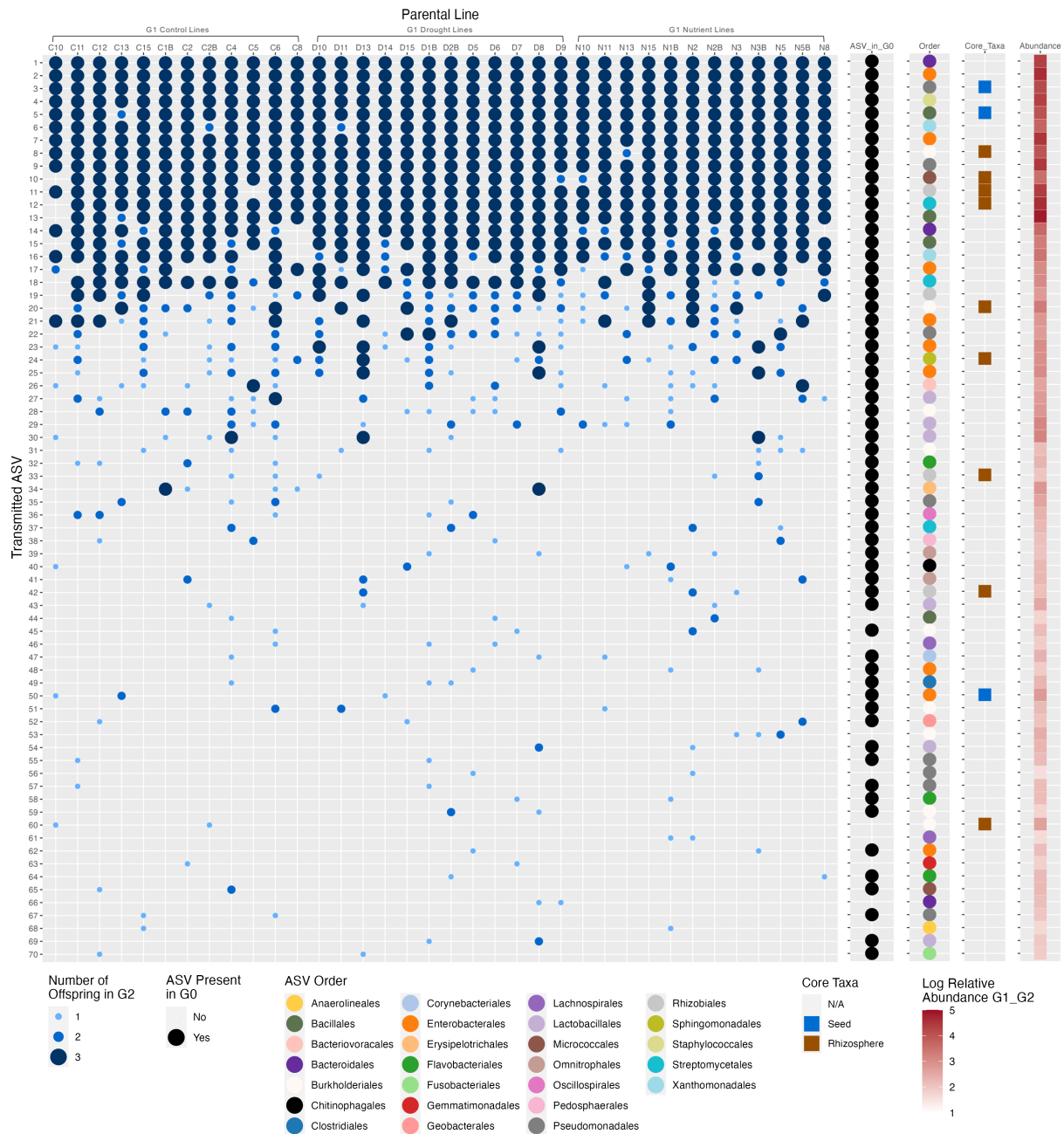
524 **Figures and Tables**



525 **Figure 1. Unique and overlapping ASVs between generations.** **A**, Number of ASVs and relative
 526 abundance of ASVs per generation intersect across all samples. G0 n=20 samples (five seeds per
 527 sample), G1 n =36, G2 n=108. **B**, Log relative abundance of ASVs based on how many
 528 generations in which they are found. Out of 658 total ASVs detected, ASVs found in all three
 529 generations are significantly more abundant in the dataset than ASVs found in only one or two
 530 generations. Black squares indicate the mean value. (Kruskal-Wallis test: test-statistic=363.59,
 531 df=2, p-value<0.0001; Post-hoc Dunn’s test with Benjamini-Hochberg correction, 1 vs 3
 532 generations: test-statistic=17.78, adjusted p<0.0001, 2 vs 3 generations: test-statistic=5.879,
 533 adjusted p<0.0001). **C**, Total number of ASVs per parent line and number of ASVs found in G1,
 534 G2, or overlapping. “G1_G2 Overlap” is defined as ASVs present in both the G1 sample and at
 535 least one G2 offspring within a parent line. 99 ASVs were identified as overlapping within
 536 parent lines, and there were overlapping ASVs identified in all 36 lines. **D**, proportion of the
 537 total ASVs per line found in G1, G2, or overlapping. Boxplots represent the median values and
 538 first and third quartiles, and whiskers represent the 95% confidence interval. C, D, and N in
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540 parent line IDs denote lines that received Control, Drought or Nutrient treatment in G1,
541 respectively.
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545 **Figure 2. Prevalence in lines, transmission, core taxa identity and relative abundance of**
 546 **overlapping ASVs in G2 offspring.** Of the 99 ASVs that were found overlapping between G1 and
 547 G2 within parent lines, 29 ASVs that were only in one parent line were removed, and the
 548 remaining 70 ASVs are listed above, ordered from presence in the highest number of lines to
 549 lowest number of lines. Blue dots represent the number of G2 offspring containing the ASV in
 550 each line. 61 of these ASVs are also found in the G0 dataset indicated by black dots. The
 551 taxonomy of each ASV identified at the Order level is indicated by colored dots in the Order
 552 column (26 Orders). Blue and brown squares in the Core_Taxa column indicate identity with
 553 seed or rhizosphere core taxa, respectively. Red boxes in the right-most column represent the

554 log relative abundance of the ASV overall across G1 and G2. There is no significant difference in
555 ASV transmission in G2 offspring between G1 treatments or parental lines (Pearson's Chi-
556 squared Test. G1 Treatment: $X^2=0.67413$, $df=4$, $p=0.9545$. Line: $X^2=63.48$, $df=70$, $p=0.6958$).
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Table 1. Stable ASVs that were detected in all three generations.

Nine ASVs are found in all 36 parental lines, two of which are core seed microbiome taxa, labeled “S”. 13 additional ASVs were present in 50% or more of the parental lines. An additional core seed microbiome member was found in three parental lines. Five of these ASVs align to bean rhizosphere core OTUs at >96% identity, labeled “R”. All ASVs listed are also found in Generation 0 seeds.

ASV	Presence	Core Taxa	Class	Order	Family	Genus
1	All lines	-	Bacteroidia	Bacteroidales	Porphyromonadaceae	<i>Porphyromonas</i>
2	All lines	-	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	
3	All lines	S	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>
4	All lines	-	Bacilli	Staphylococcales	Staphylococcaceae	<i>Staphylococcus</i>
5	All lines	S	Bacilli	Bacillales	Bacillaceae	<i>Bacillus</i>
6	All lines	-	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	<i>Stenotrophomonas</i>
7	All lines	-	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	<i>Escherichia-Shigella</i>
8	All lines	R	Gammaproteobacteria	Burkholderiales	Oxalobacteraceae	<i>Massilia</i>
9	All lines	-	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>
10	35 lines	R	Actinobacteria	Micrococcales	Micrococcaceae	<i>Arthrobacter</i>
11	35 lines	R	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	<i>Ochrobactrum</i>
12	35 lines	R	Actinobacteria	Streptomycetales	Streptomycetaceae	<i>Streptomyces</i>
13	35 lines	-	Bacilli	Bacillales	Bacillaceae	<i>Bacillus</i>
14	34 lines	-	Bacteroidia	Bacteroidales	Bacteroidaceae	<i>Bacteroides</i>
15	34 lines	-	Bacilli	Bacillales	Bacillaceae	<i>Bacillus</i>
16	32 lines	-	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	<i>Xanthomonas</i>
17	28 lines	-	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	
18	28 lines	-	Actinobacteria	Streptomycetales	Streptomycetaceae	<i>Streptomyces</i>
19	27 lines	-	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	<i>Ochrobactrum</i>
20	24 lines	R	Gammaproteobacteria	Burkholderiales	Comamonadaceae	
21	23 lines	-	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	<i>Escherichia-Shigella</i>
22	18 lines	-	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>
50	3 lines	S	Gammaproteobacteria	Enterobacterales	Erwiniaceae	<i>Pantoea</i>

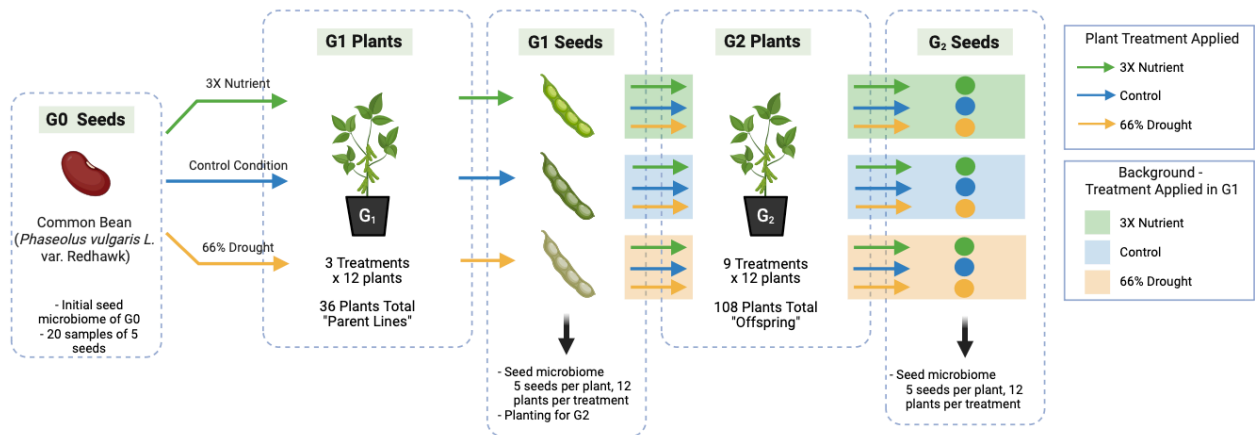
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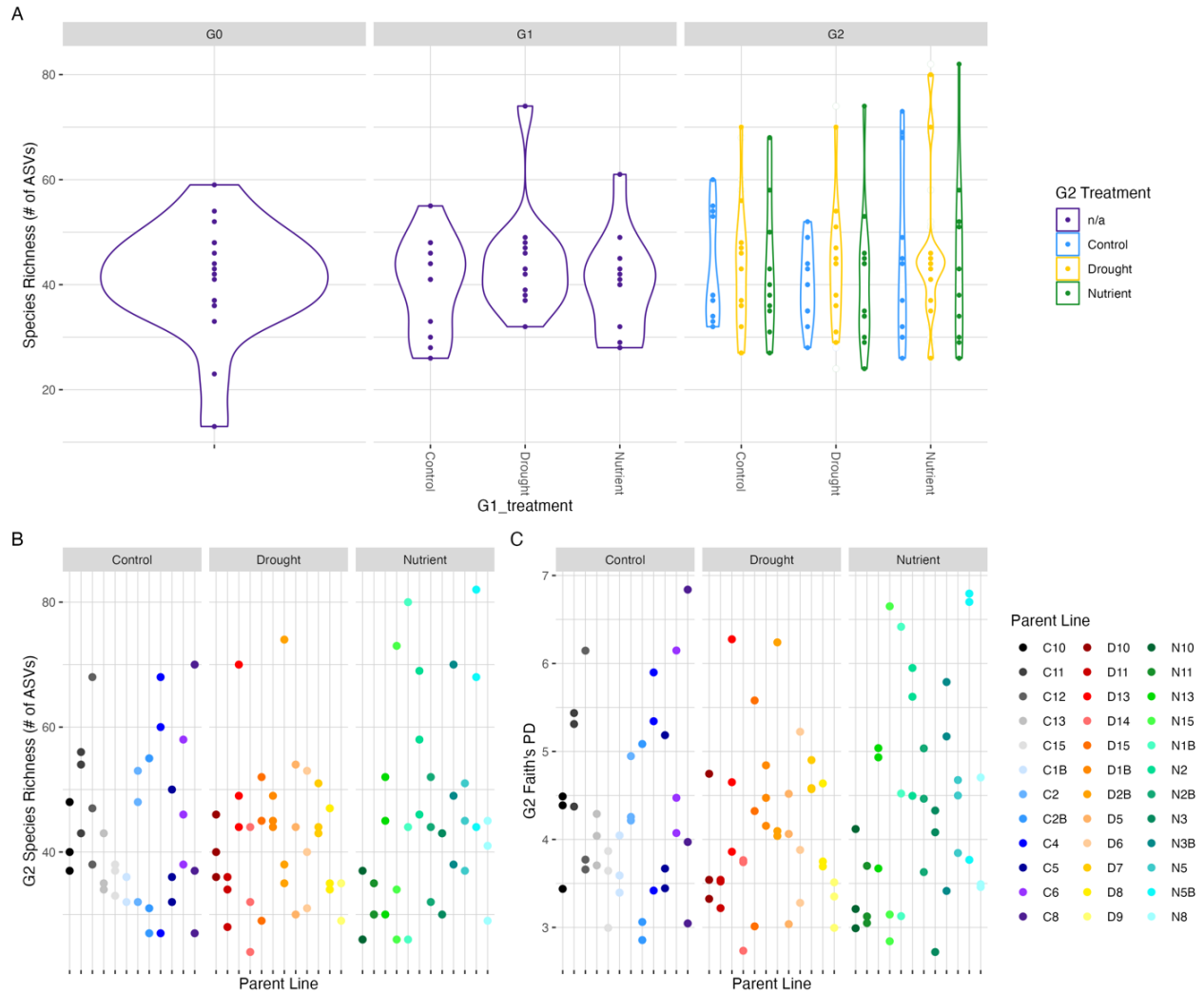
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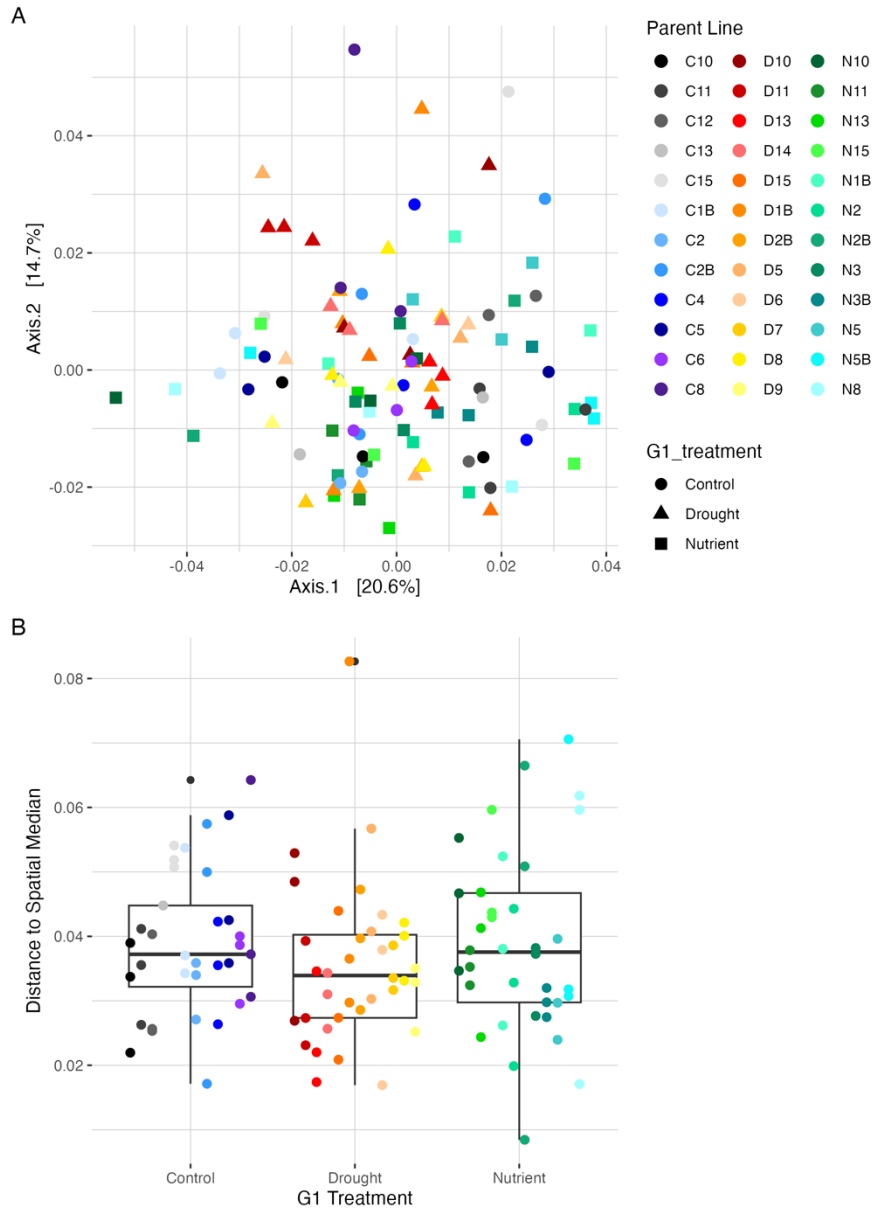
563 Supplemental Data
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566 **Fig. S1. Experimental Design.** Seed microbiome samples were taken from the G0 seed pool and
567 G1 and G2 plants. Treatments were applied in G2 in a full factorial design, where one offspring
568 from each G1 parent line was treated with each of the three treatments in G2.
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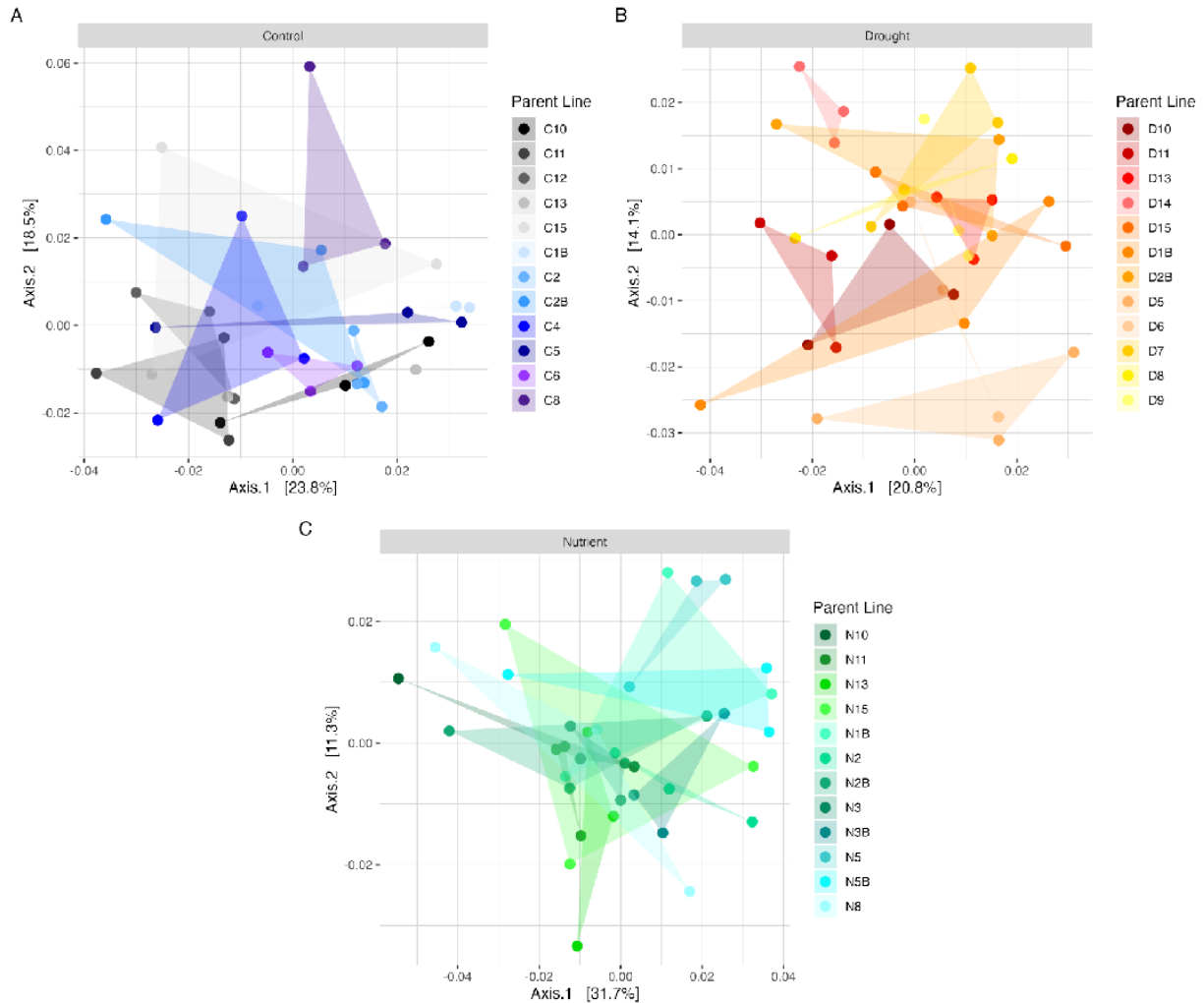


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 572 **Fig. S2. Alpha Diversity. (A)** Number of ASVs observed in each of the seed microbiome samples
 573 across all generations. Five seeds were used for each sample. G0 n=20 samples, G1 and G2 n=12
 574 samples per treatment group. There is no influence of treatment groups on the species richness
 575 observed in either G1 or G2 (ANOVA, G1_treatment: $F=0.150$, $p\text{-value}=0.861$. G1_G2: $F=0.393$,
 576 $p\text{-value}=0.923$). **(B)** Number of ASVs observed, and **(C)** Faith's Phylogenetic Diversity of G2 seed
 577 samples by parental line. Gray bars indicate treatment applied to the parent plant in G1. There
 578 are no significant differences between parent lines in either Richness or PD measure (ANOVA,
 579 Richness: $F=1.122$, $p=0.334$; PD: $F=1.111$, $p=0.346$).
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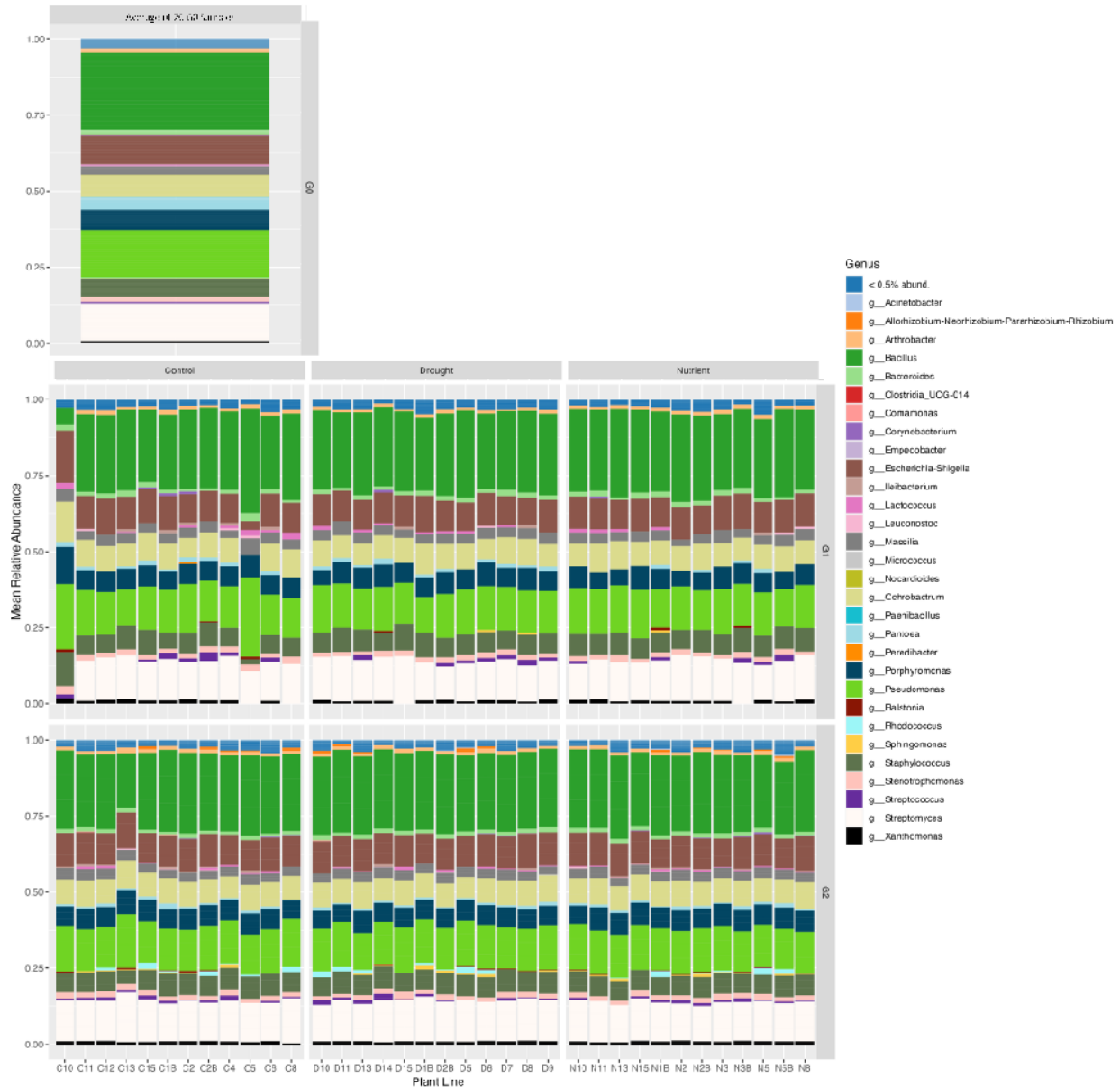
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582 **Fig. S3. Beta diversity in Generation 2 seed samples. (A)** PCoA of Weighted Unifrac distance of
583 G2 seed samples. Points represent three offspring from each parent line, each of which
584 received a different treatment in G2. Parent plant line is the only significant explanatory
585 variable in the G2 samples (PERMANOVA, $r^2=0.356$, $F= 1.2421$, $p=0.0065^{**}$). **(B)** Beta dispersion
586 around the spatial median of Weighted Unifrac distances in G2 seed samples. Lines are grouped
587 by G1 parent treatment, represented by black boxplots. G1 treatment and parent line are not
588 significant. (ANOVA, G1 Treatment: $DF=2$, $F\text{-value}= 1.2835$, $p= 0.2835$. Line: $DF: 35$, $F\text{-}$
589 $\text{value}=1.0825$, $p=0.3714$). Sample G2_9, the line C13 Nutrient offspring, was removed from the
590 figures as an outlier. However, statistics were performed with this sample included.

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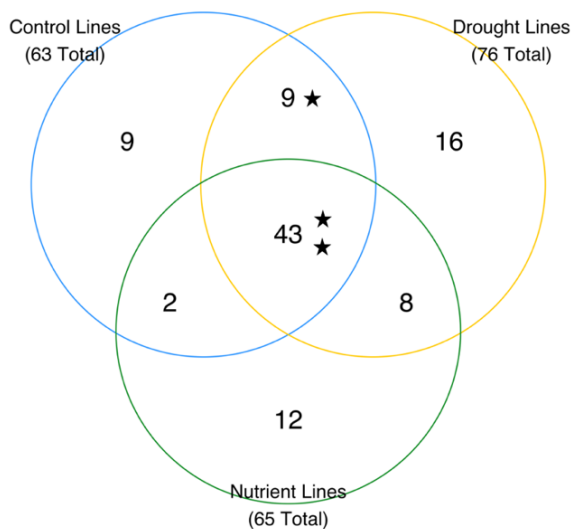
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 595 **Fig. S4. PCoA plots of Weighted Unifrac beta diversity in Generation 2, subset by parent plant**
 596 **treatment.** When plant lines are grouped by G1 parent treatment, parent plant line is not
 597 significant in the control and nutrient lines, but is significant in the drought lines (PERMANOVA,
 598 Control: $r^2= 0.35319$, $F=1.2189$, $p=0.0526$, Nutrient: $r^2= 0.34740$, $F=1.1142$, $p= 0.2687$, Drought:
 599 $r^2= 0.40311$ $F=1.4637$ $p=0.0021^{**}$). Bars above the ordinations indicated the treatment applied
 600 to the parent plants in G1: **A**, Control, **B**, Drought, **C**, Nutrient. The three offspring from each
 601 parent are connected by triangles to aid in visualization. Sample G2_9, the line C13 Nutrient
 602 offspring, was removed from figure A as an outlier. However, statistics were performed with
 603 this sample included.

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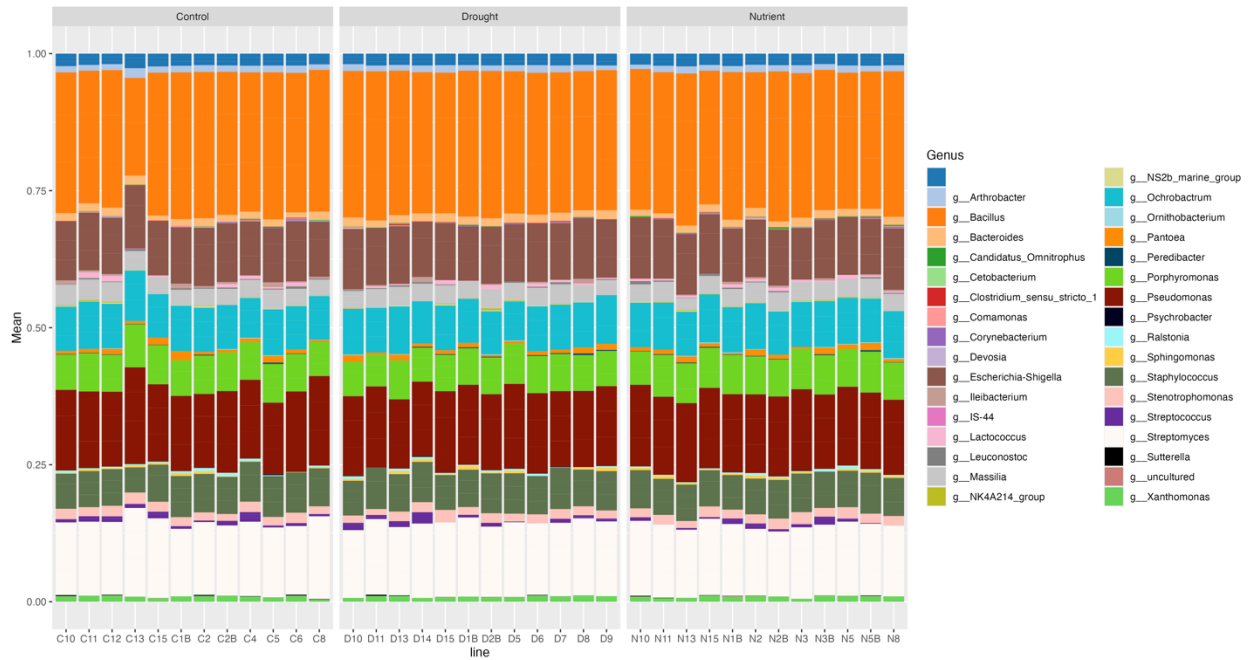
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 611 **Fig. S5. Mean relative abundance of full dataset ASVs identified at the genus level across all**
 612 **three generations.** The bar in the top row is the average of the 20 seed samples from G0. Bars
 613 in the middle row represent G1 parent samples. The bars in the bottom row represent the
 614 average of the 3 G2 offspring samples in each parent line. The “< 0.5% abund.” category
 615 comprises 263 genera less than 0.5% abundant in the dataset. “g__” indicates genus-level
 616 taxonomy.

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622 **Fig. S6. Number of ASVs found overlapping between G1 and G2 within parent lines and**
623 **shared between parent treatment groups.** Stars indicate the presence of core seed
624 microbiome taxa identified by Simonin et al. 2022. There are 99 total ASVs represented, of
625 which 85 can also be found in G0.
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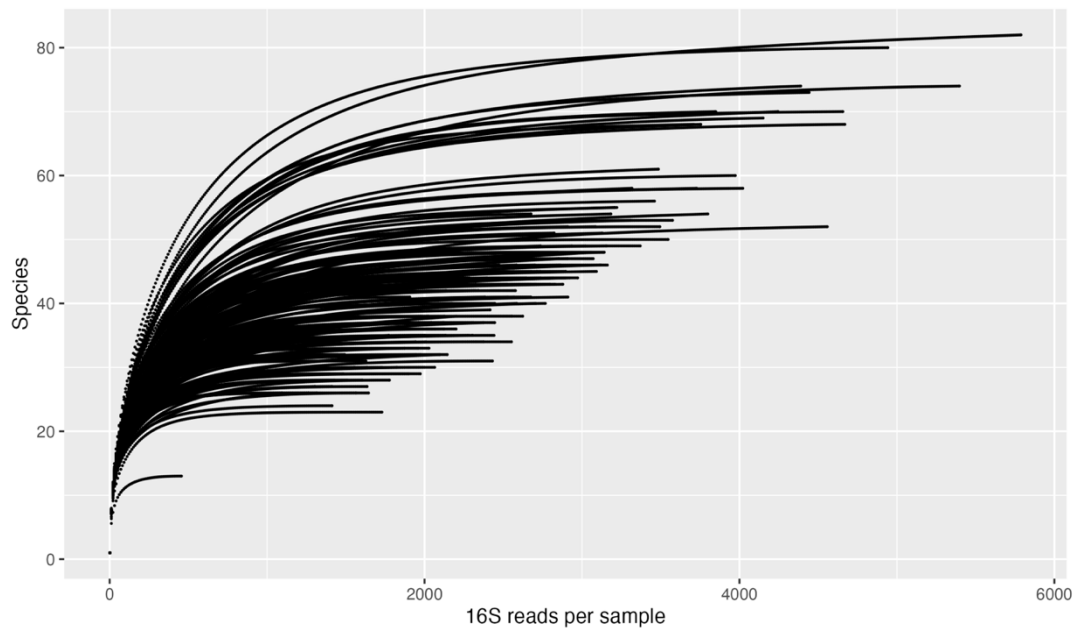
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Fig. S7. Mean relative abundance of 70 prevalent overlapping ASVs across parent lines in G2 identified at the genus level. All other ASVs have been removed from the dataset. The unlabeled legend color represents ASVs that are unresolved at the genus level.

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Fig. S8. Rarefaction curves of quality-filtered microbiome profiles (host reads removed, see methods). Each line represents one seed microbiome sample (pool of 5 seeds from the same parent plant). The DNA read range is 456-5788. All samples reached a plateau indicating a sufficient coverage to characterize seed microbiome diversity.

646 **Table S1.** Statistical tests and their results to address hypotheses of differences in the alpha
 647 and beta diversity of the endophytic seed microbiome according to environmental treatment,
 648 parental line, plant generation, and their interactions.

Analysis	Statistical Test	R command/ package	Variable of Interest	Degrees of freedom	Test Statistic	P-value
Species richness in all three generations	ANOVA	aov()/R stats	Generation	2	F = 0.456	0.634
			G1 Treatment	2	F = 0.150	0.861
			G1_G2 Combined Treatment	8	F = 0.393	0.923
Species Richness in G2	ANOVA	aov()/R stats	Parent Line	35	F = 1.122	0.334
Faith's Phylogenetic Diversity in G2	ANOVA	aov()/R stats	Parent Line	35	F = 1.111	0.346
Beta Diversity of Weighted UniFrac distance, all three generations	PERMANOVA	adonis2()/vegan	Generation	2	R-squared = 0.01600, F = 1.3173	0.1967
			Treatment	2	R-squared = 0.01823, F = 1.5008	0.1148
Beta Diversity of Weighted UniFrac distance, G1	PERMANOVA	adonis2()/vegan	G1 Treatment	2	R-squared = 0.06375, F = 1.1236	0.2948
Beta Diversity of Weighted UniFrac distance, G2	PERMANOVA	adonis2()/vegan	G1 Treatment	2	R-squared = 0.01940, F = 1.1149	0.3038
			G2 Treatment	2	R-squared = 0.01504, F = 0.8646	0.6496
			Parent Line	33	R-squared = 0.35658, F = 1.2421	0.0065 **
not enough samples per group for effective post-hoc analysis with pairwise.adonis2()						
Beta diversity dispersion around spatial median, full G2 dataset	Permutational Test for Constrained Correspondence analysis	betadisper(), permutest()/vegan	G1 Treatment	2	F = 0.5425	0.5878
			Parent Line	35	F = 1.0825	0.3714

Beta Diversity of Weighted UniFrac distance, G2 Control Lines	PERMANOVA	adonis2()/vegan	G2 Treatment	2	R-squared = 0.06729, F = 1.2773	0.1407	
			Parent Line	11	R-squared = 0.35319, F = 1.2189	0.0526	
Beta Diversity of Weighted UniFrac distance, G2 Drought Lines	PERMANOVA	adonis2()/vegan	G2 Treatment	2	R-squared = 0.4607, F = 0.9201	0.5814	
			Parent Line	11	R-squared = 0.40311, F = 1.4637	0.0021	**
Beta Diversity of Weighted UniFrac distance, G2 Nutrient Lines	PERMANOVA	adonis2()/vegan	G2 Treatment	2	R-squared = 0.02902, F = 0.5118	0.9727	
			Parent Line	11	R-squared = 0.34740, F = 1.1142	0.2687	
Relative abundance of ASVs across all generations	Kruskal-Wallis	kruskal_test()/rstatix	ASVs in 1, 2 or 3 generations across dataset	2	363.5942	1.11E-79	****
	Dunn's post-hoc with Benjamini-Hochberg correction	dunn_test()/rstatix	1 vs 2		11.29853	2.00E-29	****
			1 vs 3		17.77871	3.10E-70	****
			2 vs 3		5.87932	4.12E-09	****
Transmission rate in G2 for 70 most common ASVs	Pearson's Chi-squared Test for Count Data	chisq.test()/R stats	G1 Treatment	4	X-squared = 0.67413	0.9545	
			Parent Line	70	X-squared = 63.48	0.6958	

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652 **Table S2.** Excel file: Metadata table with MIMARKS compliant contextual information for each
 653 sample (e.g., plant treatment data, extraction batches).

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655 **Table S3.** Excel file: List of 70 ASVs from Figure 2 with taxonomic identification, core taxa
 656 membership and % identity, and 16S V4 sequence.

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