1 Stable, multigenerational transmission of the bean seed microbiome despite

2 abiotic stress

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16 Running title : Stable seed microbiome transmission across three generations

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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 conservation[3–7]. Plant microbiomes can be assembled via both

35   horizontal transmission from the environment and vertical transmission from the parent plant

36   through the seed microbiome[8–10]. 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 surfaces[10–14]. During germination, seed microbiome members that

39   first colonize the plant can determine priority effects and drive the trajectory of microbiome

40   assembly[15–17]. Additionally, these "inherited" microbiome members enriched by the parent

41   plant may provide important benefits for the offspring [18–21].

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

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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 beans[26], and salt stress in rice[28]. 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 generation[29].

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 microbiome[35].

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

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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.* 2022[36] and to the 48 "core" common bean rhizosphere OTUs identified by

107   Stopnisek and Shade 2021[37]. 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 ASVs[36] were found in the overlapping G1-G2 dataset, specifically from the

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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 taxa[37]

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,

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132   on seeds that were polled 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].

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161   **Online Methods**

162   **Bean cultivar**

163   *Phaseolus vulgaris L.* var. Red Hawk[52], 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.

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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.

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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

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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 H_2O. 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 H_2O. Petri dishes were stored in the dark at room temperature for 3-4

191   days, with an additional 2 mL sterile DI H_2O added after two days. Once seeds had sprouted

192   radicle roots, they were transferred to soil.

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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 trifoliate 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.

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226   **Seed harvest**

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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 μL TE Buffer, 10 μL 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 μL 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 buffer[53]. 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   decontamination[54].

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:GGACTACNVGGGTWTCTAAT[55–59]. Each 25 μL PCR

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271   reaction contained 9.5 µL of MO BIO PCR Water (Certified DNA-Free), 12.5 µL of QuantaBio's

272   AccuStart II PCR ToughMix (2x concentration, 1x final), 1 µL Golay barcode tagged Forward

273   Primer (5 µM concentration, 200 pM final), 1 µL Reverse Primer (5 µM concentration, 200 pM

274   final), and 1 µL of template DNA. The conditions for PCR were as follows: 94 °C for 3 minutes to

275   denature the DNA, with 35 cycles at 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s, with a final

276   extension of 10 min at 72 °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 84[61]. 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 R[62].

292   Data analyses were performed in R version 4.3.1 and R Studio version 2023.06.1+524[63].

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 1.44.0[64]. 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 group[65]. 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 2.6-4[66]. Datasets were then subset for further analysis

303   using the ps_filter() command in the microViz package version 0.10.10[67]. 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 ASVs[43].

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 3.4.2[68]. Faith's Phylogenetic Diversity was calculated with calculatePD() from

312   the biomeUtils package version 0.022[69]. ANOVAs were performed with the base R stats

313   command aov(). Weighted UniFrac distances were calculated with distance() in phyloseq and

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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 0.4.1[70]. 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 1.4.0[71] and statistical analyses were performed with leveneTest()

321   from the car package[72] and kruskal_test() and dunn_test() from the rstatix package[73]. Beta

322   diversity ordinations were created with ordinate() from phyloseq with ggplot2. Additional data

323   analysis was performed in the tidyverse package version 2.0.0[74] and dplyr package version

324   1.1.2[75]. 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.* 2022[36] were compared to transmitted ASVs,

327   and Venn Diagrams were produced with the VennDiagram package version 1.7.3[76]. To compare

328   the prevalent ASVs in this study to the 48 core bean rhizosphere microbiome taxa identified by

329   Stopnisek and Shade 2021[37], 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% identity[77].

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.

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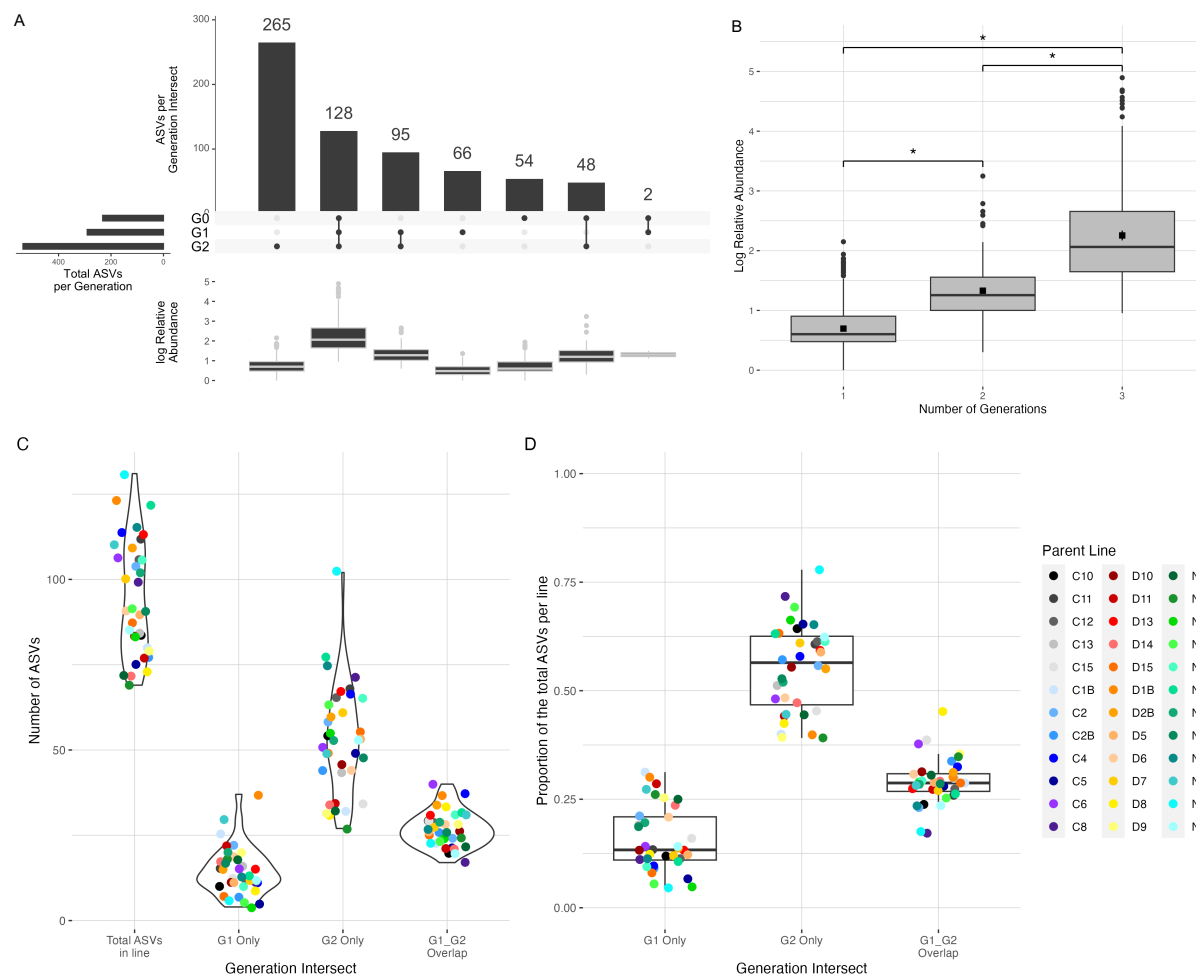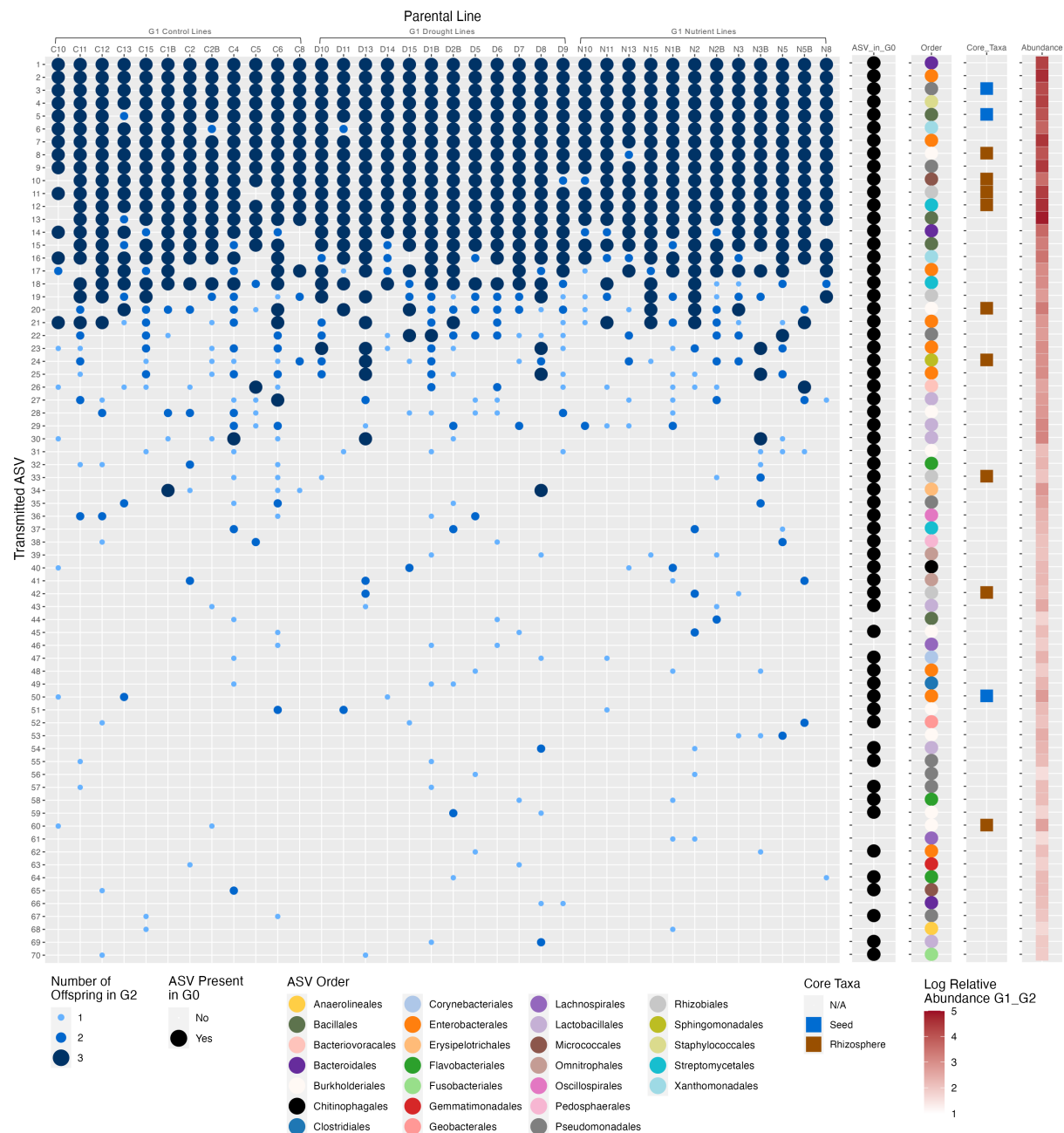523

524 Figures and Tables

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526 Figure 1. Unique and overlapping ASVs between generations. A, Number of ASVs and relative
527 abundance of ASVs per generation intersect across all samples. G0 n=20 samples (five seeds per
528 sample), G1 n =36, G2 n=108. **B,** Log relative abundance of ASVs based on how many
529 generations in which they are found. Out of 658 total ASVs detected, ASVs found in all three
530 generations are significantly more abundant in the dataset than ASVs found in only one or two
531 generations. Black squares indicate the mean value. (Kruskal-Wallis test: test-statistic=363.59,
532 df=2, p-value<0.0001; Post-hoc Dunn's test with Benjamini-Hochberg correction, 1 vs 3
533 generations: test-statistic=17.78, adjusted p<0.0001, 2 vs 3 generations: test-statistic=5.879,
534 adjusted p<0.0001). **C,** Total number of ASVs per parent line and number of ASVs found in G1,
535 G2, or overlapping. "G1_G2 Overlap" is defined as ASVs present in both the G1 sample and at
536 least one G2 offspring within a parent line. 99 ASVs were identified as overlapping within
537 parent lines, and there were overlapping ASVs identified in all 36 lines. **D,** proportion of the
538 total ASVs per line found in G1, G2, or overlapping. Boxplots represent the median values and
539 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

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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.

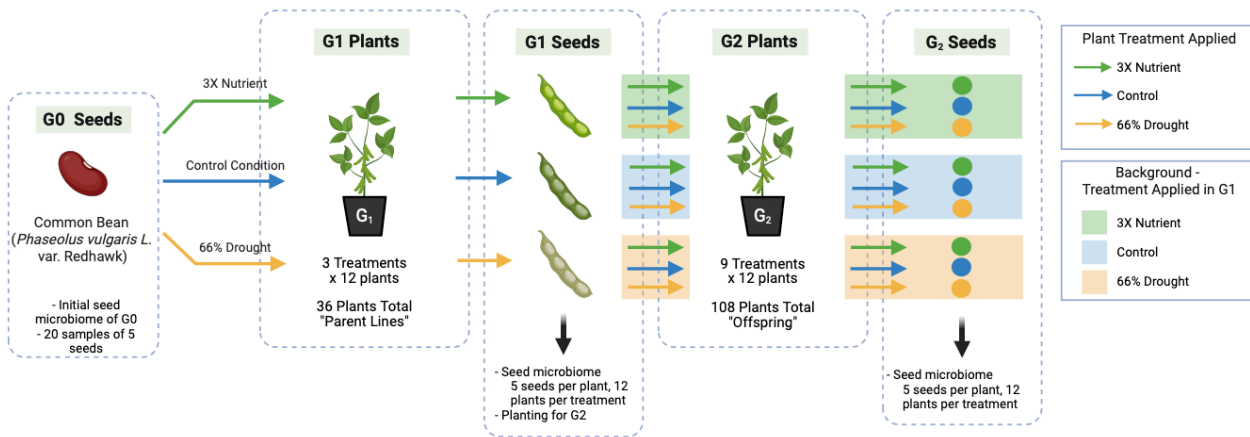| ASV	Presence	Core Taxa	Class	Order	Family	Genus
1	All lines	-	Bacteroidia	Bacteroidales	Porphyromonadaceae	*Porphyromonas*
2	All lines	-	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	
3	All lines	S	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
4	All lines	-	Bacilli	Staphylococcales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

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Supplemental Data

Fig. S1. Experimental Design. Seed microbiome samples were taken from the G0 seed pool and G1 and G2 plants. Treatments were applied in G2 in a full factorial design, where one offspring from each G1 parent line was treated with each of the three treatments in G2.
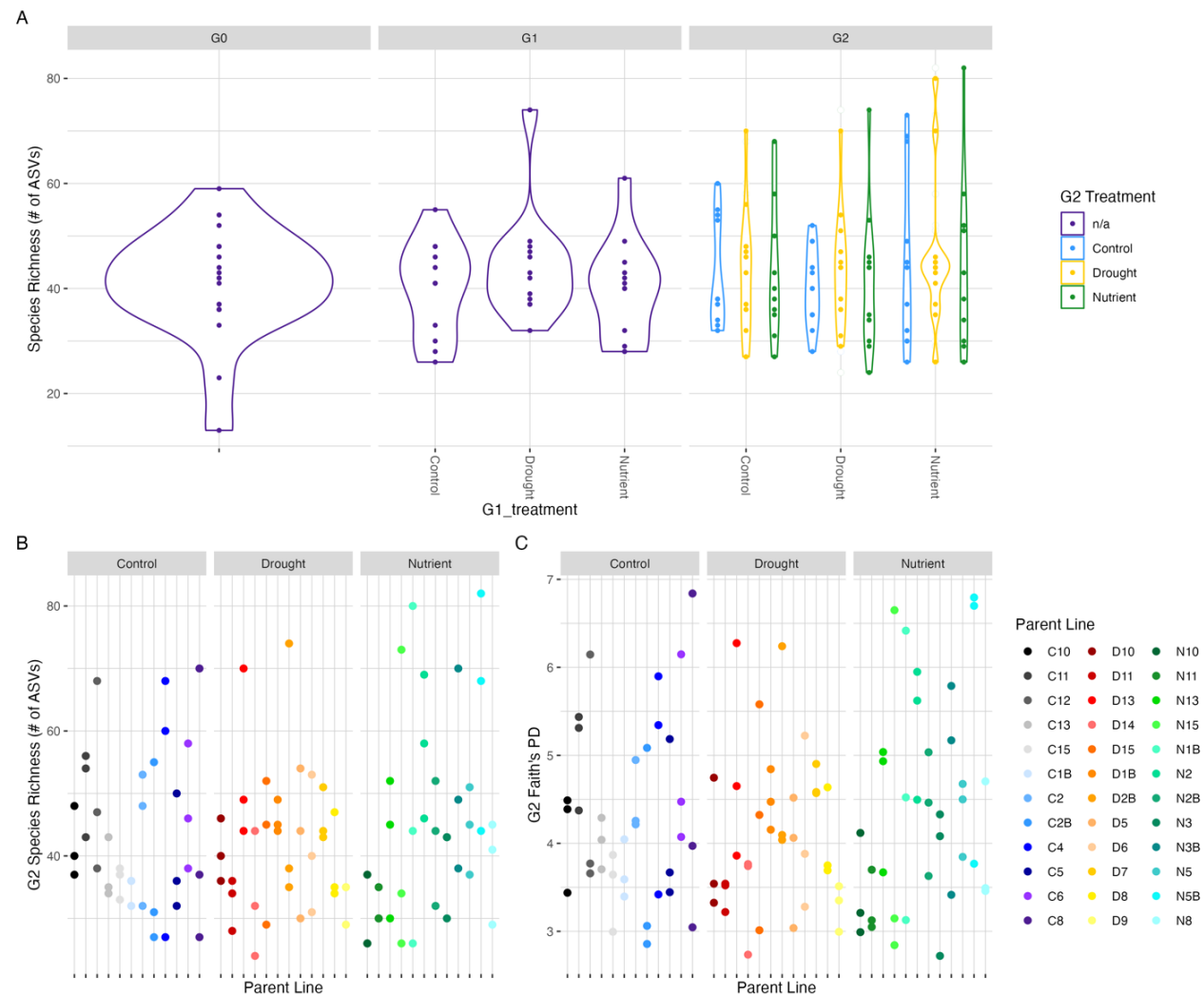
Fig. S2. Alpha Diversity. (A) Number of ASVs observed in each of the seed microbiome samples across all generations. Five seeds were used for each sample. G0 n=20 samples, G1 and G2 n=12 samples per treatment group. There is no influence of treatment groups on the species richness observed in either G1 or G2 (ANOVA, G1_treatment: F= 0.150, p-value=0.861. G1_G2: F=0.393, p-value=0.923). **(B)** Number of ASVs observed, and **(C)** Faith's Phylogenetic Diversity of G2 seed samples by parental line. Gray bars indicate treatment applied to the parent plant in G1. There are no significant differences between parent lines in either Richness or PD measure (ANOVA, Richness: F=1.122, p=0.334; PD: F=1.111, p=0.346).
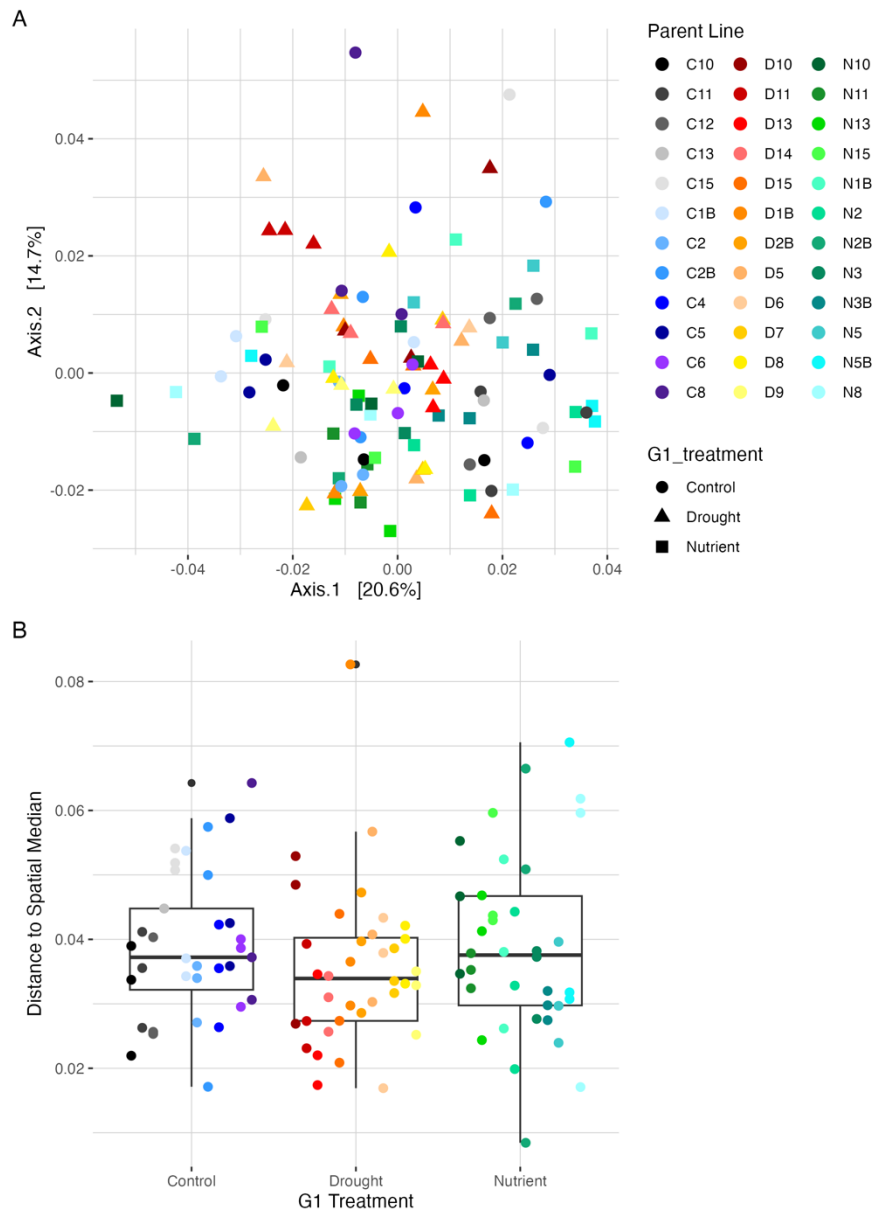
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Fig. S3. Beta diversity in Generation 2 seed samples. (A) PCoA of Weighted Unifrac distance of G2 seed samples. Points represent three offspring from each parent line, each of which received a different treatment in G2. Parent plant line is the only significant explanatory variable in the G2 samples (PERMANOVA, r^2=0.356, F= 1.2421, p=0.0065**). **(B)** Beta dispersion around the spatial median of Weighted Unifrac distances in G2 seed samples. Lines are grouped by G1 parent treatment, represented by black boxplots. G1 treatment and parent line are not significant. (ANOVA, G1 Treatment: DF=2, F-value= 1.2835, p= 0.2835. Line: DF: 35, F-value=1.0825, p=0.3714). Sample G2_9, the line C13 Nutrient offspring, was removed from the figures as an outlier. However, statistics were performed with this sample included.
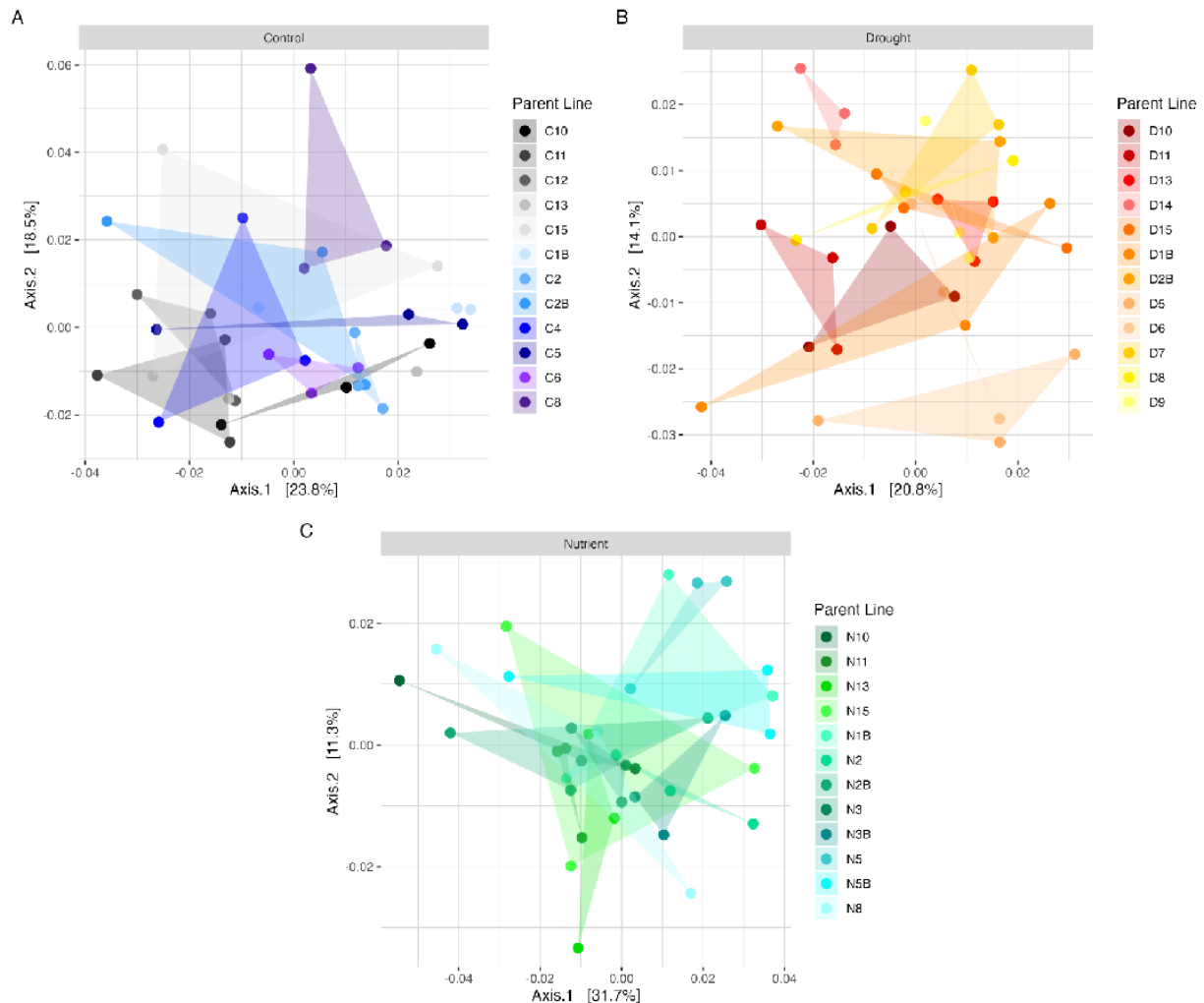
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Fig. S4. PCoA plots of Weighted Unifrac beta diversity in Generation 2, subset by parent plant treatment. When plant lines are grouped by G1 parent treatment, parent plant line is not significant in the control and nutrient lines, but is significant in the drought lines (PERMANOVA, Control: r^2= 0.35319, F=1.2189, p=0. 0526, Nutrient: r^2= 0.34740, F=1.1142, p= 0.2687, Drought: r^2= 0.40311 F=1.4637 p=0.0021**). Bars above the ordinations indicated the treatment applied to the parent plants in G1: **A,** Control, **B,** Drought, **C,** Nutrient. The three offspring from each parent are connected by triangles to aid in visualization. Sample G2_9, the line C13 Nutrient offspring, was removed from figure A as an outlier. However, statistics were performed with this sample included.
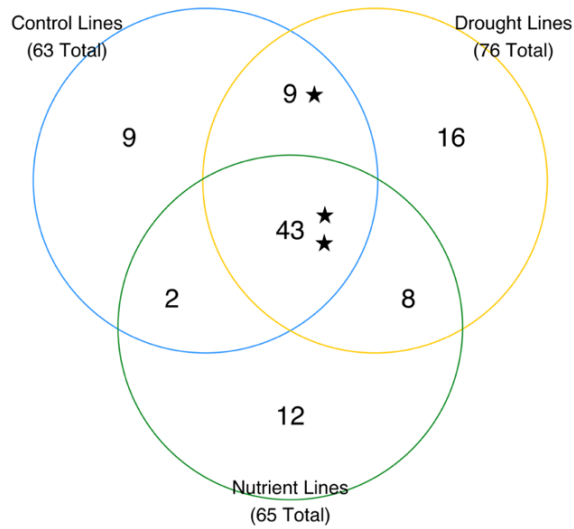
Fig. S5. Mean relative abundance of full dataset ASVs identified at the genus level across all three generations. The bar in the top row is the average of the 20 seed samples from G0. Bars in the middle row represent G1 parent samples. The bars in the bottom row represent the average of the 3 G2 offspring samples in each parent line. The "< 0.5% abund." category comprises 263 genera less than 0.5% abundant in the dataset. "g__" indicates genus-level taxonomy.

Fig. S6. Number of ASVs found overlapping between G1 and G2 within parent lines and shared between parent treatment groups. Stars indicate the presence of core seed microbiome taxa identified by Simonin et al. 2022. There are 99 total ASVs represented, of which 85 can also be found in G0.
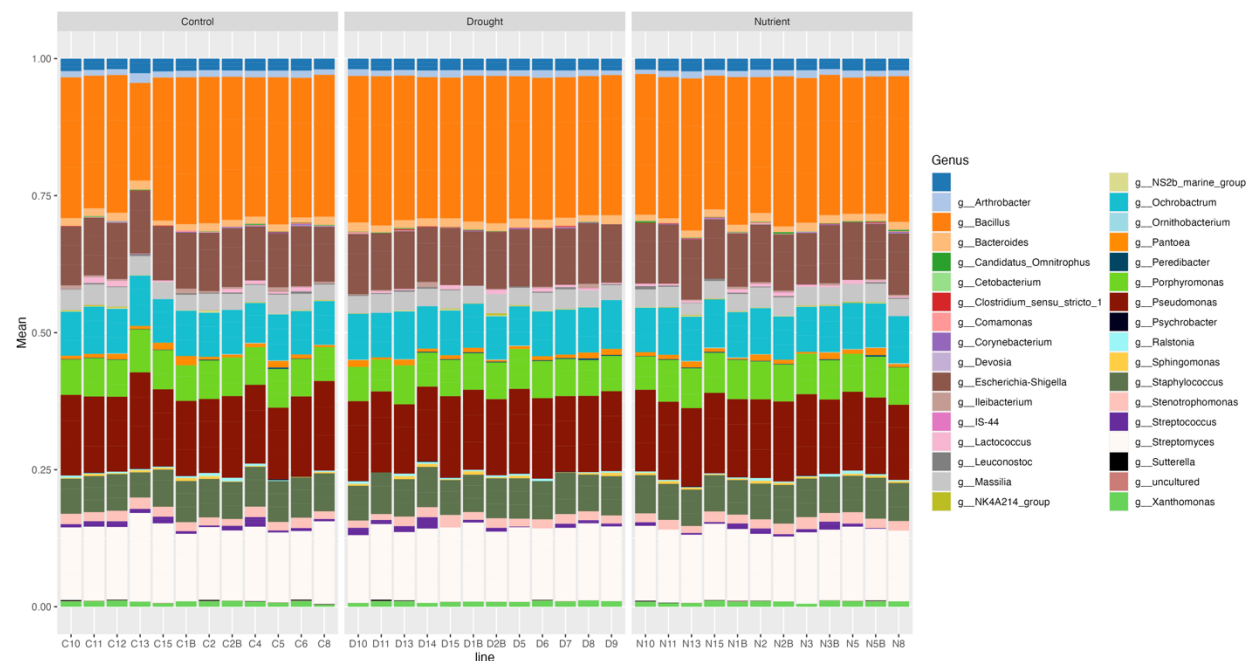
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631 **Fig. S7. Mean relative abundance of 70 prevalent overlapping ASVs across parent lines in G2**
632 **identified at the genus level.** All other ASVs have been removed from the dataset. The
633 unlabeled legend color represents ASVs that are unresolved at the genus level.
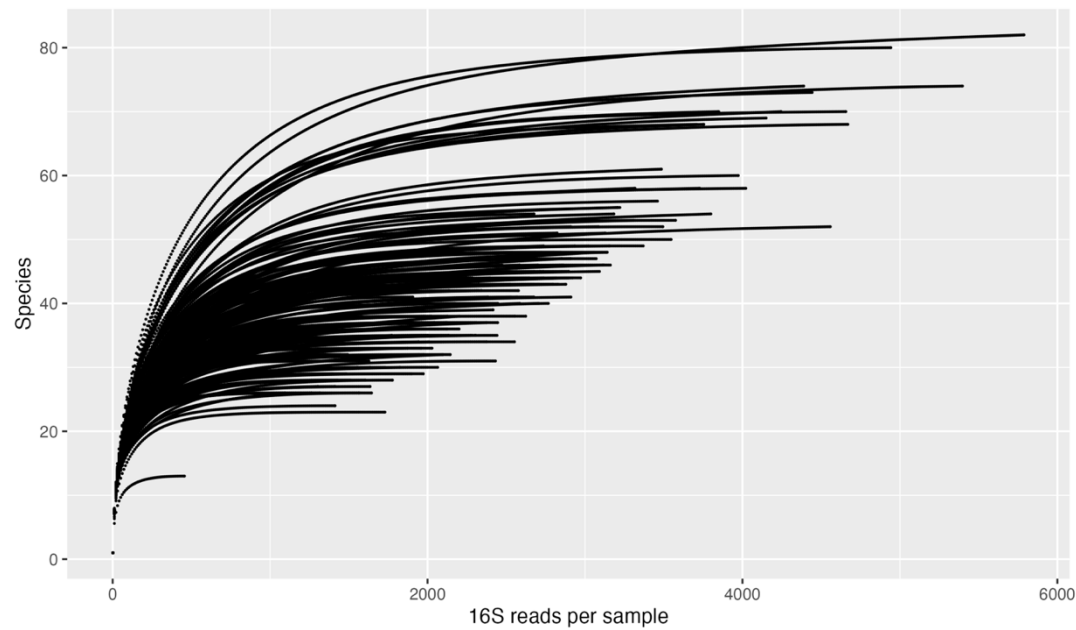634
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639 **Fig. S8. Rarefaction curves of quality-filtered microbiome profiles** (host reads removed, see
640 methods). Each line represents one seed microbiome sample (pool of 5 seeds from the same
641 parent plant). The DNA read range is 456-5788. All samples reached a plateau indicating a
642 sufficient coverage to characterize seed microbiome diversity.
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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 Banjamini-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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