Title: **Abiotic treatment to common bean plants results in an altered seed microbiome**

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Abstract

We performed a pilot study in a controlled growth chamber to investigate how the seed microbiome of the common bean (*Phaseolus vulgaris L.* (var. Redhawk)) was altered under abiotic treatments relevant for crop management with changing climate. Bean plants were subjected to one of three treatments: 66% water withholding to simulate mild drought, 50 % Hoagland nutrient solution to simulate fertilization, or control with sufficient water and baseline nutrition. We performed 16S rRNA gene amplicon sequencing and ITS amplicon sequencing of the endophytic metagenomic DNA to assess seed bacterial/archaeal and fungal community structure, respectively. We found that variability in the seed microbiome structure was high while alpha diversity was low, with tens of taxa present. Water withholding and nutrient addition altered the seed microbiome structure for bacterial/archaeal communities as compared to the untreated control, and each treatment resulted in a distinct microbiome structure. There were no statistically supported differences in the fungal microbiome across treatments. While we discuss several limitations of this study, the promising results suggest that further and more comprehensive investigation is needed to better understand abiotic stress-induced changes in

26 the seed microbiome, the mechanisms that drive those changes, and the implications of seed
27 microbiome changes for the health and stress responses of the next plant generation.
28
29 **Key words**
30 plant microbiome, 16S rRNA gene, ITS, drought, fertilizer, legume, growth chamber, endophyte, vertical
31 transmission, abiotic stress
32
33 **Introduction**
34 The plant microbiome includes bacteria, archaea, fungi, and viruses that associate with the plant
35 and inhabit different plant compartments, including the rhizosphere, phyllosphere, and endosphere
36 (Compant et al., 2019). The plant microbiome plays important roles for plant fitness, including nutrient
37 acquisition (Herrera Paredes et al., 2018), secondary metabolite production (Gargallo-Garriga et al.,
38 2016), flowering time (Panke-Buisse et al., 2015), and resistance to abiotic (Lau & Lennon, 2012; Naylor
39 & Coleman-Derr, 2018) and biotic stresses (Mendes et al., 2011; Ritpitakphong et al., 2016). Plant
40 microbiota can interact with each other as well as with the host plant, and the plant is able to shape its
41 microbiome structure and composition, for example, by producing root exudates or allelochemicals
42 (Jones et al., 2019; Sasse et al., 2018). Because plants are also influenced by the environment, any
43 stresses that a plant experiences are likely to also affect the plant microbiome. These environmental
44 stresses include abiotic stressors, such as changes in water and nutrient availability (limitation or excess)
45 and exposure to high temperatures, and biotic stressors, such as pathogen infection and herbivory.
46 Regardless of the type of stressor, as the plant responds it may alter its microbiome, either as a direct or
47 indirect consequence of the stress. Thus, both the environmental conditions and the host plant can act
48 as important filters that contribute to the ultimate composition of the plant microbiome (Hacquard et
49 al., 2017; Vacher et al., 2016).

50 Managing or manipulating the plant microbiome is one promising strategy to support plant
51 tolerance to environmental stress. We are just beginning to understand how exactly the plant
52 microbiome structure is altered during particular stressors (e.g., drought (Xu et al., 2018), with a focus
53 on the root zone and rhizosphere microbiome. We conducted a controlled study in an environmental
54 growth chamber to characterize changes in the rhizosphere microbiome of the legume common bean
55 under two different stressors of water withholding and nutrient excess. The initial purpose of our pilot
56 study was to identify members of the root microbiome that were particularly resilient either to these
57 stressors. However, the plants set pods at the end of the experiment, and we realized the opportunity to
58 also assess the seed microbiome of the treated plants as compared to control plants. The purpose of
59 this brief report is to share the seed microbiome results from the pilot study, to discuss its limitations,
60 and to suggest immediate future directions based on the most promising results.
61
62 **Methods**
63 ***Plant growth conditions and harvest.*** Common bean seeds were surface-sterilized in a 10% bleach
64 solution and planted in 24 one-gallon pots filled with a mixture of agricultural topsoil, sphagnum peat
65 and sand that had been steam-sterilized at ~100°C. The plants were grown in controlled conditions
66 in a BioChambers FLEX™ LED growth chamber with a 16-hour day/8-hour night cycle at 29°C and 22°C,
67 respectively. The plants were divided into three groups: 8 control plants received ample water (300 mL
68 every other day), 8 plants were subjected to a mild "drought" during plant development, and received
69 66% less water (100 mL every other day) (water withholding), and 8 plants received half strength
70 Hoagland solution (300 mL every other day) provided by the growth chamber facility (nutrient addition).
71 The plants were grown until the R8 stage, when plants were fully developed, and the seed pods began
72 drying.

73 Harvesting was conducted by collecting the bean pods and plant biomass. Bean seeds were
74 removed from the pods and the remaining above ground biomass from each plant was placed in a
75 brown paper bag and dried at 70°C for one week. The root system was gently pulled from the pot,
76 cleaned of excess soil with deionized water and dried at 70°C for one week. Once dried, the above and
77 below ground dry weight was measured for each plant. The remaining soil was collected for soil
78 chemical analysis. One hundred grams of each soil sample was sent to the Michigan State University Soil
79 and Plant Nutrient Laboratory (SPNL) for soil chemical testing. Soil parameters including pH, lime index,
80 phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), nitrate (NO_3^-), ammonium (NH_4^+), and
81 organic matter (OM) were measured from all soil samples.
82
83 ***DNA extraction and amplicon sequencing.*** Twenty seeds from each plant were collected for DNA
84 extraction following the protocol of the previous study (Barret et al., 2015). Seeds were surfaced
85 sterilized in 10% bleach and placed in sterile 50 mL centrifuge tubes with 30 mL of sterile 1X PBS with
86 0.05% Tween 20 and shaken at 140 rpm at room temperature for 4 hours. After shaking, tubes were
87 centrifuged at 500 x g for 15 minutes and the supernatant and seeds were discarded. The pellet was
88 resuspended with 2 mL of sterile 1X PBS-Tween and transferred to a microcentrifuge tube and spun at
89 20,000 x g for 10 min. The supernatant was discarded, and the pellet was used for DNA extraction with
90 the PowerSoil®DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, United States) following
91 manufacturer's instructions. DNA extracted from seeds were quantified with Qubit™dsDNA BR Assay Kit
92 (ThermoFisher Scientific, Waltham, MA, United States) and verified with Polymerase Chain Reaction
93 (PCR) using 515f/806r universal primers (Caporaso et al., 2011) for amplification of the V4 region of the
94 16S rRNA gene for bacterial/archaeal community analysis. The 16S rRNA gene amplification was
95 conducted under the following conditions: 95°C for 5 min, followed by 30 cycles of 95°C (30 s), 54°C (45
96 s), and 72°C (90 s), with a final extension at 72°C (5 min). The amplification was performed in 12.5 µl

97 mixtures containing 6.25 µl GoTaq®Green Master Mix (Promega, Madison, WI, United States), 0.125 µl
98 of each primer (20 mM), 1 µl of DNA template (final concentration of 0.02 - 0.626 ng per µl), and 4.5 µl
99 nuclease free water. Seed DNA (concentration range of 5 - 20 ng per µl) was sequenced at the Research
100 Technology Support Facility (RTSF) Genomics Core, Michigan State sequencing facility using Illumina
101 MiSeq platform.

102 Fungal communities were assessed using PCR amplification of the Internal Transcribed Spacer 1
103 (ITS1) region with the ITS1f/ITS2 primer pair (Walters et al., 2015) with the addition of index adapters as
104 required by the RTSF Genomics Core, (https://rtsf.natsci.msu.edu/genomics/sample-
105 requirements/illumina-sequencing-sample-requirements/). The PCR conditions of ITS gene amplification
106 were as follows: 95°C for 5 min, followed by 30 cycles of 95°C (30 s), 54°C (45 s), and 72°C (90 s), with a
107 final extension at 72°C (5 min). The amplification was performed in 50 µl mixtures containing 20 µl
108 GoTaq®Green Master Mix (Promega, Madison, WI, United States), 1 µl of each primer (20 mM), 4 µl of
109 DNA template (final concentration of 0.02 – 0.626 ng per µl), and 26 µl nuclease free water. The product
110 of the ITS gene amplification was cleaned and purified using the Wizard®SV Gel and PCR Clean-Up
111 System (Promega, Madison, WI, United States), following the manufacturer's protocol. Purified ITS gene
112 amplification products with the concentration range of 5 - 50 ng per µl were sequenced at the RTSF
113 Genomics Core using the Ilumina MiSeq platform. The 16S and ITS libraries were prepared using the
114 Illumina TruSeq®Nano DNA Library Prep Kit. Ilumina MiSeq was run using v2 Standard and paired end
115 reads sequencing format (2 x 250 bp).
116

117 ***Sequencing data analysis and OTU clustering*.** Bacterial/archaeal raw reads produced from Illumina
118 MiSeq were processed including merging the paired end reads, filtering the low-quality sequences,
119 dereplication to find unique sequence, singleton removal, denoising, and chimera checking using the
120 USEARCH pipeline (v.10.0.240) (Edgar & Flyvbjerg, 2015). Operational taxonomic unit (OTU) clustering

121 was conducted using an open reference strategy (Lee et al., 2017). First, closed reference OTU picking
122 was performed at 97 % identity by clustering quality filtered reads against the SILVA database (v.132)
123 (Quast et al., 2013) using USEARCH algorithm (-*usearch_global* command) (Edgar, 2010). Reads that
124 failed to match the SILVA reference were subsequently clustered *de novo* at 97% identity using UPARSE-
125 OTU algorithm (-*cluster_otus* command) (Edgar, 2013). Closed reference and *de novo* OTUs were
126 combined into a full set of representative sequences, and then all merged sequences were mapped back
127 to that set using the -*usearch_global* command.

128 The set of representative sequences were aligned on QIIME 1.9.1 (Caporaso et al., 2010b) using
129 PyNAST (Caporaso et al., 2010a) against the SILVA (v.132) reference database. The unaligned OTU
130 sequences were excluded from the OTU table and the representative sequences. Taxonomic assignment
131 was conducted on QIIME 1.9.1 using the SILVA (v.132) database and the UCLUST default classifier at a
132 minimum confidence of 0.9 (Edgar, 2010). Plant contaminants such as chloroplast and mitochondria;
133 and unassigned taxa and sequences were removed from the OTU table as well as the representative
134 sequences using *filter_taxa_from_otu_table.py* and *filter_fasta.py* command on QIIME. Rarefaction to
135 the lowest sequencing depth (Gihring et al., 2012; Weiss et al., 2017) (11,137 bacterial/archaeal reads)
136 was conducted on QIIME.

137 The processing of fungal ITS raw reads was also conducted using the USEARCH (v.10.0.240)
138 pipeline. Read processing included paired end read merging, primer removal using cutadapt (v.2.0)
139 (Martin, 2011), filtering the low-quality sequences, and dereplication to find unique sequence.
140 Operational taxonomic unit clustering was conducted using an open reference OTU picking strategy.
141 First, closed reference OTU picking was performed by clustering quality filtered reads against the UNITE
142 fungal ITS database (v.8.0) (Kõljalg et al., 2013) at 97% identity threshold using the USEARCH algorithm.
143 Reads that failed to match the reference were clustered *de novo* at 97% identity using the UPARSE-OTU
144 algorithm. Closed reference and *de novo* OTUs were combined into a full set of representative

145 sequences, and then all merged sequences were mapped back to that set using *-usearch_global*
146 command. Fungal taxonomic classification was performed in CONSTAX tool (Gdanetz et al., 2017) at a
147 minimum confidence of 0.8 using the UNITE reference database release 01-12-2017. Assigning
148 taxonomy in CONSTAX was conducted using three classifiers, including RDP Classifier (v.11.5) (Cole et al.,
149 2014; Wang et al., 2007), UTAX from USEARCH (v.8.1.1831) (Edgar, 2013), and SINTAX from USEARCH
150 (v.9.2) (Edgar, 2016). Any contaminants including mitochondria, chloroplast and other unwanted
151 lineages of eukaryotes were removed from the OTU table. Rarefaction was conducted to the lowest
152 number of sequences (21,329 fungal reads) on QIIME.
153
154 ***Microbial community analysis.*** Microbial community analyses were conducted in R (v.3.6.1) (R Core
155 Development Team). Microbial composition and relative abundance were analyzed using Phyloseq
156 package (v.1.28.0) on R (McMurdie & Holmes, 2013). Microbial richness (the number of taxa present)
157 was calculated on the rarefied OTU table using the vegan package (v.2.5-6) (Oksanen et al., 2019). The
158 normality and homoscedasticity of the data were tested using Saphiro-Wilk and Levene's test,
159 respectively. The one-way analysis of variance (one-way ANOVA) or non-parametric Kruskal-Wallis test
160 was then performed to analyze the data. Post hoc Dunn's test with false discovery rate (FDR) correction
161 using the Benjamini-Hochberg adjustment for multiple comparisons was performed to compare plant
162 biomass data among treatments.
163 Beta diversity was calculated on the rarefied OTU table using the vegan package using Jaccard
164 dissimilarity indices and visualized with a principal coordinate analysis (PCoA) plot. We used the Jaccard
165 index, which is based on presence-absence counts rather than relative abundance data, because we
166 reasoned that the seed microbiome members are unlikely to be actively growing inside the seed and
167 that any differences in relative abundances in the seed endophyte are unlikely attributable to
168 competitive growth outcomes *in situ*. Permutational multivariate analysis of variance (PERMANOVA)

169 using the function adonis (Oksanen et al., 2019) was performed to assess the effects of the treatments
170 to the microbial community structure. We performed multivariate analysis to check the homogeneity of
171 dispersion (variance) among groups using the function betadisper (Oksanen et al., 2019).
172
173 ***Data and code availability.*** The computational workflows for sequence processing and ecological
174 statistics are available on GitHub (https://github.com/ShadeLab/BioRxiv_Seed_Microbiome_2020). Raw
175 sequence data of bacteria/archaea and fungi have been deposited in the Sequence Read Archive (SRA)
176 NCBI database under Bioproject accession number PRJNA635871.
177
178 **Results**
179 There were overall differences in plant biomass among treatments (Kruskal-Wallis results Table
180 1). Specifically, plants receiving nutrient-addition were larger in shoot and root biomass than control or
181 mildly droughted plants (Fig. 1, Table 1). Nutrient-addition plants also had higher pod number and pod
182 mass compared to the water withholding and control plants (Fig. 1, Table 1). Rhizosphere soil from the
183 nutrient addition treatment had higher phosphorus and potassium content than the other two
184 treatments (Fig. 2, Table 2). Nutrient addition plants also had rhizosphere soils with higher nitrate
185 relative to control plants (Fig. 2, Table 2).
186 Analysis of bacterial/archaeal and fungal sequences from seed samples resulted in a total of 81
187 and 226 OTUs (97% sequence identity), respectively. Bacterial/archaeal communities in control, water
188 withholding, and nutrient addition seeds had different taxonomic compositions (Fig. 3A).
189 Bacterial/archaeal communities in the control seeds were almost exclusively dominated by the OTUs
190 within the genus *Bacillus,* with a mean relative genus-level abundance of more than 99%. Although the
191 bacterial/archaeal community in the water withholding and nutrient addition seeds were also
192 dominated by *Bacillus*, genus-level taxonomic diversity increased with the addition of other, non-

193 dominating lineages. Specifically, water with-holding and nutrient addition seed communities were also
194 composed of *Virgibacillus*, *Pseudomonas*, and several other bacterial/archaeal genera.
195 Similarly, different plant treatments had different seed fungal community compositions. Even
196 though *Aspergillus* dominated the fungal community in the control and treated seeds, in the treated
197 seeds there was a shift to include other fungal taxa, including some identified as *Penicillium* and
198 *Wallemia* (Fig. 3B). These observations indicate that the seed microbiome is altered when maternal
199 plants are exposed to abiotic stress or environmental alternation. Since the seed microbiomes contained
200 a relatively simple community of tens to dozens of taxa, even alterations in the composition or
201 abundances of few taxa may have consequence for microbiome assembly of the next plant generation.
202 Because we do not expect microbiome members to be actively doubling inside the seed, we
203 used a presence-absence assessment (Jaccard index) of beta diversity. There was a statistically
204 supported difference in bacterial/archaeal microbiome composition between treated seeds and control
205 seeds (Fig. 4A, permuted multivariate analysis of variance, PERMANOVA, F-stat = 4.73, R^2 = 0.31, P-val =
206 0.001). In contrast, there was no distinct clustering of fungal communities associated with different
207 treatments (Fig. 4B, PERMANOVA P-val > 0.05). These results indicate that abiotic treatments alter the
208 bacterial/archaeal but not fungal community composition in the common bean seed. Also, there were
209 differences variability in composition (multivariate dispersion) among treatments for bacterial/archaeal
210 communities (permutated analysis of multivariate dispersion, PERMDISP, F=7.553, P-val=0.0033), but
211 not for fungal composition (PERMDISP F=0.491, P-val=0.619). This provide additional evidence that
212 abiotic treatments can lead to increased variability in seed microbiome composition. Notably,
213 PERMANOVA was found to be largely unaffected by heterogeneity for balanced designs (Anderson &
214 Walsh, 2013).
215
216 **Discussion**

217 Seed microbiomes are an endpoint of microbiome assembly for the maternal plant's
218 reproductive compartment, but also the starting point of microbiome assembly for the new seedling
219 (Shade et al., 2017). Seed microbiomes are composed of early colonizers that are acquired from the
220 maternal plant either through the vascular system or floral stigma, and also of late colonizers that are
221 acquired via seed contact with its environment (Maude, 1996). Moreover, the seed microbiome also can
222 directly impact the seed and seedling in ways that are important for crop establishment, such as by
223 releasing the seed from dormancy and promoting seed germination and seedling emergence (Goggin et
224 al., 2015; Lamichhane et al., 2018). While the vast majority of plant-associated microorganisms are
225 acquired from the surrounding environment, (e.g., aerosols (Vacher et al., 2016) and soils (Edwards et
226 al., 2015)), vertical transmission of microbes via the seed has been reported for a variety of plant
227 species, as recently summarized (Shade et al., 2017) and reported (Bergna et al., 2018; Rodríguez et al.,
228 2020). For the majority of seed microbiome members, their identities, functions and persistence are
229 either not known or not well-understood. However, it is plausible that some of members of the seed
230 microbiome that are altered as a result of stress may have consequence for the health or resilience of
231 the next plant generation. Specifically, a depletion of beneficial members or enrichment of pathogens
232 could disadvantage the plant, while an enrichment of beneficial members could advantage the plant.
233 We highlight three important observations from this study. First, treatment of the parent plant
234 altered the seed microbiome structure and composition compared to control plants, especially for the
235 bacterial/archaeal community. Second, bacterial/archaeal community from the seeds of treated plants
236 had more variation as compared to the seeds from control plants. This suggests that abiotic stress
237 results in changes analogous to those observed during other types of microbiome "dysbiosis" (aka Anna
238 Karenina effects: higher variability across replicates, increased beta-dispersion, and higher contribution
239 of stochastic assembly processes (Zaneveld et al., 2017)). The expectation of high variability should be
240 taken into consideration for future studies, as sufficient replication will be needed to power statistical

241 tests. Third and finally, the fungal community was, on balance, stable relative to the bacterial/archaeal
242 community, suggesting that the persistence of fungal members is less sensitive to water-withholding
243 and nutrient addition.

244 We acknowledge that this is a pilot study and that these results are preliminary. We offer a
245 discussion of some of the major considerations and limitations in interpreting the results and for
246 planning future seed microbiome studies.

247 A first consideration is that there is an apparent maximum stress to plants that can be applied
248 when investigating its consequence for a seed microbiome. After stress exposure is released, plants
249 must be healthy enough to produce pods and seeds, and a balance must be achieved in which plants are
250 stressed but still able to become fully mature. This constraint in stress exposure will never
251 accommodate an experimental design of severe or prolonged stress. However, the investigation of a
252 mild or moderate stress is still valuable because it is pertinent to agriculture. There many situations in
253 which non-lethal stress occurs over part of a growing season, but then crops recover fully or partially to
254 produce some yield. Therefore, the result of mild or moderate stress for seed microbiomes has real-
255 world relevance.

256 Another consideration is the definition and directness of abiotic treatment, and whether an
257 abiotic treatment is expected to act on the plant, the microbiome, or both. In this pilot study, we applied
258 two different abiotic treatments: one that was expected to stress the plant directly (water-withholding
259 to simulate mild drought) and one that was expected to weaken a legume's relationship with its root-
260 associated microbiome and symbiotic nitrogen fixers as nitrogen fixers are down-regulated by nitrogen
261 application (Müller & Pereira, 1995; Wilker et al., 2019) (nutrient addition). Thus, the addition of
262 nutrients was a benefit to the plant, rather than a stress, as indicated by the increased root and shoot
263 masses. However, nutrient addition caused a clear shift in the seed microbiome, demonstrating the
264 potential of fertilizer use to have multi-generational for plant microbiome assembly. Therefore,

265 management practices that advantage the plant as far as yield and health in the short term could have
266 long-term consequences for plant-microbiome relationships.
267 A clear limitation of the study is the substrate used for plant growth, which, with the microbes in
268 and on the original seeds, serves as a starting source for the assembly of the new plant's microbiome
269 (Barret et al., 2015; Shade et al., 2017). For the pilot, used agricultural topsoil mixed with sphagnum
270 peat and sand and sterilized, and this mix is provided by the growth chamber facility. The exact origin
271 and physical/chemical characteristics of facility topsoil us unknown, and so it is unclear how
272 representative this soil may be of bean field soil. Additionally, the initial microbial community in the soil
273 was not analyzed before planting the common bean plants, so we cannot determine the origin of the
274 observed microbial consortia in the seeds and to what extent they overlap with the potting substrate.
275 Previous work suggests that soil type can have a large influence on the seed endophytic bacteria in rice
276 (Hardoim et al., 2012), and this is likely also true for other plant seeds. We observed a dominance of a
277 specific taxon from genus *Bacillus* in common bean seeds in all three treatments. While *Bacillus* taxa
278 were reported to be enriched in the green bean seedling (Barret et al., 2015), steam sterilization of the
279 growth chamber soil may have killed most of the indigenous microbial taxa that were not spore-formers
280 or otherwise resistant to heat (and, bacilli are known to be resistant to such treatments, as per
281 (Nicholson et al., 2000)). Therefore, the microbial composition that were observed in these bean seeds
282 may not be representative of or similar to those in bean seeds grown in the field, or even in different
283 substrates. We urge caution in generalizing from the compositional changes, but rather to focus on the
284 larger changes in beta-diversity and dispersion that were consistently observed across very different
285 abiotic treatments and may be more characteristic of seed microbiome responses. Future work should
286 focus efforts on using soil that is representative of the typical agricultural environment of common
287 bean, and the existing microbial community in the soil should be sequenced prior to planting for
288 comparison to the seed microbiome.

289 Another limitation of this study the absence of negative controls for DNA extraction controls.
290 Seed endophytes contain a very low total biomass of microbial cells. Here, we pooled twenty seeds to
291 use for one extraction to increase the microbial biomass yield for microbiome interrogation. However,
292 we did not perform a buffer-only control, which would allow for assessment of contaminants from the
293 DNA extraction process. While the surface-sterilization of the seeds prior to extraction and negative PCR
294 controls provide confidence that the starting material was not compromised and that we did not
295 unintentionally amplify contaminants from the PCR reagents, we cannot know if there were or
296 extraction kit or buffer contaminants that contributed to the observed seed microbiome composition.
297 A final minor limitation is in the choice of bacterial marker gene. We performed amplicon
298 sequencing of the 16S rRNA gene for the bacterial/archaeal community analysis in the seed. It is well
299 known that the variability of rRNA copy numbers among bacterial species can lead to an inflation of
300 species richness and obfuscate relative abundances of taxa (Větrovský & Baldrian, 2013). Moreover, the
301 most resolved taxonomic level achieved from 16S rRNA amplicon gene sequences often is genus, rather
302 than species or strain. Using a single-copy marker gene that has higher precision and sensitivity at the
303 species level, like the *gyr*B gene, may be a valuable alternative (Barret et al., 2015). The *gyr*B gene has
304 been successfully applied to other seed microbiome studies (Barret et al., 2015; Rezki et al., 2016; Rezki
305 et al., 2017). It may be valuable to consider use of both marker genes to the same seed microbiome
306 samples, so that taxonomic precision can be maximized and compared across seeds with *gyrB*, while
307 also maintaining an ability to source-track and compare composition across the many plant-microbiome
308 16S rRNA amplicon datasets that have been deposited publicly.
309 In summary, while this pilot study provides a key insight into the response of the seed
310 microbiome structure to abiotic treatment in the host plant, there is much more work to be done. Next
311 steps include exposing the plants to more severe drought and nutrient excess conditions, quantifying
312 the physiological status of plants to determine their experience of stress, using representative field soil

313 for plants and assessing the field soil microbiome to deduce seed taxon origins, using negative controls
314 for both DNA extractions to identify contaminants, and considering use of an alternative marker gene
315 for improved precision in microbial taxonomy and taxon abundances.
316 Despite noted considerations and limitations, we posit that this pilot study revealed an
317 important insight regarding how seed microbiomes may be altered after abiotic treatment of a plant.
318 Next, we need to understand the implications of this change for both the host plant and the microbial
319 community. An altered seed microbiome may have positive, negative, or entirely neutral outcomes for
320 the next plant generation. Additional work is needed to understand these outcomes over consecutive
321 plant generations to determine the effects on plant fitness and resilience. If positive or negative
322 outcomes are detected, this work opens a new direction of research that could spur exciting
323 applications in plant-microbiome management.
324

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332
333

334 **Tables**

335 Table 1. Comparison of plant biomass among treatments. Bolded rows are significantly different as

336 determined by Kruskal-Wallis test and post-hoc Dunn's test with Benjamini-Hochberg false discovery

337 rate (FDR) correction

	Kruskal-Wallis test			**Post-hoc Dunn's test with Benjamini-Hochberg FDR correction**			
Parameter	**Chi-squared**	**df**	**p-val**	**Comparison**	**Z**	**P. unadj**	**P. adj**
Shoot mass	20.165	2	**0.00004**	Control – Nutrient addition	-2.29810	0.02156	**0.03233**
				Control – Water withholding	2.19203	0.02838	**0.02838**
				Nutrient addition – Water withholding	4.49013	0.00001	**0.00002**
Root mass	15.365	2	**0.00046**	Control – Nutrient addition	-3.42947	0.00060	**0.00181**
				Control – Water withholding	-0.07071	0.94363	0.94363
				Nutrient addition – Water withholding	3.35876	0.00078	**0.00117**
Pod mass	18.305	2	**0.00010**	Control – Nutrient addition	-2.08597	0.03698	**0.03698**
				Control –Water withholding	2.19203	0.02838	**0.04257**
				Nutrient addition – Water withholding	4.27800	0.00002	**0.00006**
Pod number	17.973	2	**0.00013**	Control – Nutrient addition	-2.53347	0.01129	**0.01694**
				Control – Water withholding	1.67708	0.09353	0.09353
				Nutrient addition – Water withholding	4.21055	0.00003	**0.00008**

338

339

340 Table 2. Comparison of rhizosphere soil chemistry of the three treatments using one-way ANOVA or

341 Kruskal-Wallis test. Bolded rows are significantly different as determined by Kruskal-Wallis test and post-

342 hoc Dunn's test with Benjamini-Hochberg false discovery rate (FDR) correction.

	Kruskal-Wallis test			**Post-hoc Dunn's test with Benjamini-Hochberg FDR correction**			
Parameter	**Chi-squared**	**df**	**p-value**	**Comparison**	**Z**	**P. unadj**	**P. adj**
pH	14.634	2	**0.00066**	Control – Nutrient addition	3.824263	0.00013	**0.00039**
				Control – Water withholding	1.83057	0.06716	0.06716
				Nutrient addition – Water withholding	-1.99369	0.04619	0.06928
P	15.613	2	**0.00041**	Control – Nutrient addition	-3.60033	0.00032	**0.00095**
				Control – Water withholding	-0.39018	0.69640	0.69640
				Nutrient addition – Water withholding	3.21014	0.00133	**0.00199**
K	19.172	2	**0.00007**	Control – Nutrient addition	-4.34149	0.00001	**0.00004**
				Control –Water withholding	-1.67822	0.09330	0.09330
				Nutrient addition – Water withholding	2.66327	0.00774	**0.01161**
$NO3^-$	14.261	2	**0.0008**	Control – Nutrient addition	-3.67776	0.00024	**0.00071**
				Control –Water withholding	-2.58150	0.00984	**0.01476**
				Nutrient addition – Water withholding	1.09625	0.27297	0.27297
Mg	4.8514	2	0.08842				
OM	0.53985	2	0.7634				
	One-way ANOVA test						
Parameter	**F-value**	**df**	**p-value**				
Ca	2.035	2	0.156				
$NH4^+$	0.343	2	0.713				

343

344

345 **Figure legends**

346 **Fig 1**. Plant aboveground (shoot) and belowground (root) biomass for control, water withholding, and
347 nutrient addition treatments of common bean. Plant biomasses were calculated on eight plant
348 replicates for each treatment. For each box plot, circles represent a single plant measurement within a
349 treatment. The central horizontal lines represent the mean, the outer horizontal lines of the box
350 represent the 25th and 75th percentiles. Boxes labelled with different letters were significantly different
351 by a Kruskal-Wallis and post-hoc Dunn's test with a Benjamini-Hochberg false discovery rate correction.

352

353 **Fig 2**. Plant rhizosphere soil chemistry for control, water with-holding, and nutrient addition treatments
354 of common bean. For each box plot, circles represent one rhizosphere measurement within a treatment.
355 The central horizontal lines represent the mean of measurement, the outer horizontal lines of the box
356 represent the 25th and 75th percentiles. Boxes labelled with different letters are identified as
357 significantly different by a Kruskal-Wallis and post-hoc Dunn's test with a Benjamini-Hochberg false
358 discovery rate correction.

359

360 **Fig 3.** Mean relative abundances of genera of bacterial/archaeal (A) and fungal (B) detected in the seed
361 across control, water with-holding and nutrient addition treatments. Each bar represents the endophyte
362 microbiome from DNA extracted from 20 seeds collected from one plant replicate within a treatment.
363 Bacterial/archaeal and fungal genera with mean relative abundances of less than 1 and 10 %,
364 respectively, were grouped into the 'Other' classification, which includes many lineages (not
365 monophyletic).

366

367 **Fig. 4** Principal coordinate analysis (PCoA) plot of the bacterial/archaeal (A) and fungal community (B) in

368 the common bean seed based on the Jaccard index. Symbol colors and shapes represent different

369 abiotic treatments.

370

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Figure 1

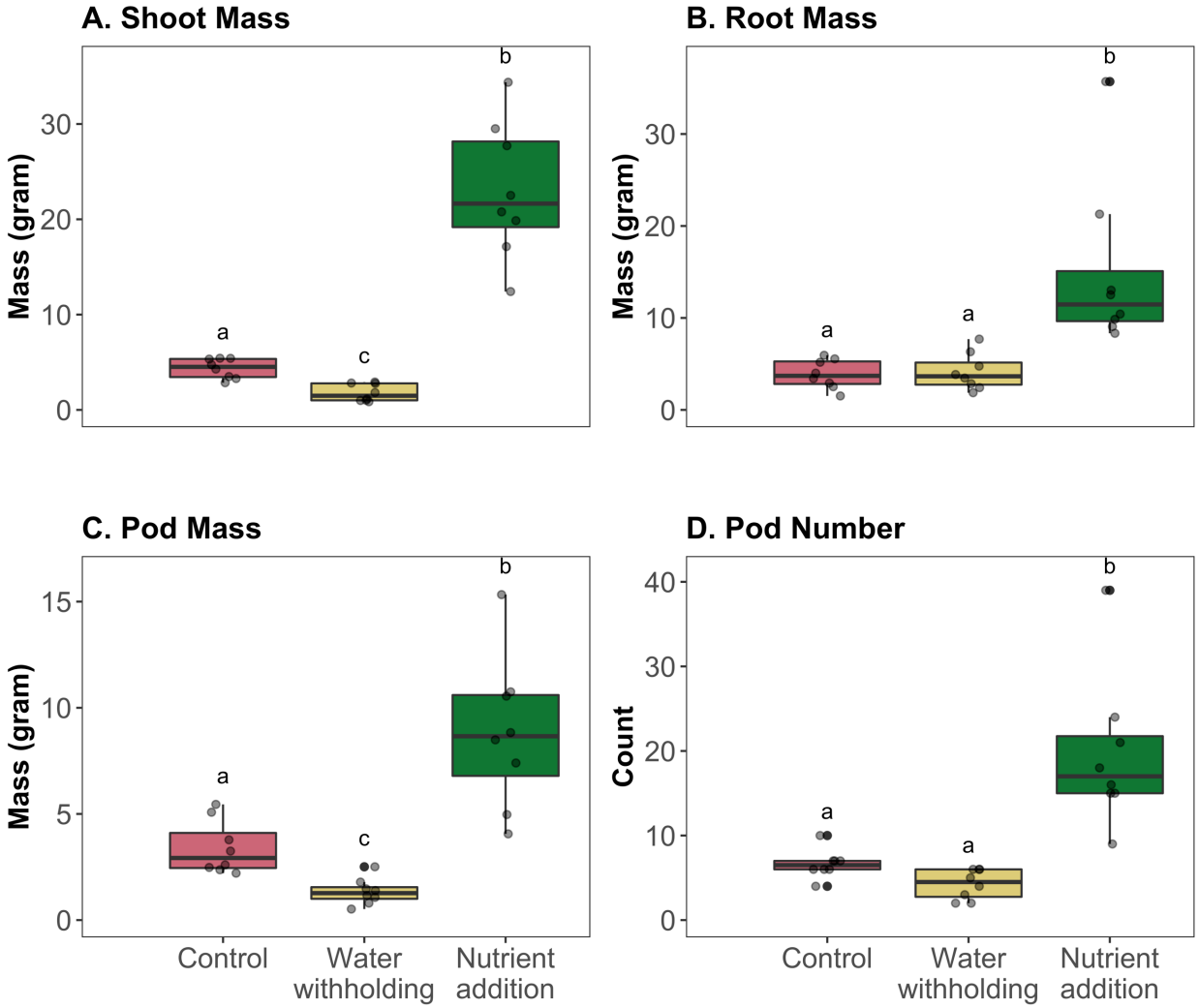
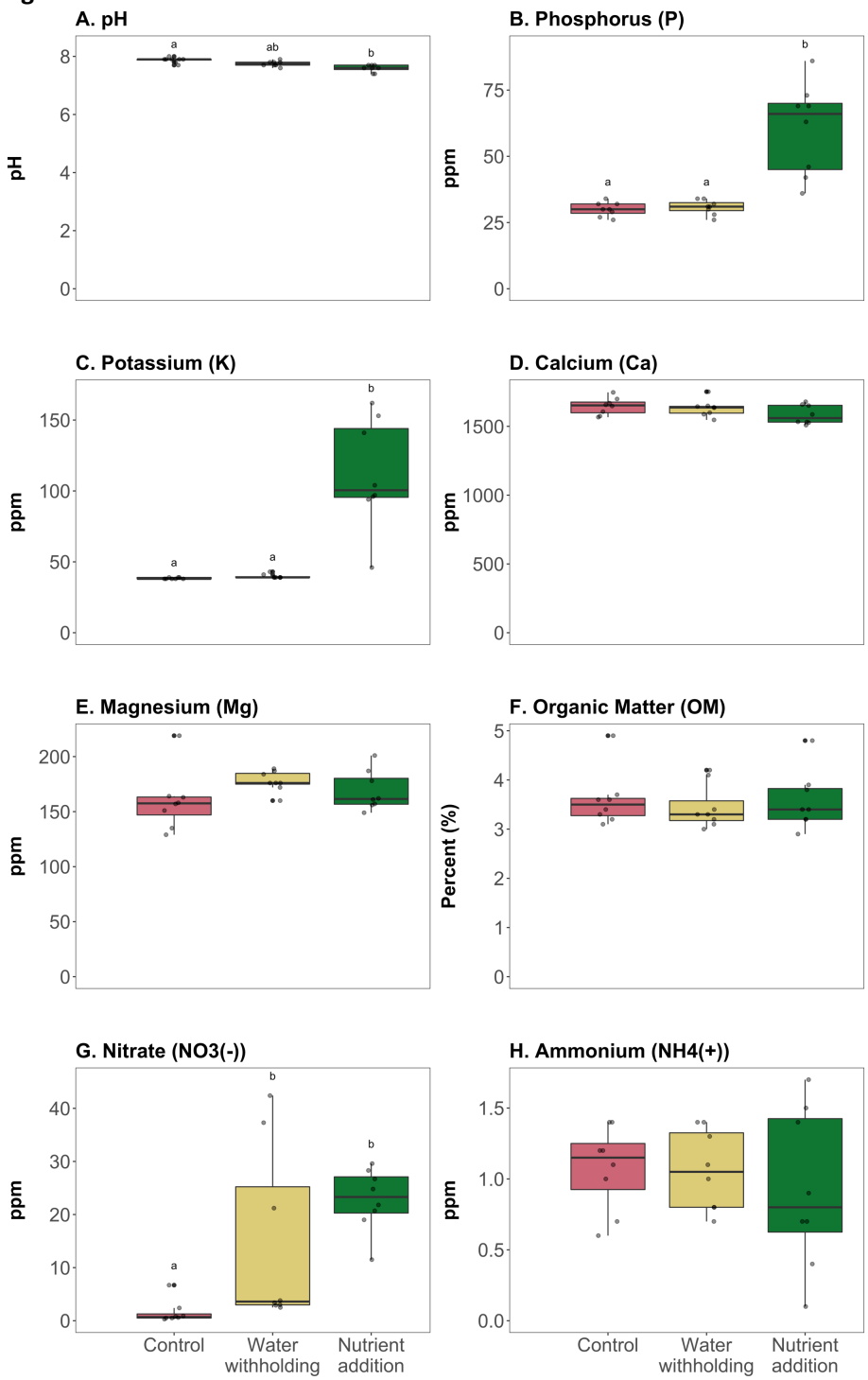
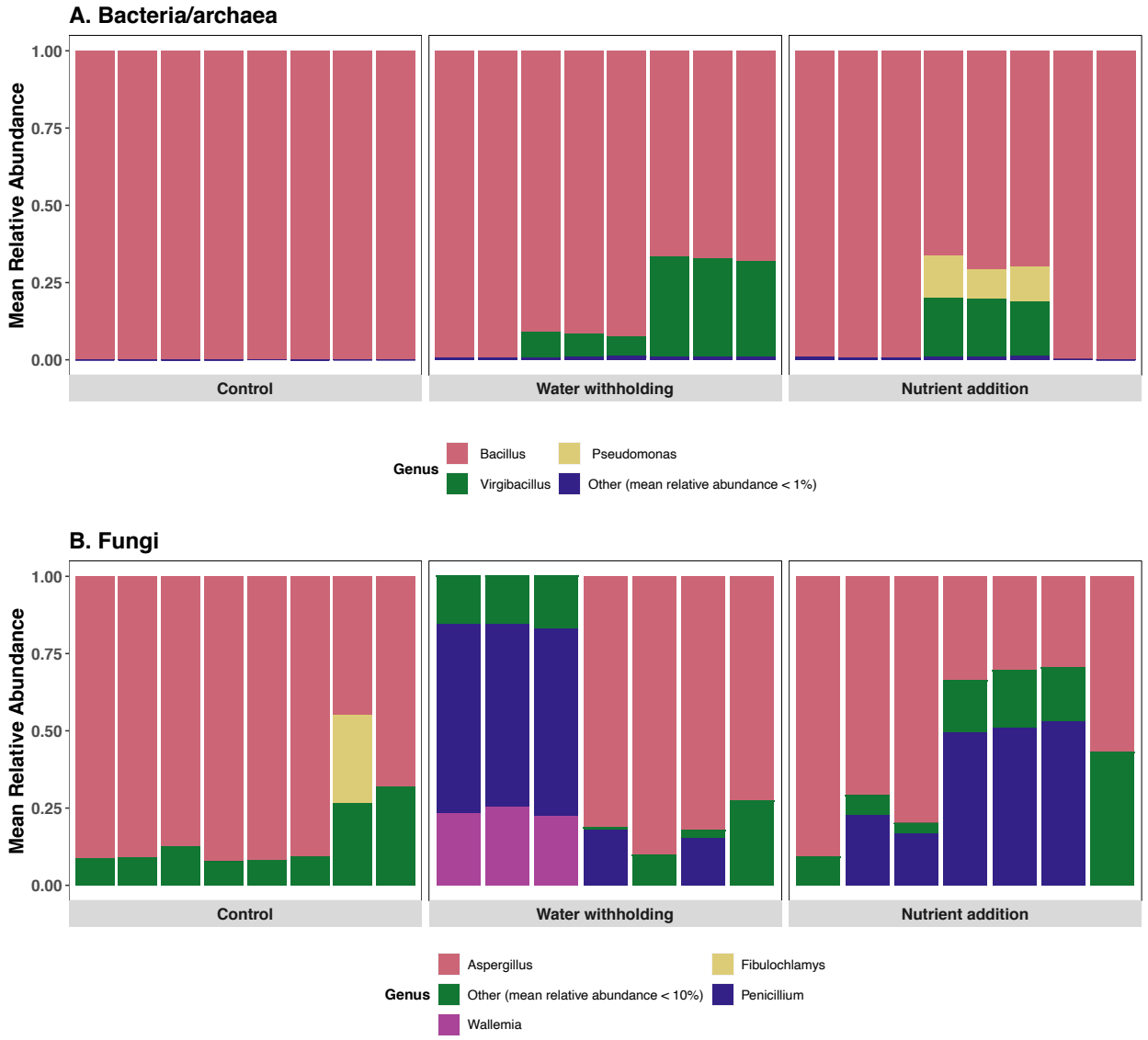

A. Shoot Mass
B. Root Mass
C. Pod Mass
D. Pod Number
Mass (gram)
Count
a
c
b
Control
Water withholding
Nutrient addition

Figure 2


A. pH
pH
B. Phosphorus (P)
ppm
C. Potassium (K)
ppm
D. Calcium (Ca)
ppm
E. Magnesium (Mg)
ppm
F. Organic Matter (OM)
Percent (%)
G. Nitrate (NO3(-))
ppm
H. Ammonium (NH4(+))
ppm
Control
Water withholding
Nutrient addition

519 **Figure 3**

520



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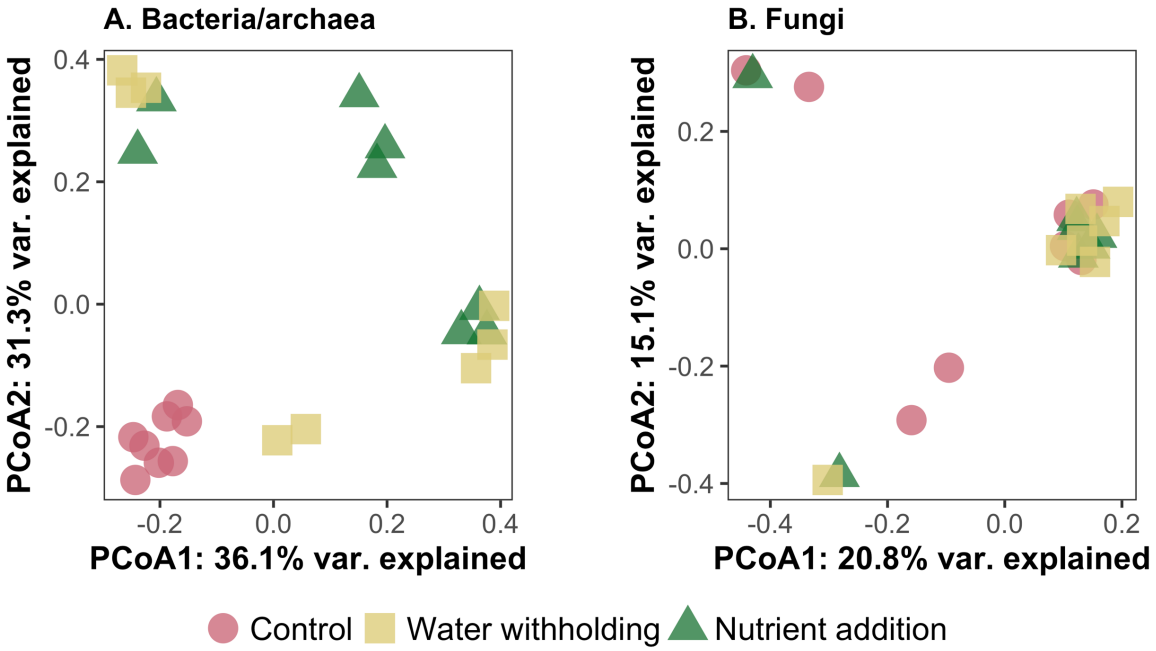
524 **Figure 4**



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