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1 Plant phenology influences rhizosphere microbial community and is accelerated by serpentine
2 microorganisms in *Plantago erecta*.
3
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12 Keywords: serpentine; plant-microbe interaction; rhizosphere; plant development; Plantago
13 erecta
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17 ABSTRACT

18 Serpentine soils are drought-prone and rich in heavy metals, and plants growing on serpentine
19 soils host distinct microbial communities that may affect plant survival and phenotype. However,
20 whether the rhizosphere communities of plants from different soil chemistries are initially
21 distinct or diverge over time may help us understand drivers of microbial community structure
22 and function in stressful soils. Here, we test the hypothesis that rhizosphere microbial
23 communities will converge over time (plant development), independent of soil chemistry and
24 microbial source. We grew *Plantago erecta* in serpentine or nonserpentine soil, with serpentine
25 or nonserpentine microbes and tracked plant growth and root phenotypes. We used 16S rRNA
26 barcoding to compare bacterial species composition at seedling, vegetative, early-, and late-
27 flowering phases. Plant phenotype and rhizosphere bacterial communities were mainly structured
28 by soil type, with minor contributions by plant development, microbe source and their
29 interactions. Serpentine microorganisms promoted early flowering in plants on non-serpentine
30 soils. Despite strong effects of soil chemistry, the convergence in bacterial community
31 composition across development demonstrates the importance of the plant-microbe interactions
32 in shaping microbial assembly processes across soil types.

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36 INTRODUCTION

37 Plant-microbe associations occur on a small scale, but can impact global patterns, including plant
38 and microbial biodiversity (Cui and He 2009; Ravichandran and Thangavelu 2017; Kandlikar et
39 al. 2019). Plants associate with distinct microbial communities that can benefit plants by
40 enhancing nutrient acquisition (Emami et al. 2018; Fei et al. 2020) and protection against
41 pathogens (De Curtis et al. 2010; Akhtar et al. 2011). These associations generate plant-soil
42 feedbacks which can influence plant community structure (Van Der Heijden et al. 2006). Plant-
43 microbe associations have also been explored for their ability to impact the phenotypes of
44 agricultural plants (Gouda et al. 2018). As a result, microbial amendments are being developed
45 for their ability to influence plant yield (Murgese et al. 2020) and stress tolerance (Orlandini et
46 al. 2014; Kwak et al. 2018). However, the extent to which soil community members establish in
47 the rhizosphere, and when during a plant's development, remain poorly understood and may
48 affect the efficacy of microbial amendments.
49
50 Soil chemistry and plant species both influence the composition of rhizosphere microbial
51 communities (Haichar et al. 2008; Berg and Smalla 2009). Plant development, or phenology, has
52 been shown to correlate with distinct microbial associations. For example, seedlings of
53 *Arabidopsis thaliana* showed distinct microbial communities from later-phase plants (Chaparro
54 et al. 2014). In *Oryza sativa*, rhizosphere microbial communities are dynamic during vegetative
55 growth and can represent particular life phases (Edwards et al. 2018). One mechanism for plant
56 effects on rhizosphere communities is through the rhizodeposition of root exudates, which can
57 change over time and correlates with distinct rhizosphere microbial communities observed at
58 each phase of plant development (Chaparro et al. 2013; Zhalnina et al. 2018). However, whether
59 the same plant species assemble microbial communities similarly in distinct soil backgrounds
60 remains unexplored.
61
62 Serpentine soils are characterized by low water-holding-capacity, elevated concentrations of
63 heavy metals, low concentrations of essential plant nutrients, and high Mg to Ca ratios (O'Dell et
64 al. 2006). These characteristics are partially responsible for the low plant productivity and
65 endemism observed on serpentine soils (Anacker 2014). While most plants cannot grow on
66 serpentine soils and other plants can only grow on serpentine soil, serpentine-indifferent plants

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67 are able to thrive on serpentine soils and compete on non-serpentine soils (Safford et al. 2005).
68 Serpentine-indifferent plants, with their ability to grow both on and off serpentine soils, are an
69 excellent tool with which to study how soil chemistry influences microbial composition and
70 phenology (Igwe and Vannette 2019). In addition, by utilizing soil treatments with non-adapted
71 microorganisms we can understand how phenology is influenced by different microbial
72 communities.
73
74 Abiotic and biotic factors including soil chemistry and soil moisture have also been shown to
75 influence plant phenology; for example, plants growing on serpentine and drought-prone soils
76 have generally been shown to flower sooner than those growing on non-serpentine and non-
77 droughted soils (Sherrard and Maherali 2006; Wright et al. 2006; Rossington et al. 2018;
78 Sakaguchi et al. 2019), but this is not always the case (Schneider 2017). Therefore, an additional
79 goal of our experiments was to investigate the abiotic vs biotic control on phenology, especially
80 as it relates to flowering time.
81
82 In this study, we aimed to answer the following broad ecological question: Do plant rhizosphere
83 microbial community grown in disparate soil chemistries converge or diverge over time? More
84 specifically, we test the hypothesis is that soil chemistry influences how microbial communities
85 change over plant development. Further, we predict that rhizosphere microbial communities
86 associated with serpentine-indifferent plants growing on serpentine and non-serpentine soils will
87 become more similar as the plant develops. We also hypothesize that serpentine components
88 introduced to nonserpentine soils, including serpentine microbes, nickel or simulated drought,
89 will change microbial communities and plant characteristics to be similar to those of live
90 serpentine soils. For example, if soil chemistry is the major driver of flowering time, then we can
91 expect that treatments with the same soil origin, regardless of microbial community, will not
92 significantly differ in phenology. If the microbial community influences plant phenology to a
93 greater extent than soil chemistry, we can expect to see significant differences in plant
94 development in soil treatments with non-adapted microorganisms relative to soil treatments with
95 adapted microbes. It is important to understand the relative influence of these factors on plant
96 phenology because the reproductive success of an individual plant and the plant community

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97 structure is directly related to plant phenology (Fenner 1998; Rodríguez-Pérez and Traveset
98 2016; Hidalgo-Triana and Pérez-Latorre 2018).
99
100 METHODS
101 *Study system and soil collection*
102 Soils were collected from McLaughlin Natural Reserve in June 2018 from three serpentine and
103 three nonserpentine sites (Sites 1, 2, and 3 from Igwe and Vannette 2019). McLaughlin Natural
104 Reserve is characterized by a Mediterranean climate with hot and dry summers from April to
105 October. Gallon-sized plastic bags of soil were collected every 5-meters across a 20-meter
106 transect at each site at an average depth of 10 cm. These soils were placed on ice in the field and
107 then in Sterilite plastic containers at 4ºC until the start of the experiment in July 2018. We used
108 *Plantago erecta* (Serpentine Affinity Mean = 1.0), which is common in serpentine and non-
109 serpentine sites locally (Safford et al. 2005). Seeds used in the experiment were purchased from
110 S&S Seeds in 2016 (Carpinteria, CA) after field-collected seeds germinated poorly.
111
112 *Growth chamber experiment*
113 We conducted an experiments aimed to examine how background soil chemistry and soil
114 microbial community jointly influence plant growth and microbial community assembly in the
115 rhizosphere. All soils included an autoclaved soil background (serpentine or nonserpentine), to
116 which live (unautoclaved) soils from either serpentine or nonserpentine soils were added at
117 approximately 16% (v/w) to create the microbial amendments (Farrer and Suding 2016;
118 Calderón et al. 2017). Soils were autoclaved at 120ºC at 15 psi for two 30-min periods with 24-
119 hours between sterilizations (Ishaq et al. 2017). Using this method, four factorial treatments were
120 created: autoclaved serpentine soil with serpentine microbes (S+Sm), autoclaved nonserpentine
121 soil with nonserpentine microbes (NS+NSm), autoclaved serpentine soil with nonserpentine
122 microbes (S+NSm), autoclaved nonserpentine soil with serpentine microbes (NS+Sm). In
123 addition, to explore which dimensions of serpentine soils shape plant-microbe-soil interactions
124 (Wright et al. 2006), we amended some NS+Sm treatments with either nickel (final
125 concentration of 25 ppm; NS+Sm+Ni), or grew plants in conditions simulating drought stress
126 (NS+Sm+Drought).
127

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128 *Plantago erecta* seeds were added to D16 Deeppots (volume: 16 in^3 and 262 mL; Stuewe and
129 Sons., Inc, Tangent, OR) containing ~100 g of soil in one of six soil chemistries above. Plants
130 were grown in a growth chamber under 12:12 light/dark regime at 20ºC at the UC Davis
131 Environmental Horticulture Greenhouse Complex and were grown to senescence, with 15
132 replicates per treatment and 3 non-planted controls per treatment. The simulated drought soil
133 treatment was watered until the soil was saturated once a week while all other soil treatments
134 were watered daily with DI water. Leaf number and plant height were recorded weekly until
135 senescence. Plants in each treatment were harvested at seedling, vegetative, early flowering, and
136 late flowering phases and a random subset (N=6) were used for rhizosphere soil collection,
137 microbial DNA extraction, and 16S rRNA sequencing. Plants were classified as 'seedlings' upon
138 emergence from the soil. When true leaves were present, plants were classified as 'vegetative'.
139 'Early flowering' was characterized by shoot development and the presence of an undeveloped
140 terminal protuberance. Once the plant began to bloom, the plant was characterized as 'late
141 flowering'. Once the plant became brittle to the touch, they were classified as 'senescing'. For
142 two treatments (NS+Sm+Ni) and (NS+Sm+Drought), only seedling and late flowering phases
143 were harvested due to limitation in growth chamber space.
144
145 *Rhizosphere soil collection*
146 For each harvested plant, roots were shaken to remove loosely adhering soil. An ethanol-
147 sterilized razor was used to separate the stem from the roots. Above-ground plants were dried at
148 80ºC for 48 hours and then weighed.
149 Roots were separated from rhizosphere soils. Briefly, roots were sonicated in 0.9% NaCl/0.01%
150 Tween 80 (v/v) solution for 180 seconds to remove the tightly adhering soil particles (Barillot et
151 al. 2013). Centrifuge tubes containing NaCl/Tween (without roots) were then centrifuged for 20
152 minutes at 4ºC at 3234 x g. The pellet was frozen at -20ºC until DNA extraction using a Zymo
153 fecal/soil DNA extraction kit according to enclosed directions (Zymo Research, Irvine, CA).
154 After sonication, root samples were stored in 50% ethanol solution until root image analysis.
155 DNA extraction was confirmed using a Nanodrop 1000 spectrophotometer (ThermoScientific,
156 Waltham, MA, USA) then samples were submitted to the Centre for Comparative Genomics and
157 Evolutionary Bioinformatics Integrated Microbiome Resource at Dalhousie University for PCR
158 and sequencing. The V6-V8 subregion of the 16S SSU rRNA was amplified using B969F

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159 (ACGCGHNRAACCTTACC) and BA1406R (ACGGGCRGTGWGTRCAA) primers (Comeau
160 et al. 2011). DNA was amplified using Phusion High-Fidelity DNA polymerase (NEB) and
161 MiSeq (300+300 bp PE) for final amplicon lengths that were 508 bp. Raw sequences are
162 archived at www.ncbi.nlm.nih.gov/sra/PRJNA623253.
163
164 *Root imaging*
165 To analyze root length, volume, surface area and diameter, samples were scanned using
166 WinRHIZO optical scanner and software (Regent Instruments Inc., Canada). Each root sample
167 was imaged individually by laying them flat onto a tray containing 50% ethanol to cover the
168 entire root. Tangled roots were carefully separated with forceps and any roots broken off were
169 also imaged. Before root parameters were measured, any residual soil particles and foreign root
170 fragments scanned by the imaging software were eliminated from the selected root image. To do
171 this, the entire root was selected by drawing a box around the imaged root. Next, foreign
172 particles were excluded from analysis by selecting *Regions - Exclusion Regions* and drawing a
173 box around each individual target region. Once this step was completed, root parameters
174 including root length, root diameter, root surface area, and root volume were measured by
175 selecting *Image - Image with Analysis*.
176
177 *Bioinformatics*
178 Amplicon sequence variants (ASVs) from 16S rRNA amplicons were identified using DADA2
179 (v1.7.2) (Callahan et al. 2016a). Briefly, paired-end fastq files were processed by filtering and
180 truncating forward reads at position 250 and reverse reads at position 200. Sequences were
181 dereplicated, merged and error-corrected according to code archived on Dryad. Chimeras were
182 removed, and the taxonomy assigned using the SILVA database (v128) (Quast et al. 2012;
183 Yilmaz et al. 2014; Glöckner et al. 2017). A phylogenetic tree based on 16S sequences was
184 created using the DECIPHER package (v2.8.1) in R to perform multi-step alignment and
185 phangorn (v2.4.0) to construct the tree using neighbor-joining (Wright et al. 2006; Schliep 2011).
186 The sequence table, taxonomy, and metadata were combined into a phyloseq object and used for
187 further analysis (phyloseq v1.30.0) (McMurdie and Holmes 2013; Callahan et al. 2016b).
188 Mitochondrial and chloroplast sequences as well as any sequences that were not assigned to
189 bacteria were removed from the ASV table.

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190

191 *Statistical analysis*

192 To visualize the relative abundance of each phylum, ASVs were aggregated to the phylum level
193 and taxa representing less than 2% of relative abundance were filtered out. To determine effect
194 of soil treatment and plant developmental phase on alpha diversity of rhizosphere communities,
195 Shannon diversity was calculated on the full dataset using the estimate_richness function in the
196 phyloseq package (1.30.0) and used as a response variable in ANOVA with plant developmental
197 phase, soil chemistry (S, NS, NS+Sm+Ni, and NS+Sm+D), and microbe source (S or NS) as
198 predictors. Shannon index was used because it accounts for both abundance and evenness in
199 samples (Kaisermann et al. 2017).
200 To examine differences in rhizosphere bacterial species composition due to soil chemistry,
201 microbe source, and plant developmental phases, Bray-Curtis dissimilarities were calculated and
202 visualized using non-metric multidimensional analysis (NMDS). To determine which predictors
203 were associated with variation in rhizosphere bacterial composition, we used the 'adonis'
204 function from the vegan package with Bray-Curtis dissimilarities as the response variable and
205 plant developmental phase, soil chemistry, and microbe source as predictors. To test for
206 differences in multivariate dispersion among rhizosphere communities, the 'betadisper' function
207 from the vegan (v2.5.3) package was used (Oksanen et al. 2019) with soil chemistry, plant
208 developmental phase, and microbe source as predictors.
209 To determine the effects of soil treatments on plant growth, each plant trait (leaf number, plant
210 height, root length, root diameter, root surface area, and root volume), was analyzed using a
211 general linearized model with soil chemistry, microbe source, and plant developmental phase as
212 the predictor and differences between group means were identified using likelihood ratio tests.
213 Tukey HSD was used as a post-hoc test to identify differences among groups.
214 To determine the effects of soil treatments on time to flowering, survival analysis was conducted
215 on binary flowering data (yes/no) using Kaplan-Meier curves and a Cox proportional hazards
216 regression model ('coxph') on a survival object ('Surv') in the survival package (v2.42.6) to
217 describe the soil treatment impacts the probability of flowering over time (Therneau 2015). The
218 time to event (late flowering), was measured in days from the onset of seedling phase. The model
219 provides a hazard ratio (HR) where a $HR > 1$ indicates an increased likelihood of development,
220 while an $HR < 1$ indicates a decreased likelihood of development. Differences in the hazard ratio

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221 were visualized using 'ggforest' in the survminer package (v0.4.3) (Kassambara and Kosinski
222 2018).
223 To determine if the relative abundance of bacteria ASVs differed among plant developmental
224 phases or soil chemistries, differential abundance analysis using DESeq2 (1.26.0) was used with
225 soil chemistry as the predictor. DESeq2 analysis was conducted with all soil treatments for the
226 seedling and late flowering plant developmental phase and another analysis was conducted with
227 S+Sm, NS+NSm, S+NSm, and NS+Sm for all plant developmental phases to represent the
228 experimental design.
229
230 RESULTS
231 After quality filtering and removal of non-target sequences, we recovered 1,608,688 reads
232 (average 9,353 reads per sample) that were grouped into 23,894 amplicon sequence variants.
233 Sampling curves within most samples were saturating (NS+NSm, NS+Sm+Ni, NS+Sm+D)
234 indicating a robust sampling of the microbial diversity associated with individual plants while
235 serpentine soils did not fully saturate (S+Sm, S+NSm) (Supplemental Figures 1-6). To compare
236 among samples, count data were normalized by relative abundance in a sample.
237
238 *Soil treatment and plant developmental phase influence species richness and community*
239 *similarity*
240 Bacteria from the phyla Proteobacteria or Acidobacteria comprised nearly 90% of reads from
241 most samples depending on soil chemistry (Figure 1a). Alpha diversity differed among soil
242 chemistries (Figure 1b; $F_{3,100} = 124.05$, $P < 0.001$) and plant developmental phase ($F_{4,100} = 5.74$,
243 $P = <0.001$), but not microbe source ($F_{1,100} = 2.64$, $P = 0.107$). There was a significant
244 interaction between soil chemistry and microbe source ($F_{1,100} = 6.80$, $P = 0.011$) as well as soil
245 chemistry, microbe source, and plant developmental phase ($F_{5,100} = 4.08$, $P = 0.004$) on bacterial
246 composition. The serpentine soil treatments (S+Sm and S+NSm) had lower alpha diversity at all
247 time points compared to all treatments with nonserpentine soils (NS+Sm, NS+Sm+Ni,
248 NS+Sm+D). The treatment with simulated drought (NS+Sm+D) treatment had higher species
249 richness than either the live nonserpentine treatment (NS+NSm) or the treatment with nickel
250 added (NS+Sm+Ni).
251

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252 Bacterial community composition in the rhizosphere varied with soil chemistry and plant
253 developmental phase, with surprisingly minimal contribution from the microbial source (Figure
254 2; Table 1). Variability in bacterial communities among plants (beta diversity) was associated
255 with soil chemistry (Betadisper: $F_{3,128} = 93.67$, $P = 0.001$) and plant developmental phases
256 (Betadisper: $F_{4,127} = 5.79$, $P = 0.004$), but only weakly with microbe source (Betadisper: $F_{1,130} =$
257 3.42, $P = 0.068$). Microbial communities from the seedling phase were less variable than all
258 other plant developmental phases, but there were no significant differences between the
259 variability of other phases. Both serpentine soil treatments were less variable than that of either
260 nonserpentine soil treatments.
261
262 *Plant growth responses to soil chemistry and microbial source*
263 In general, the height, leaf number, aboveground dried biomass, root length, root diameter, root
264 surface area, and root volume were all impacted by soil chemistries (Figures 3 and 4; Table 2 and
265 3). The interaction between plant developmental phase and microbe source as well as soil
266 chemistry and microbe source also influenced these plant traits. Microbe source, alone, only
267 significantly influenced root diameter.
268
269 *Serpentine microbes alter plant vegetative and flowering phenology*
270 Cox proportional hazards regression models showed differences in plant progression through
271 developmental phases among treatments (Table 4). Plants associated with serpentine microbes
272 reached the post-seedling vegetative phase and flowered earlier than those associated with
273 nonserpentine microorganisms (Table 4).
274
275
276 *Bacterial taxa*
277 DESeq2 identified taxa that were differentially abundant according to soil type (Figure 5,
278 Supplemental Figure 7-8). *Solirubrobacter*, *Lactobacillus*, and *Methylobacterium* were genera
279 that were of particular interest. *Solirubrobacter* were present in all treatments with nonserpentine
280 soils and absent in both treatments with serpentine soils. *Lactobacillus* were present in both
281 serpentine soil treatments. *Methylobacterium* were most abundant in the NS+NSm, NS+Sm, and
282 S+Sm treatments.

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283

284 DISCUSSION

285 The role of rhizosphere microbes in plant health is increasingly recognized, but efforts to manage
286 or alter rhizosphere composition require understanding the relative importance of soil chemistry,
287 microbial species pools and plant development in assembly processes. In comparing serpentine
288 to nonserpentine soils and microbial sources, we found that soil chemistry exerts that strongest
289 influence on microbial community composition, with more minor changes with plant phenology
290 and with microbial source. Plant phenology was also impacted by soil chemistry and microbe
291 source with plants growing in serpentine soils having a delayed vegetative and flowering
292 phenology. Changes in flowering phenology can have an impact on life-history traits and
293 population dynamics (Dorji et al. 2013; Yang et al. 2020).

294

295

296 *Soil chemistry and plant developmental phase both significantly impact microbial diversity and*
297 *community composition*

298 Here, rhizosphere alpha diversity generally increased with plant developmental phase and was
299 generally higher when plants were grown in nonserpentine soils, which allowed plants to grow
300 larger. Previous research found no or minimal difference in bacterial alpha diversity between
301 serpentine and nonserpentine soils (Oline 2006; Igwe and Vannette 2019). Consistent with our
302 previous research, plants grown on nonserpentine soils showed increased alpha diversity,
303 suggesting that plant growth rather than the diversity of microbes in the species pool is more
304 important in determining bacterial diversity in the rhizosphere. This may be due to accumulation
305 of microbes simply due to the amount of time the plants spent in soil (Dombrowski et al. 2017)
306 or changes in the amount or type of exudates deposited in the rhizosphere (Chaparro et al. 2013;
307 Zhalnina et al. 2018).

308

309 The largest change between microbial composition occurred between the start of the experiment
310 and the seedling phase. After the seedling phase, the microbial community composition
311 stabilized. This occurrence is in line with previous research in rice that showed similar results
312 (Edwards et al. 2018). Some microbial communities in the serpentine soil treatments at the
313 flowering phases shift to look more similar to those associated with nonserpentine *P. erecta*.

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314 Therefore, some convergence is occurring; however, in this experiment, soil chemistry
315 contributed more to the observed beta diversity in the microbial community than plant
316 developmental phase, even at later phases.
317
318 Various mechanisms could contribute to the observed results. For example, the presence of DNA
319 from dead cells that saturated sequencing efforts relative to new DNA. The soil could also exert
320 a selective pressure that is stronger than that of *P. erecta* rhizosphere. Alternatively, is possible
321 that some serpentine microbes that grow well in nonserpentine soils and vice versa. It has been
322 shown that serpentine and nonserpentine soils can host the same microbes with varying
323 accessory genomes (Porter et al. 2017). Shotgun sequencing or whole genome sequencing could
324 identify if the same microbes with distinct genotypes grew in reciprocal soil chemistries. By
325 exuding carbon compounds, phenolic acids, and amino acids, plants can enhance the growth of
326 specific beneficial or pathogenic members of the soil microbial community, which can enhance
327 plant growth in some cases (Paterson et al. 2007). Root exudates change over the course of plant
328 development where younger plants exude more sugars while older plants exude more complex
329 carbon compounds. Characterizing root exudation of *P. erecta* over plant development and
330 correlating the results with changes in microbial community composition can provide greater
331 insight into the role of dynamic plant exudation on survival in serpentine and nonserpentine
332 soils.
333
334 Our study cannot disentangle the possible mechanisms that contribute to the observed results.
335 We sampled plants at distinct development phases irrespective of soil residence time which is an
336 experimental design that considers that plant development phase was shown to influence the
337 rhizosphere microbial community separately from chronological age (Edwards et al. 2018). In
338 addition, our study was performed in a growth chamber, preventing the opportunity for microbial
339 immigration from the soil, which could affect microbial diversity and composition in the field.
340
341 *Soil microbial community and soil chemistry influence time to vegetative growth and flowering*
342 Our reciprocal transplants revealed that serpentine microorganisms, when in non-serpentine
343 soils, accelerate vegetative, early flowering, and flowering phenology (Table 4). The importance
344 of the microbial community for time to flowering has been previously demonstrated in

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345 *Arabidopsis* (Wagner et al. 2014) and *Ipomea purpurea* (Chaney and Baucom 2020). Drought-
346 adapted microbes accelerated flowering in *Brassica* when compared to non-drought-adapted
347 microbes (Lau and Lennon 2012). A few mechanisms for microbial effects on phenology have
348 been proposed including nutrient availability, production of plant hormones or their precursors,
349 or by exacerbating stress. However, flowering was delayed in the presence of serpentine
350 microbes grown in nonserpentine soils (Table 4), which have been previously demonstrated to be
351 more nutrient-rich than serpentine soils (Brady et al. 2005), suggesting another mechanism may
352 underlie microbial effects in this experiment. It may be that microbes produce plant hormones
353 such as indole acetic acid (IAA) which plays a significant role in flowering time. Nitrogen can be
354 converted to tryptophan and then to IAA and increases time to flowering (Lu et al. 2018).
355 Alternatively, flowering time has been shown to be impacted by biotic stress (Kazan and Lyons
356 2016). It is possible that introducing non-adapted microorganisms to non-serpentine soils may
357 constitute a biotic stressor that can induce changes in plant phenology.
358
359 Thirty-four genera across 5 phyla were shown to be differentially abundant between soil
360 treatments by DESeq2 analysis. Of particular interest are *Solirubrobacter* which were only
361 detected in nonserpentine soil treatments, *Lactobacillus* which characterized serpentine soil
362 treatments, and *Methylobacterium* which were most abundant in NS+NSm, S+Sm, and NS+Sm
363 treatments. *Solirubrobacter* are gram-positive, non-motile bacteria that has been identified and
364 isolated from bulk soil, rhizosphere, and endosphere environments (Wei et al. 2014;
365 Albuquerque and Da Costa 2014). In general, it has been shown to associate with high soil
366 quality (Gravuer and Eskelinen 2017; Lopez et al. 2017; Sánchez-Marañón et al. 2017).
367 Nonserpentine soils are generally more nutrient-rich than serpentine soils and this may influence
368 the abundance of *Solirubrobacter* observed in the nonserpentine soil treatments. *Lactobacillus*
369 are lactic acid bacteria (LAB) that are gram-positive and microaerophilic. Plasmids comprise up
370 to 4.8% of LAB total gene content and are important for growth in the diverse, yet specific
371 environments where these bacteria are found (Makarova et al. 2006). *Lactobacillus* have been
372 shown to be metallotolerant and have the ability to bind heavy metals and protect against metal-
373 induced oxidative stress (Li et al. 2017; Liu et al. 2019; Barman et al. 2020). Their abundance in
374 serpentine soil treatments may reflect these phenotypic properties as serpentine soils have high
375 concentrations of heavy metals. *Enterobacter*, which was most abundant in the NS+Sm+Drought

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376 treatment, has been shown to have plant-growth-promoting properties such as phosphorus
377 solubility and ACC deaminase activity (Danish et al. 2020). Its application to *Sorghum bicolor*
378 (L.) Moench increased the plants root architecture and ability to tolerate stress (Govindasamy et
379 al. 2020). *Methylobacterium* (order *Rhizobiales*) belong to the same family as *Microvirga,* which
380 has previously been shown to associate with legumes and non-legumes on serpentine soils (Igwe
381 and Vannette 2019). In addition members of *Methylobacterium* can produce auxins and induce
382 root nodulation (Kelly et al. 2014) and can promote plant growth through the production of ACC
383 deaminase (Belimov et al. 2019; Sharma et al. 2021).
384
385 *Serpentine soils increase root diameter, but have no impact or decrease other plant growth*
386 *metrics*
387 *Plantago erecta* grown in serpentine soils were shorter, and generally smaller than those grown
388 in nonserpentine soils as has been documented previously (O'Dell and Rajakaruna 2004;
389 Kayama et al. 2005). Root length, surface area, and volume were smallest in serpentine soils
390 while root diameter was the largest in this soil chemistry aligning with previous work that
391 demonstrated that heavy-metal tolerant species of *Arabidopsis arenosa* and *Arabidopsis halleri*
392 have thicker roots than the heavy-metal sensitive *Arabidopsis thaliana* (Staňová et al. 2012).
393 Although all *P. erecta* growing in nonserpentine soils were larger than those in serpentine soils,
394 only the plants in the NS+Sm soil treatment flowered sooner relative to the live serpentine
395 (S+Sm) treatment. Collectively, differences in plant size and vegetative and flowering phenology
396 between *P. erecta* on serpentine or nonserpentine soils are important for a plant's life history
397 (Metcalf et al. 2019). Plants that flower, set seed, and then die generally flower at the size that
398 will ensure the best reproductive success (Metcalf et al. 2003). Continued research could
399 determine how local adaptation of microbial communities influence reproductive success in
400 plants growing in serpentine and nonserpentine soils.
401
402 Ecologically, competition for resources impact plant size. Soil nutrients, in particular, impact
403 root architecture. Additionally, smaller plants are less vulnerable to drought (Olson et al. 2018).
404 Phosphate deficiency, for example, produces plants that increase lateral root production over
405 primary root production (López-Bucio et al. 2003). Plant-growth-promoting bacteria (PFPB) is
406 one mechanism by which plants can access nutrients and defend against pathogenic bacteria

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407 (Glick 2012). A few direct plant-growth promoting methods that would be important in
408 serpentine soils include phosphorus solubilization, metal chelation, and the production of extra-
409 polymeric substances (EPS). Together, these traits would increase nutrient availability, decrease
410 metal availability, and increase the water-holding capacity of the soil for the plant. Still, the
411 ability of the microbes to confer benefits to plants growing on serpentine soils is dependent on
412 local adaption of the microbes to serpentine (Rúa et al. 2016; Porter et al. 2016, 2019). Root
413 diameter was the only plant trait that was larger is serpentine soils relative to nonserpentine soils
414 and removing microbes that were locally adapted to serpentine soils removed this advantage.
415 Conversely, replacing microbes that were locally adapted to nonserpentine with microbes that
416 were locally adapted to serpentine contributed to plants that flowered sooner than other
417 treatments.
418
419 CONCLUSIONS
420 The root-associated microbial communities of *Plantago erecta* grown in serpentine and
421 nonserpentine soils with adapted or non-adapted microorganisms have differing alpha and beta
422 diversities. Notably, *P. erecta* grown in nonserpentine soils with serpentine microorganisms
423 experienced accelerated vegetative and flowering phenology as they entered the vegetative, early
424 flowering and late flowering phase before any plants that were grown in live serpentine soils
425 (S+Sm) or live nonserpentine soil (NS+NSm). Above- and below-ground development on *P.*
426 *erecta* on serpentine soil treatments were less than those grown on nonserpentine soil treatments.
427 Overall, our results support a role of locally adapted microorganisms in impacting plant
428 phenology despite minimal effects on other measurable aspects of plant phenotype.
429
430 CONFLICT OF INTERESTS
431 Authors declare no conflict of interests in this project
432
433 ACKNOWLEDGEMENTS
434 This work was made possible by the University of California Natural Reserve System
435 (McLaughlin Natural Reserve) Reserve DOI: (https://doi.org/10.21973/N3W08D). We thank
436 Cathy Koehler for assistance at McLaughlin Natural Reserve. We would like to thank Imade Ojo
437 and Shenwen Gu who helped set up the experiment. Thanks to the California Native Plant
438 Society, Davis Botanical Society Student Research Grant, Jastro Research Scholarship Award,
439 and Natural Reserve System Graduate Student Grant Program for providing funds for the

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440 research. We are thankful to members of the Vannette Lab who provided feedback on
441 manuscript drafts.
442

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443 REFERENCES

444 Akhtar MS, Siddiqui ZA, Wiemken A (2011) Arbuscular Mycorrhizal Fungi and Rhizobium to
445 Control Plant Fungal Diseases. In: Lichtfouse E (ed) Alternative Farming Systems,
446 Biotechnology, Drought Stress and Ecological Fertilisation. Springer Netherlands,
447 Dordrecht, pp 263–292
448 Albuquerque L, Da Costa MS (2014) The families Conexibacteraceae, Patulibacteraceae and
449 Solirubrobacteraceae. In: The Prokaryotes: Actinobacteria. Springer-Verlag Berlin
450 Heidelberg, pp 185–200
451 Anacker BL (2014) The nature of serpentine endemism. Am J Bot 101:219–224. doi:
452 10.3732/ajb.1300349
453 Barillot CDC, Sarde C-O, Bert V, et al (2013) A standardized method for the sampling of
454 rhizosphere and rhizoplan soil bacteria associated to a herbaceous root system. Ann
455 Microbiol 63:471–476. doi: 10.1007/s13213-012-0491-y
456 Barman D, Jha DK, Bhattacharjee K (2020) Metallotolerant bacteria: Insights into bacteria
457 thriving in metal-contaminated areas. In: Microbial Versatility in Varied Environments:
458 Microbes in Sensitive Environments. Springer Singapore, pp 135–164
459 Belimov AA, Zinovkina NY, Safronova VI, et al (2019) Rhizobial ACC deaminase contributes
460 to efficient symbiosis with pea (Pisum sativum L.) under single and combined cadmium and
461 water deficit stress. Environ Exp Bot 167:103859. doi: 10.1016/j.envexpbot.2019.103859
462 Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and
463 function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68:1–13. doi:
464 10.1111/j.1574-6941.2009.00654.x
465 Brady KU, Kruckeberg AR, Bradshaw Jr. HD (2005) Evolutionary ecology of plant adaptation to
466 serpentine soils. Annu Rev Ecol Evol Syst 36:243–266. doi:
467 10.1146/annurev.ecolsys.35.021103.105730
468 Calderón K, Spor A, Breuil MC, et al (2017) Effectiveness of ecological rescue for altered soil
469 microbial communities and functions. ISME J 11:272–283. doi: 10.1038/ismej.2016.86
470 Callahan BJ, McMurdie PJ, Rosen MJ, et al (2016a) DADA2: High-resolution sample inference
471 from Illumina amplicon data. Nat Methods 13:581–583. doi: 10.1038/nmeth.3869
472 Callahan BJ, Sankaran K, Fukuyama JA, et al (2016b) Bioconductor workflow for microbiome
473 data analysis: from raw reads to community analyses. F1000Research 5:1492. doi:
474 10.12688/f1000research.8986.2
475 Chaney L, Baucom RS (2020) The soil microbial community alters patterns of selection on
476 flowering time and fitness-related traits in Ipomoea purpurea. Am J Bot 107:186–194. doi:
477 10.1002/ajb2.1426
478 Chaparro JM, Badri D V, Bakker MG, et al (2013) Root exudation of phytochemicals in
479 Arabidopsis follows specific patterns that are developmentally programmed and correlate
480 with soil microbial functions. PLoS One 8:e55731. doi: 10.1371/journal.pone.0055731
481 Chaparro JM, Badri D V, Vivanco JM (2014) Rhizosphere microbiome assemblage is affected
482 by plant development. ISME J 8:790–803. doi: 10.1038/ismej.2013.196
483 Comeau AM, Li WKW, Tremblay JÉ, et al (2011) Arctic ocean microbial community structure
484 before and after the 2007 record sea ice minimum. PLoS One 6:. doi:
485 10.1371/journal.pone.0027492
486 Cui QG, He WM (2009) Soil biota, but not soil nutrients, facilitate the invasion of Bidens pilosa
487 relative to a native species Saussurea deltoidea. Weed Res 49:201–206. doi:
488 10.1111/j.1365-3180.2008.00679.x

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489 Danish S, Zafar-Ul-Hye M, Hussain S, et al (2020) Mitigation of drought stress in maize through
490 inoculation with drought tolerant ACC deaminase containing PGPR under axenic
491 conditions. Pakistan J Bot 52:49–60. doi: 10.30848/PJB2020-1(7)
492 De Curtis F, Lima G, Vitullo D, De Cicco V (2010) Biocontrol of Rhizoctonia solani and
493 Sclerotium rolfsii on tomato by delivering antagonistic bacteria through a drip irrigation
494 system. Crop Prot 29:663–670. doi: 10.1016/j.cropro.2010.01.012
495 Dombrowski N, Schlaeppi K, Agler MT, et al (2017) Root microbiota dynamics of perennial
496 Arabis alpina are dependent on soil residence time but independent of flowering time. ISME
497 J 11:43–55. doi: 10.1038/ismej.2016.109
498 Dorji T, Totland Ø, Moe SR, et al (2013) Plant functional traits mediate reproductive phenology
499 and success in response to experimental warming and snow addition in Tibet. Glob Chang
500 Biol 19:459–472. doi: 10.1111/gcb.12059
501 Edwards JA, Santos-Medellín CM, Liechty ZS, et al (2018) Compositional shifts in root-
502 associated bacterial and archaeal microbiota track the plant life cycle in field-grown rice.
503 PLoS Biol 16:. doi: 10.1371/journal.pbio.2003862
504 Emami S, Alikhani HA, Pourbabaei AA, et al (2018) Improved growth and nutrient acquisition
505 of wheat genotypes in phosphorus deficient soils by plant growth-promoting rhizospheric
506 and endophytic bacteria. Soil Sci Plant Nutr 64:719–727. doi:
507 10.1080/00380768.2018.1510284
508 Farrer EC, Suding KN (2016) Teasing apart plant community responses to N enrichment: the
509 roles of resource limitation, competition and soil microbes. Ecol Lett 19:1287–1296. doi:
510 10.1111/ele.12665
511 Fei H, Crouse M, Papadopoulos YA, Vessey JK (2020) Improving biomass yield of giant
512 Miscanthus by application of beneficial soil microbes and a plant biostimulant. Can J Plant
513 Sci 100:29–39. doi: 10.1139/cjps-2019-0012
514 Fenner M (1998) The phenology of growth and reproduction in plants. Perspect Plant Ecol Evol
515 Syst 1:78–91. doi: https://doi.org/10.1078/1433-8319-00053
516 Glick BR (2012) Plant Growth-Promoting Bacteria: Mechanisms and Applications. 963401:. doi:
517 10.6064/2012/963401
518 Glöckner FO, Yilmaz P, Quast C, et al (2017) 25 years of serving the community with ribosomal
519 RNA gene reference databases and tools. J Biotechnol 261:169–176. doi:
520 10.1016/j.jbiotec.2017.06.1198
521 Gouda S, Kerry RG, Das G, et al (2018) Revitalization of plant growth promoting rhizobacteria
522 for sustainable development in agriculture. Microbiol Res 206:131–140. doi:
523 10.1016/j.micres.2017.08.016
524 Govindasamy V, George P, Kumar M, et al (2020) Multi-trait PGP rhizobacterial endophytes
525 alleviate drought stress in a senescent genotype of sorghum [Sorghum bicolor (L.) Moench].
526 3 Biotech 10:13. doi: 10.1007/s13205-019-2001-4
527 Gravuer K, Eskelinen A (2017) Nutrient and rainfall additions shift phylogenetically estimated
528 traits of soil microbial communities. Front Microbiol 8:1–16. doi:
529 10.3389/fmicb.2017.01271
530 Haichar FEZ, Marol C, Berge O, et al (2008) Plant host habitat and root exudates shape soil
531 bacterial community structure. ISME J 2:1221–1230. doi: 10.1038/ismej.2008.80
532 Hidalgo-Triana N, Pérez-Latorre A V. (2018) Phenological patterns in Mediterranean south
533 Iberian serpentine flora. Nord J Bot 36:1–11. doi: 10.1111/njb.02028
534 Igwe AN, Vannette RL (2019) Bacterial communities differ between plant species and soil type,

bioRxiv preprint doi: https://doi.org/10.1101/2021.03.29.437500; this version posted March 29, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

535 and differentially influence seedling establishment on serpentine soils. Plant Soil 441:423–
536 437. doi: 10.1007/s11104-019-04135-5
537 Kaisermann A, de Vries FT, Griffiths RI, Bardgett RD (2017) Legacy effects of drought on
538 plant–soil feedbacks and plant–plant interactions. New Phytol 215:1413–1424. doi:
539 10.1111/nph.14661
540 Kandlikar GS, Johnson CA, Yan X, et al (2019) Winning and losing with microbes: how
541 microbially mediated fitness differences influence plant diversity. Ecol Lett 22:1178–1191.
542 doi: 10.1111/ele.13280
543 Kassambara A, Kosinski M (2018) survminer: Drawing Survival Curves using “ggplot2”. R
544 package version 0.4.3
545 Kayama M, Quoreshi AM, Uemura S, Koike T (2005) Differences in growth characteristics and
546 dynamics of elements absorbed in seedlings of three spruce species raised on serpentine soil
547 in northern Japan. Ann Bot 95:661–672. doi: 10.1093/aob/mci063
548 Kazan K, Lyons R (2016) The link between flowering time and stress tolerance. J Exp Bot
549 67:47–60. doi: 10.1093/jxb/erv441
550 Kelly DP, McDonald IR, Wood AP (2014) The Family Methylobacteriaceae. In: Rosenberg E,
551 DeLong EF, Lory S, et al. (eds) The Prokaryotes. Spring, Berlin, Heidelberg, pp 313–340
552 Kwak MJ, Kong HG, Choi K, et al (2018) Rhizosphere microbiome structure alters to enable
553 wilt resistance in tomato. Nat Biotechnol 36:1100–1116. doi: 10.1038/nbt.4232
554 Lau JA, Lennon JT (2012) Rapid responses of soil microorganisms improve plant fitness in
555 novel environments. Proc Natl Acad Sci 109:14058–14062. doi: 10.1073/pnas.1202319109
556 Li B, Jin D, Yu S, et al (2017) In Vitro and in Vivo evaluation of Lactobacillus delbrueckii
557 subsp. Bulgaricus KLDS1.0207 for the alleviative effect on lead toxicity. Nutrients 9:. doi:
558 10.3390/nu9080845
559 Liu, Zheng, Ma, et al (2019) Evaluation and Proteomic Analysis of Lead Adsorption by Lactic
560 Acid Bacteria. Int J Mol Sci 20:5540. doi: 10.3390/ijms20225540
561 López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L (2003) The role of nutrient availability in
562 regulating root architecture. Curr Opin Plant Biol 6:280–287. doi: 10.1016/S1369-
563 5266(03)00035-9
564 Lopez S, Piutti S, Vallance J, et al (2017) Nickel drives bacterial community diversity in the
565 rhizosphere of the hyperaccumulator Alyssum murale. Soil Biol Biochem 114:121–130.
566 doi: 10.1016/j.soilbio.2017.07.010
567 Lu T, Ke M, Lavoie M, et al (2018) Rhizosphere microorganisms can influence the timing of
568 plant flowering. Microbiome 6:. doi: 10.1186/s40168-018-0615-0
569 Makarova K, Slesarev A, Wolf Y, et al (2006) Comparative genomics of the lactic acid bacteria.
570 Proc Natl Acad Sci U S A 103:15611–15616. doi: 10.1073/pnas.0607117103
571 McMurdie PJ, Holmes S (2013) Phyloseq: An R Package for Reproducible Interactive Analysis
572 and Graphics of Microbiome Census Data. PLoS One 8:. doi:
573 10.1371/journal.pone.0061217
574 Metcalf CJE, Henry LP, Rebolleda-Gómez M, Koskella B (2019) Why evolve reliance on the
575 microbiome for timing of ontogeny? MBio 10:1–10. doi: 10.1128/mBio.01496-19
576 Metcalf JC, Rose KE, Rees M (2003) Evolutionary demography of monocarpic perennials.
577 Trends Ecol Evol 18:471–480. doi: 10.1016/S0169-5347(03)00162-9
578 Murgese P, Santamaria P, Leoni B, Crecchio C (2020) Ameliorative Effects of PGPB on Yield,
579 Physiological Parameters, and Nutrient Transporter Genes Expression in Barattiere
580 (Cucumis melo L.). J Soil Sci Plant Nutr 20:784–793. doi: 10.1007/s42729-019-00165-1

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581 O'Dell RE, James JJ, Richards JH (2006) Congeneric serpentine and nonserpentine shrubs differ
582 more in leaf Ca:Mg than in tolerance of low N, low P, or heavy metals. Plant Soil 280:49–
583 64. doi: 10.1007/s11104-005-3502-y
584 O'Dell RE, Rajakaruna N (2004) Intraspecific Variation, Adaptation, and Evolution. In: Harrison
585 S, Rajakaruna N (eds) Serpentine: The Evolution and Ecology of a Model System. pp 97–
586 138
587 Oksanen J, Blanchet FG, Friendly M, et al (2019) Vegan: Community Ecology Package (version
588 2.5-6)
589 Oline DK (2006) Phylogenetic comparisons of bacterial communities from serpentine and
590 nonserpentine soils. Appl Environ Microbiol 72:6965–6971. doi: 10.1128/AEM.00690-06
591 Olson ME, Soriano D, Rosell JA, et al (2018) Plant height and hydraulic vulnerability to drought
592 and cold. Proc Natl Acad Sci U S A 115:7551–7556. doi: 10.1073/pnas.1721728115
593 Orlandini V, Emiliani G, Fondi M, et al (2014) Network Analysis of Plasmidomes: The
594 Azospirillum brasilense Sp245 Case . Int J Evol Biol 2014:1–14. doi: 10.1155/2014/951035
595 Paterson E, Gebbing T, Abel C, et al (2007) Rhizodeposition shapes rhizosphere microbial
596 community structure in organic soil. New Phytol 173:600–610. doi: 10.1111/j.1469-
597 8137.2006.01931.x
598 Porter SS, Bantay R, Friel CA, et al (2019) Beneficial microbes ameliorate abiotic and biotic
599 sources of stress on plants. doi: 10.1111/1365-2435.13499
600 Porter SS, Chang PL, Conow CA, et al (2017) Association mapping reveals novel serpentine
601 adaptation gene clusters in a population of symbiotic Mesorhizobium. ISME J 11:248–262.
602 doi: 10.1038/ismej.2016.88
603 Porter SS, Chang PL, Conow CA, et al (2016) Association mapping reveals novel serpentine
604 adaptation gene clusters in a population of symbiotic Mesorhizobium. Isme J 11:248
605 Quast C, Pruesse E, Yilmaz P, et al (2012) The SILVA ribosomal RNA gene database project:
606 improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596. doi:
607 10.1093/nar/gks1219
608 Ravichandran KR, Thangavelu M (2017) Role and influence of soil microbial communities on
609 plant invasion. Ecol Quest 27:9–23. doi: 10.12775/EQ.2017.024
610 Rodríguez-Pérez J, Traveset A (2016) Effects of flowering phenology and synchrony on the
611 reproductive success of a long-flowering shrub. AoB Plants 8:. doi: 10.1093/aobpla/plw007
612 Rossington N, Yost J, Ritter M (2018) Water Availability Influences Species Distributions on
613 Serpentine Soils. Madroño 65:68–79. doi: 10.3120/0024-9637-65.2.68
614 Rúa MA, Antoninka A, Antunes PM, et al (2016) Home-field advantage? evidence of local
615 adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis.
616 BMC Evol Biol 16:1–15. doi: 10.1186/s12862-016-0698-9
617 Safford AHD, Viers JH, Harrison SP (2005) Serpentine endemism in the California flora: a
618 database of serpentine affinity. Madrono 52:222–257
619 Sakaguchi S, Horie K, Ishikawa N, et al (2019) Maintenance of soil ecotypes of Solidago
620 virgaurea in close parapatry via divergent flowering time and selection against immigrants.
621 J Ecol 107:418–435. doi: 10.1111/1365-2745.13034
622 Sánchez-Marañón M, Miralles I, Aguirre-Garrido JF, et al (2017) Changes in the soil bacterial
623 community along a pedogenic gradient. Sci Rep 7:1–11. doi: 10.1038/s41598-017-15133-x
624 Schliep KP (2011) phangorn: Phylogenetic analysis in R. Bioinformatics 27:592–593. doi:
625 10.1093/bioinformatics/btq706
626 Schneider A (2017) Flowering time evolution is independent of serpentine tolerance in the

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627 California flora. Ecosphere 8:. doi: 10.1002/ecs2.1767
628 Sharma S, Chandra D, Sharma AK (2021) Rhizosphere Plant–Microbe Interactions Under
629 Abiotic Stress. Springer, Singapore, pp 195–216
630 Sherrard ME, Maherali H (2006) the Adaptive Significance of Drought Escape in Avena
631 Barbata, an Annual Grass. Evolution (N Y) 60:2478. doi: 10.1554/06-150.1
632 Staňová A, Ďurišová E, Banásová V, et al (2012) Root system morphology and primary root
633 anatomy in natural non-metallicolous and metallicolous populations of three Arabidopsis
634 species differing in heavy metal tolerance. Biologia (Bratisl) 67:505–516. doi:
635 10.2478/s11756-012-0040-y
636 Therneau TM (2015) A Package for Survival Analysis in S. version 2.38
637 Van Der Heijden MGA, Bakker R, Verwaal J, et al (2006) Symbiotic bacteria as a determinant
638 of plant community structure and plant productivity in dune grassland. FEMS Microbiol
639 Ecol 56:178–187. doi: 10.1111/j.1574-6941.2006.00086.x
640 Wagner MR, Lundberg DS, Coleman-Derr D, et al (2014) Natural soil microbes alter flowering
641 phenology and the intensity of selection on flowering time in a wild Arabidopsis relative.
642 Ecol Lett 17:717–726. doi: 10.1111/ele.12276
643 Wei L, Ouyang S, Wang Y, et al (2014) Solirubrobacter phytolaccae sp. nov., an endophytic
644 bacterium isolated from roots of Phytolacca acinosa Roxb. Int J Syst Evol Microbiol
645 64:858–862. doi: 10.1099/ijs.0.057554-0
646 Wright JW, Stanton ML, Scherson R (2006) Local adaptation to serpentine and non-serpentine
647 soils in Collinsia sparsiflora. Evol Ecol Res 8:1–21
648 Yang X, Guo R, Knops JMH, et al (2020) Shifts in plant phenology induced by environmental
649 changes are small relative to annual phenological variation. Agric For Meteorol
650 294:108144. doi: 10.1016/j.agrformet.2020.108144
651 Yilmaz P, Parfrey LW, Yarza P, et al (2014) The SILVA and "all-species Living Tree Project
652 (LTP)" taxonomic frameworks. Nucleic Acids Res 42:643–648. doi: 10.1093/nar/gkt1209
653 Zhalnina K, Louie KB, Hao Z, et al (2018) Dynamic root exudate chemistry and microbial
654 substrate preferences drive patterns in rhizosphere microbial community assembly. Nat
655 Microbiol 3:470–480. doi: 10.1038/s41564-018-0129-3

656

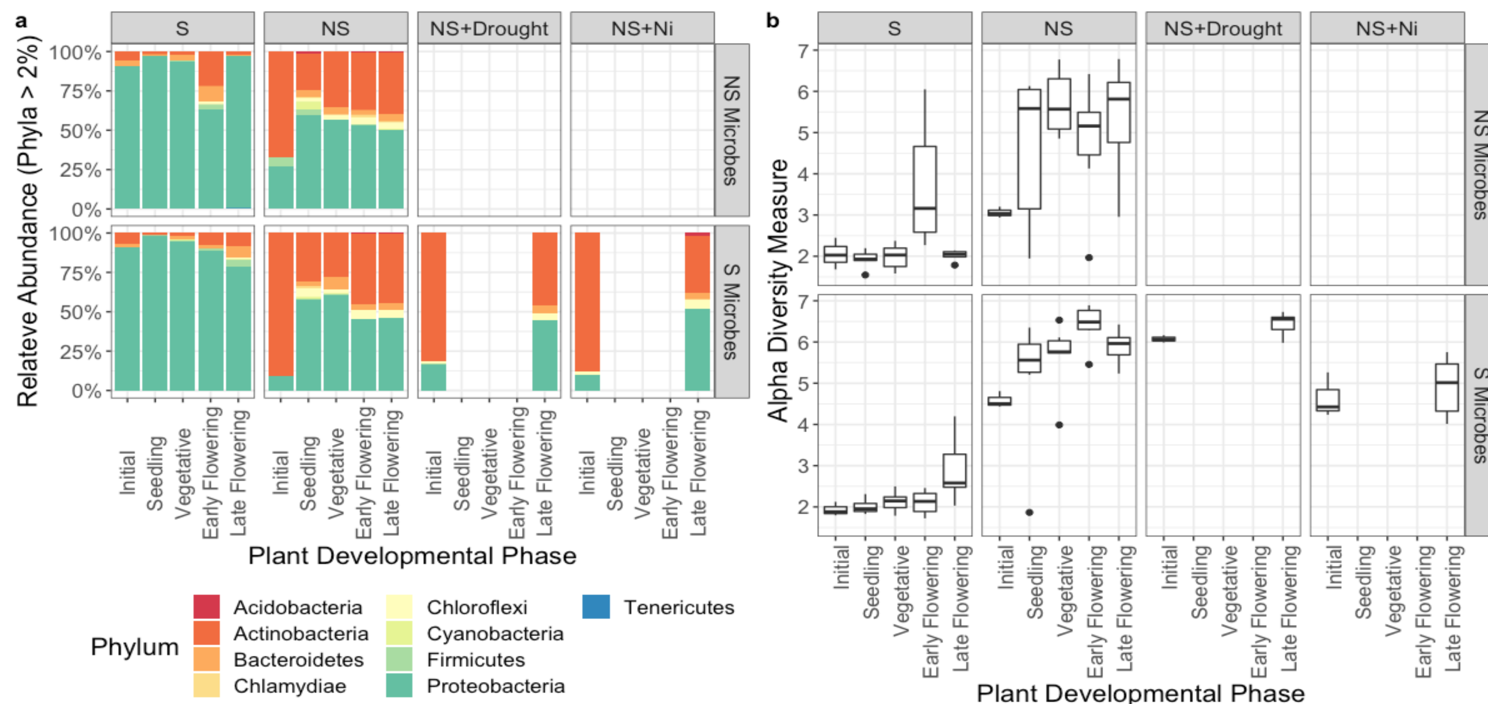

Figure 1 - Relative abundance and alpha diversity of bacterial community composition on roots of *Plantago erecta* across soil treatments and plant phenology.

(a) Bacterial phyla with a relative abundance of at least 2% are visualized in a bar graph facetted by plant developmental phase and soil treatment (S = Serpentine and NS = Nonserpentine). (b) Shannon diversity is significantly different between soil chemistries ($F_{3,100}$ = 124.05, P <0.001) and plant developmental phase ($F_{4,100}$ = 5.74, P <0.001), but not microbe source ($F_{1,100}$ = 2.64, P = 0.11). There was a significant interaction between soil chemistry and microbe source ($F_{1,100}$ = 6.80, P = 0.011) as well as soil chemistry, microbe source, and plant developmental phase ($F_{5,100}$ = 4.08, P = 0.004).

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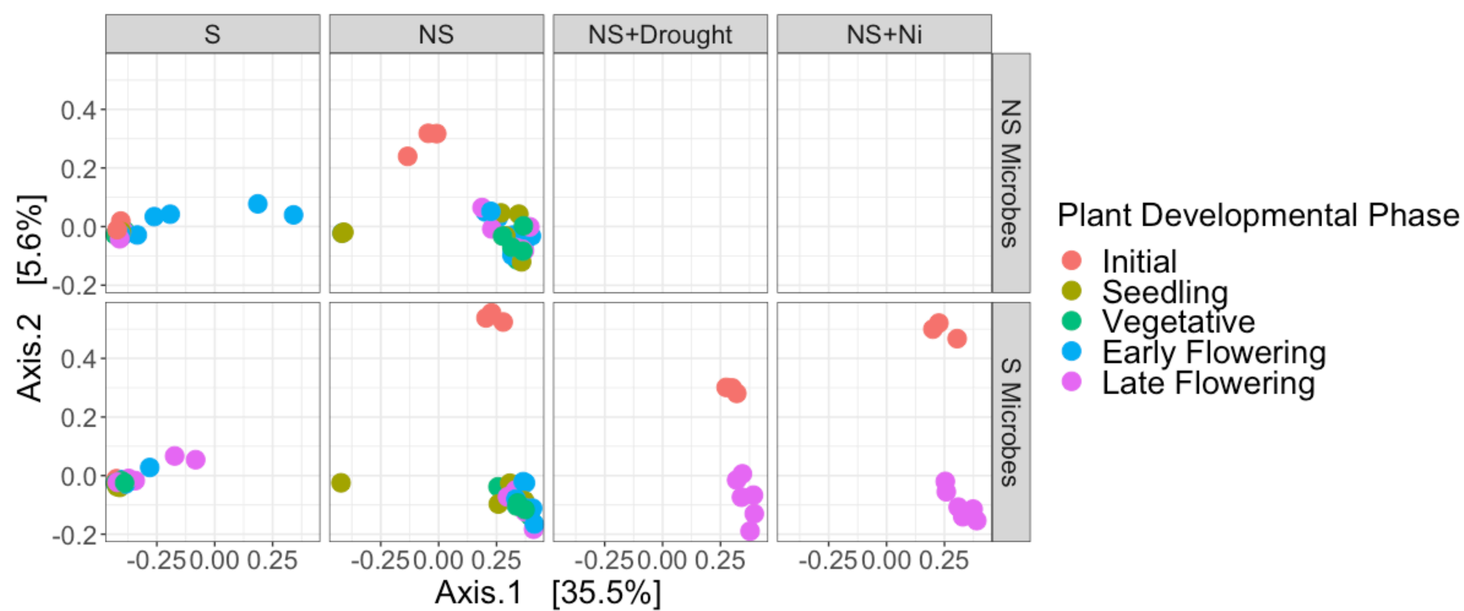

Figure 2 - PCoA of *Plantago erecta* rhizosphere bacterial communities across soil treatments and plant phenology using Bray-Curtis dissimilarity. Point color indicates plant developmental phase and panels indicate distinct soil treatments.

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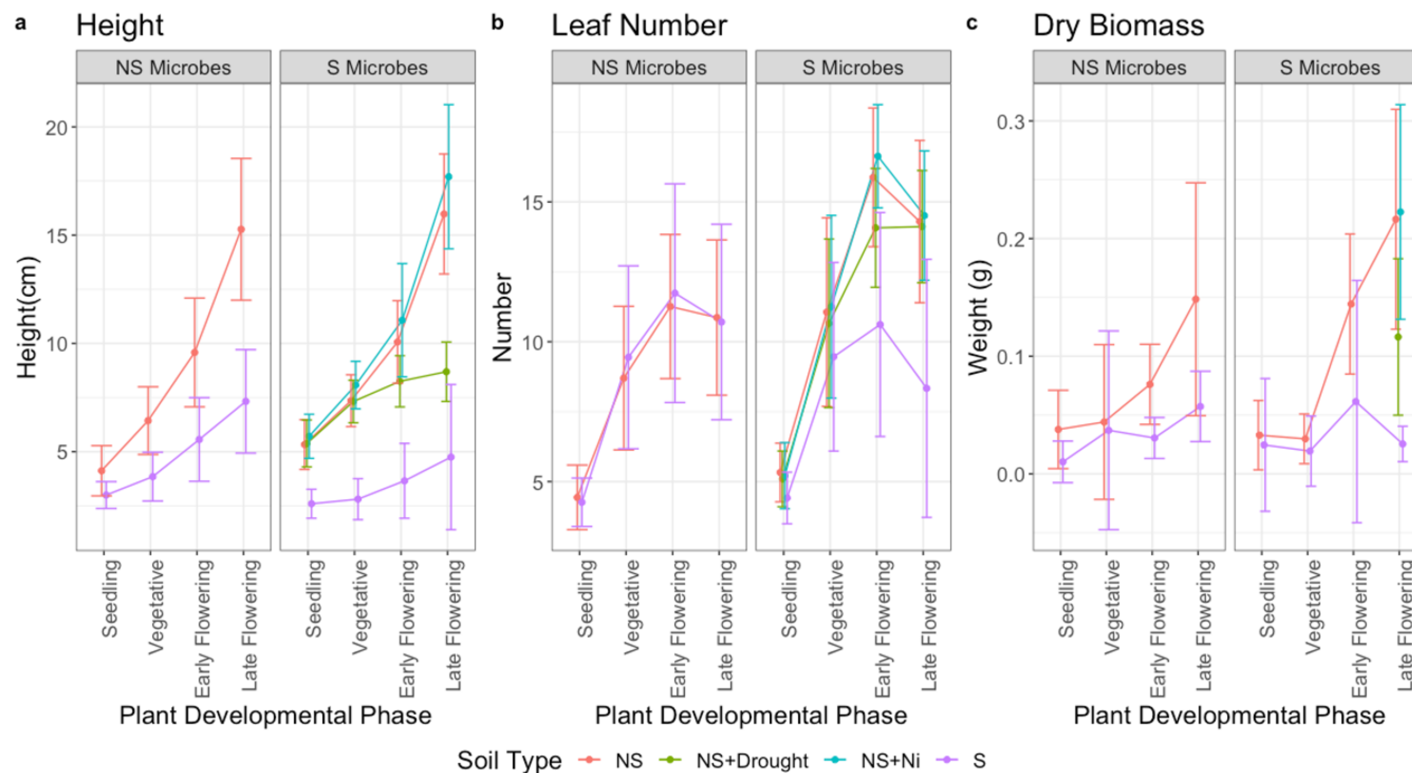

Figure 3 - Growth traits of *Plantago erecta* vary among soil treatments and plant developmental phase. Points indicate mean +/- 1SD and points from soil treatments are connected, showing there is a significant difference between (a) height and (b) leaf number between soil treatment and plant developmental phase. Dry biomass (c) was significantly different between plant developmental phase, but not soil treatment.

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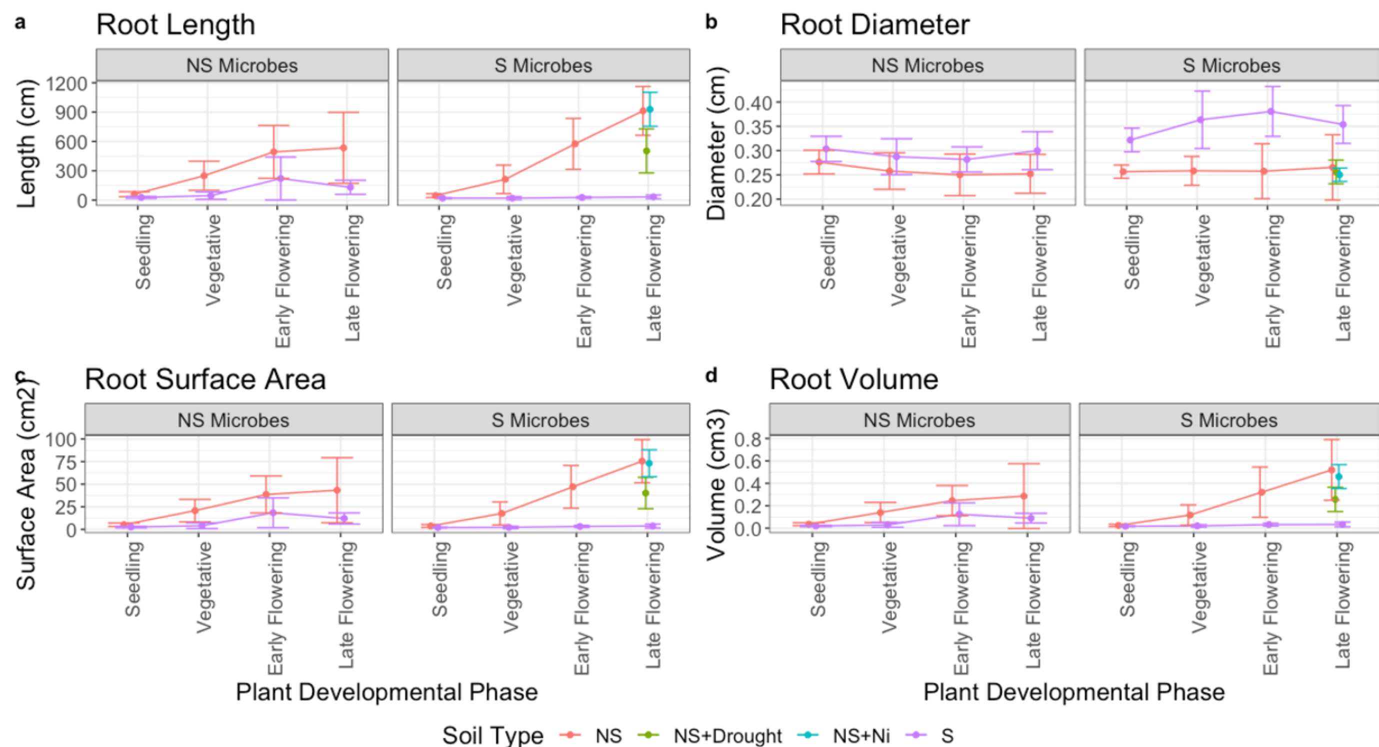

Figure 4 - Root growth traits vary among soil treatments and plant developmental phase.

Mean value for root traits and standard deviation show that (a) root length was significantly different across soil treatment and plant developmental phase. Root diameter (b) was different between soil treatments, but not plant developmental phase. Root surface area (c) and root volume (d) showed significant differences between plant developmental phases and soil treatments. The interaction between soil treatment and plant developmental phase influenced all root metrics.

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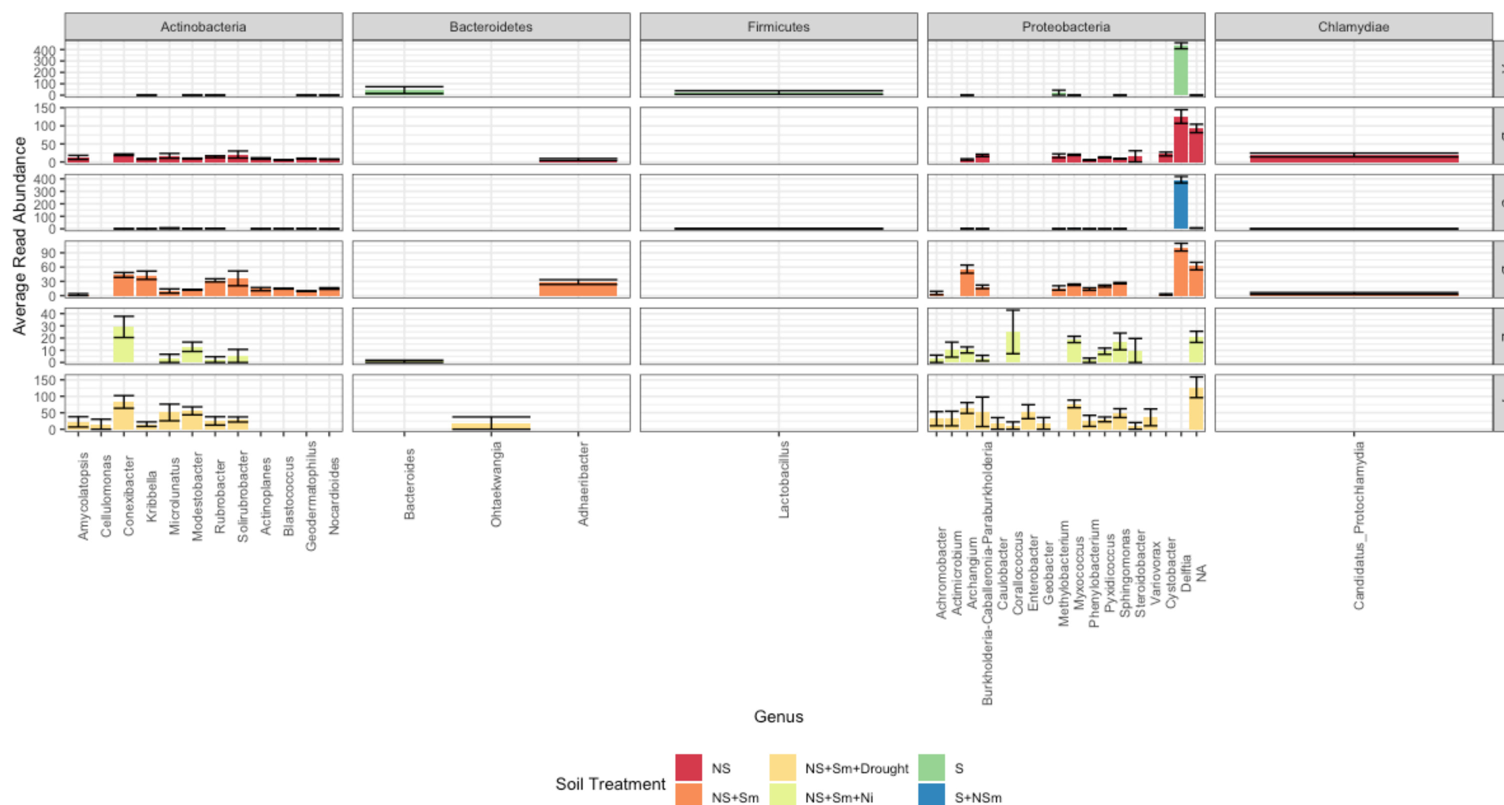

Figure 5 - Differentially abundant genera across soil treatments.

DESEq2 analysis showing ASVs that were differentially abundant between soil treatments (FDR <0.01). Bacterial genus is on the x-axis and relative average read abundance on the y-axis. Colors represent soil treatments (A=Serpentine soil and serpentine microbes, B=Nonserpentine soil and nonserpentine microbes, C=Serpentine soil and nonserpentine microbes, D=Nonserpentine soil and serpentine microbes, E=Nonserpentine soil and serpentine microbes and nickel stress, F=Nonserpentine soil and serpentine microbes and drought stress). Bars represent means +/- 1SE.

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Table 1 - Statistical analysis of microbial community dissimilarity using ANOVA. The predictors, degrees of freedom (df), number of samples (N), F-Value (F), Variation, and P-Value are listed

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Predictor	df	N	F	R^2	P
Soil type	3	100	22.23	0.304	0.001
Developmental phase	4	100	3.21	0.059	0.001
Microbe source	1	100	3.24	0.015	0.008
Soil type*Developmental phase	6	100	2.21	0.06	0.001
Microbe source*Developmental phase	4	100	1.36	0.025	0.089
Soil type*Microbe source	1	100	3.04	0.014	0.011
Soil type*Microbe source*Developmental phase	4	100	1.58	0.029	0.031

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Table 2 - Statistical results for aboveground plant metrics. Linear mixed-effects model was used to determine the impact of various predictors on plant height, leaf number, and dry biomass. The tray where plants were grown was used as a random variable.

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			Plant Height		Leaf Number		Dry Biomass	
Predictor	df	N	X2	*P*	X2	*P*	X2	*P*
Soil chemistry	3	3372	4890.83	<0.0001	78.44	<0.0001	60.40	<0.0001
Developmental phase	3	3372	7012.10	<0.0001	3064.16	<0.0001	79.84	<0.0001
Microbe source	1	3372	2.58	0.108	137.12	<0.0001	2.45	0.11784
Soil chemistry*Developmental phase	9	3372	1294.27	<0.0001	57.13	<0.0001	35.69	<0.0001
Microbe source*Developmental phase	3	3372	40.84	<0.0001	20.45	0.0001	8.81	0.03193
Soil chemistry*Microbe source	1	3372	296.64	<0.0001	142.12	<0.0001	2.34	0.12583
Soil chemistry*Microbe source*Developmental phase	3	3372	11.79	0.008	53.86	<0.0001	6.72	0.08134

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Table 3 - Statistical results for belowground plant metrics. Linear mixed-effects model was used to determine the impact of various predictors on root length, diameter, surface area, and volume. The tray where plants were grown was used as a random variable.

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			Root Length		Root Diameter		Root Surface Area		Root Volume	
Predictor	df	N	X2	*P*	X2	*P*	X2	*P*	X2	*P*
Soil chemistry	3	242	197.34	<0.0001	145.73	<0.0001	154.25	<0.0001	89.19	<0.0001
Developmental phase	3	242	160.15	<0.0001	0.24	0.971	140.32	<0.0001	94.90	<0.0001
Microbe source	1	242	1.40	0.235	22.25	<0.0001	1.66	0.198	1.86	0.173
Soil chemistry*Developmental phase	3	242	79.24	<0.0001	3.46	0.326	66.09	<0.0001	42.79	<0.0001
Microbe source*Developmental phase	3	242	10.75	0.013	9.81	0.02	9.60	0.022	6.88	0.076
Soil chemistry*Microbe source	1	242	9.65	0.002	29.46	<0.0001	8.62	0.003	5.97	0.015
Soil chemistry*Microbe source*Developmental phase	3	242	17.88	0.001	3.50	0.321	16.63	0.001	12.64	0.005

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Table 4 - Cox proportional hazards model to determine differences in *Plantago erecta* phenology. Cox proportional hazards model was used to determine the likelihood of *P. erecta* reaching a particular plant development phase in distinct soil types. A HR=1 indicates the treatment was used as a reference to which other treatments were compared. A HR > 1 indicates an increased likelihood of development, while an HR < 1 indicates a decreased likelihood of development. For example, *P. erecta* grown in Serp+NSmic is 3.1 times more likely to reach the flowering phase than those grown in live serpentine soil (Serp). Values in parentheses are the confidence intervals for the hazard ratio and * indicates p-value ≤ 0.001.

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	Plant Developmental Phase		
Soil Type	Vegetative	Early Flowering	Late Flowering
S+Sm	1	1	1
NS+NSm	1.38 (0.94-2.0)	1.7 (0.99-3.1)	1.3 (0.68-2.6)
S+NSm	0.84 (0.56-1.2)	1.2 (0.65-2.1)	1 (0.52-2.1)
NS+Sm	6.23* (4.03-9.6)	4.2* (2.36-7.6)	3.1* (1.56-6.1)
NS+Sm+Ni	7.45* (4.02-13.8)	6.3* (3.02-16.1)	2.1 (0.99-4.5)
NS+Sm+D	4.74* (2.61-8.6)	9.9* (4.78-20.3)	30.9* (11.52-83.0)

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