Invasive grass dominance over native forbs is linked to shifts in the bacterial rhizosphere microbiome

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Abstract

Rhizosphere microbiomes have received growing attention in recent years for their role in plant health, stress tolerance, soil nutrition, and invasion. Still, relatively little is known about how these microbial communities are altered under plant competition, and even less about whether these shifts are tied to competitive outcomes between native and invasive plants. We investigated the structure and diversity of rhizosphere bacterial and fungal microbiomes of native annual forbs and invasive annual grasses individually and in competition using high-throughput amplicon sequencing of the bacterial 16S rRNA gene and the fungal ITS region. We assessed how differentially abundant microbial families correlate to plant biomass under competition We find that bacterial diversity and structure differ between native forbs and invasive grasses, but fungal diversity and structure do not. Further, bacterial community structures under competition are distinct from individual bacterial community structures. We also identified five bacterial families that varied in normalized abundance between treatments and that were correlated with plant biomass under competition. We speculate that invasive grass dominance over these natives may be partially due to effects on the rhizosphere community, with changes in specific bacterial families potentially benefiting invaders at the expense of natives.

Keywords: rhizosphere microbiome, competition, invasive species, plant-microbe interactions, annual grassland

Declarations

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Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Availability of data and material

The datasets and scripts supporting the conclusions of this article are available in the GitHub repository, DOI:10.5281/zenodo.4422181. The sequence reads generated for this 16S rRNA gene and ITS region amplicon project were deposited at Genbank under accession no. PRJNA666893.

Code availability

No custom software was used. All R scripts used to process the data are available in the GitHub repository, DOI:10.5281/zenodo.4422181

Authors' contributions

MLL, CLE, and HK conceived of, planned, and carried out the experiment. MLL and CLE processed and analyzed the data and wrote the paper. All authors contributed to the final version of the manuscript.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Introduction

Plants and their associated soil microbial communities have coevolved complex dynamic relationships through time. The soil directly surrounding plant roots, known as the rhizosphere, is home to a diversity of microbes that play vital roles in plant health [1], nutrition [2], and stress tolerance [3,4] and is distinct from the surrounding bulk soil [1,5–7]. Though rhizosphere composition is driven predominantly by abiotic factors [8–10], these communities can be highly host plant-specific [11,12], with plants exhibiting strong local adaptations to their home microbial community [13–15]. Further, there is growing evidence that microbial communities vary in the presence of certain neighbors [16], and can even affect competitive outcomes between plants [17–19] with the potential to drive either coexistence or exclusion [20,21].

Microbes can affect plant-host competition both directly via resource availability and indirectly through community interactions. For example, microbes can make nitrogen more readily available to plants [22,23], which could preferentially benefit hosts with faster uptake

83 rates [24,25] thereby increasing their competitive success [26]. Plants may also indirectly
84 compete for beneficial plant-growth-promoting microbes [7,27,28] or come into contact with
85 novel pathogens through competition with a novel plant, such as an invader [29]. These
86 interactions can lead to the loss of specialized microbes in the inferior competitor [30] and drive
87 the resulting microbial community to resemble that of the dominant competitor [18,19].

88 Shifts in the microbiome are a potentially important contributor to the dominance of
89 invasive plants. Although there is strong evidence for local adaptation between plants and their
90 home microbiome, invasive plants are relatively novel players, causing disruptions in important
91 plant-microbe interactions by increasing soil microbial activity [31], reducing microbial biomass
92 [32] and diversity [33], increasing nitrification rates [24], and changing microbiome composition
93 [34,35]. These plant-soil feedbacks can benefit the invader [35,36], selectively harm natives [37],
94 and/or benefit other invaders [38]. While it is known that invaders and natives can harbor distinct
95 microbial communities, and that competition between host plants can affect microbiome
96 composition (and vice versa), little is known of how changes in the abundance of specific
97 microbial taxa may be tied to the competitive dominance of invaders and/or the competitive
98 inferiority of natives.

99 Serpentine soils in California annual grasslands are characterized by high heavy metal
100 content and low levels of essential plant nutrients, and as a result, are home to a unique
101 community of native forbs. While some of these soils are rocky and shallow, other serpentine
102 areas are deeper and more finely textured ("lush" serpentine) and therefore hold more water and
103 nitrogen [39], making them more favorable to invasive annual grass establishment and growth.
104 These fast-growing invasive grasses outcompete the less abundant and competitively inferior
105 native forbs, contributing to their declines across California grasslands [40–42]. In native-

106 dominated serpentine areas, microbes have been shown to increase seedling survival [43] and
107 facilitate heavy metal tolerance [44]. With the invasion of grasses, microbiome changes may
108 strengthen the advantage grasses have over natives if they recruit beneficial microbes away from
109 natives or harbor novel microbes harmful to natives. Alternatively, locally adapted microbes may
110 be harming invasive grasses, helping native forbs to persist in these areas.

111 We sought to understand how competition between native forbs and invasive grasses
112 affects the bacterial and fungal rhizosphere microbiomes, and how microbes may be shaping
113 competitive outcomes in this community. Our main questions were:

114 (Q1) Do microbiomes differ between invasive grasses and native forbs?

115 (Q2) Do microbiomes of grass-forb pairs (i.e. under competition) differ from the
116 microbiomes of (a) invasive grasses and (b) native forbs?

117 (Q3) If so, are microbiomes of grass-forb pairs sourced more from invasive grasses,
118 native forbs, or equally from both?

119 (Q4) Given compositional changes in the microbiome in grass-forb pairs, which specific
120 microbial families are driving these changes?

121 (Q5) Are abundances of these microbial families correlated with plant performance in
122 grass-forb pairs?

123 To answer these questions, we conducted a manipulative shade-house experiment in
124 which forbs and grasses were grown in field-collected soil both individually and in grass-forb
125 pairs. For each question, we looked at effects in both the bacterial and the fungal rhizosphere
126 measured through high throughput amplicon sequencing, focusing our analysis on group-level
127 results (i.e. forb, grass, and grass-forb pairs).

128 **Methods**

129 *Experimental set-up*

130 In spring and summer 2017, we collected soil and seed from a lush serpentine annual
131 grassland at the University of California McLaughlin Natural Reserve [45] (N 38°52', W
132 122°26'). Seeds were collected from >10 individuals each of six common native annual forb
133 species (Fig. 1a; *Lasthenia californica, Clarkia purpurea, Agoseris heterophylla, Calycadenia*
134 *pauciflora, Hemizonia congesta,* and *Plantago erecta)* and three dominant invasive annual
135 grasses (Fig. 1b; *Avena fatua, Taeniatherum caput-medusae,* and *Bromus hordeaceus*). In
136 December 2017, seeds were sown into physan-washed pots with a mixture of 65% field-collected
137 soil and 35% sterile autoclaved sand to improve drainage. Each species was grown alone (one
138 individual per pot, five replicate pots per species, 45 total pots: 30 forbs, 15 grasses) to assess the
139 microbial communities of individual plant species. To assess the effects of competition between
140 natives and invasives, each forb species was grown with each grass species in a pairwise factorial
141 design for a total of 18 different species pairs (one individual of each grass and forb per pot, five
142 replicate pots per combination, 90 total pots). We chose to focus on grass-forb pairs for our
143 competition treatment as this treatment is the most relevant to understanding how microbial
144 changes in the rhizosphere may contribute to invasive grass dominance. Due to plant mortality
145 and a sample mix-up, final numbers were as follows: grass-forb pairs = 84, forbs = 27, grasses =
146 15. Pots were placed in a shade-house open to natural temperature variation in the UC Davis
147 Orchard Park Greenhouse and soil moisture was maintained with an automatic drip irrigation
148 system (Fig. 1c).

149 *Sample collection*

150 In April 2018, we sampled rhizosphere microbial communities by gently shaking off soil
151 from plant roots, then submerging the roots in autoclaved nanopure water in a 50 mL conical

152 tube and vortexing to obtain the rhizosphere soil similar to Edwards et al. [1]. Conical tubes were
153 then centrifuged at 4000 g for 1 minute to obtain rhizosphere soil pellets. In grass-forb pairs, we
154 sampled the joint rhizosphere microbiome, similar to Sun et al. [46], as roots were completely
155 interwoven.

156 To assess how the microbial community may aid in invasive grass dominance over native
157 forbs, we harvested, dried, and weighed aboveground plant biomass. Roots were not included
158 due to the difficulty of identifying roots to species in the competition treatment. Previous work in
159 this system has shown that competition with invasive grasses lowers fitness of native forbs
160 through decreased seed production[41,47]. While we were not able to measure seed production
161 of plants in this study due to rhizosphere sampling prior to seed set, aboveground biomass in
162 annual plants is often used as a surrogate for competitive ability [48,49] and is well correlated
163 with seed production [50,51] and is therefore a reasonable estimate of plant performance.

164 *Molecular methods and sequence generation*

165 Briefly, we extracted DNA from the 135 rhizosphere soil pellets using the MoBio
166 PowerSoil DNA Extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). Samples were
167 then sent to the Integrated Microbiome Resource (IMR) at Dalhousie University to amplify and
168 sequence the 16S rRNA gene using the 515FB-806RB primer set [52,53] and ITS2 region using
169 the ITS86F-ITS4R primer set [54]. The sequence reads generated for this 16S rRNA gene and
170 ITS region amplicon project were deposited at Genbank under accession no. PRJNA666893.

171 *Sequence processing*

172 Sequence data was processed using the DADA2 workflow [55] in R [56] to create
173 Amplicon Sequence Variant tables (ASV). Chimeras, contaminants, mitochondria and
174 chloroplasts were removed from these tables prior to further analysis. For questions 1-3, the 16S

175 rRNA gene dataset was rarefied to 9434 reads per sample and the ITS region dataset was rarefied
176 to 7557 reads per sample. These rarefaction levels were chosen, after examining rarefaction
177 curves for saturation, based on the size of the sample with the smallest number of reads in order
178 to retain the maximum number of samples for downstream analysis.

179 Further methodological details related to experimental set-up, sample collection and
180 processing, sequence generation, and data processing prior to downstream analyses can be found
181 in the Supplemental Materials.

182 *Data analysis (Q1-Q2)*

183 To evaluate how rhizosphere communities varied in structure (beta diversity) between
184 grasses, forbs, and grass-forb pairs, we conducted a Principal Coordinates Analysis (PCoA) on
185 weighted UniFrac of each sample for rarefied 16S rRNA gene data and on Bray-Curtis
186 dissimilarities for rarefied ITS region data. We tested for significant differences in mean
187 centroids between groups (native forbs, invasive grasses, grass-forb pairs) using a permutational
188 multivariate analysis of variance (PERMANOVA) and between mean dispersions using the
189 betadisper function in vegan [57]. We then conducted post-hoc analyses with Benjamini-
190 Hochberg p-value corrections [58]. We also investigated whether these groups varied compared
191 to background soil (i.e. the community in the soil-sand mix at the start of the experiment) by
192 performing PERMANOVA tests. Finally, we investigated the degree of species-level variation
193 within both grasses and forbs by running PERMANOVA tests on each group separately to
194 evaluate whether there was more variation within or between groups.

195 To assess how rhizosphere communities varied in alpha diversity between grasses, forbs
196 and grass-forb pairs, we calculated Shannon diversity of each sample and conducted a Kruskal-

197 Wallis test to test whether alpha diversity varied between these groups. We then conducted post-
198 hoc analyses using a Dunn Test with Benjamini-Hochberg p-value corrections.

199 *Data analysis (Q3)*

200 To evaluate whether a larger proportion of the joint microbiome community was
201 predicted to be sourced from forbs or grasses, we used SourceTracker, a Bayesian source
202 tracking classifier [59]. SourceTracker uses Gibbs sampling and Dirichlet distributions to
203 estimate the proportional abundance of an ASV originating from provided source populations.
204 First, we trained SourceTracker on the rarified ASV tables of forbs, grasses, and background soil
205 and then tested the trained Bayesian model on the grass-forb pair joint microbiomes to estimate
206 the proportion of ASVs originating from each of these sources. The model assumes that joint
207 microbiomes contain a combination of colonists from known and unknown sources (i.e., any
208 ASVs that are absent from the provided source datasets, and thus any sources not sampled here
209 such as air, water, etc.) and estimates the fraction of ASVs detected in the joint microbiome that
210 originated from grasses, forbs, background soil or unknown habitats. We used Wilcoxon signed-
211 rank tests to assess differences in the SourceTracker predicted proportions between different
212 sources for the bacterial and fungal communities, and then also tested for differences in the
213 proportions of each individual source between the two communities.

214 *Data Analysis (Q4)*

215 We used differential abundance analysis to identify microbial families whose abundance
216 varied between groups in order to investigate which families were driving observed differences
217 in community structure and composition. To do this, we first summed the raw read counts of
218 each full data set (i.e. not rarefied), then used the DESeq2 R package [60] on counts to examine
219 the $\log_2$fold change (i.e. differential abundance) of families between pairwise contrasts of groups

220 (forbs, grasses, and grass-forb pairs). We chose to investigate differences at the family-level to
221 maximize the potential for understanding the functional role of microbes while minimizing zero-
222 inflation observed at higher resolutions. Only 1% of bacterial ASVs were unable to be
223 taxonomically classified at the family level, while 49% of fungal ASVs could not be assigned to
224 a family. Families with Bonferroni corrected p-values < 0.01 were classified as differentially
225 abundant. In subsequent analyses, we focus on families that varied between both grass and forb
226 individual microbiomes and between individual microbiomes and joint grass-forb pair
227 microbiomes to highlight how baseline differences between groups are related to differences
228 observed during competition.

229 *Data analysis (Q5)*

230 To understand whether the abundances of these microbial families may be tied to the
231 dominance of grasses over forbs, we first assessed how biomass varied between forbs and
232 grasses both alone and in pairs, then we investigated the relationship between plant biomass in
233 pairs and microbial family abundance. To compare biomass of forbs and grasses grown alone,
234 we conducted a linear mixed effects model with biomass of plants grown alone as the response
235 variable and functional group (grass or forb) as the predictor variable. Biomass was log-
236 transformed to fit model assumptions and we included a plant species random intercept to
237 account for species-level differences. To compare biomass of grasses and forbs in grass-forb
238 pairs we ran a similar model on log biomass of plants in paired pots, again with a random
239 intercept for each species. We explored other random effect structures that included a term
240 accounting for non-independence of pots, however ultimately did not include this term as it did
241 not improve the model based on the log-likelihood.

242 If grass dominance over forbs was controlled wholly by density dependent effects and
243 competitive hierarchies rather than microbial interactions, we would expect an inverse
244 relationship between grass biomass and forb biomass. To investigate the degree to which forb
245 biomass was linked to grass biomass in pairs we used similar models with log-transformed forb
246 biomass as the response variable and log-transformed grass biomass as the predictor variable and
247 a random intercept for forb species.

248 To investigate the relationship between performance in grass-forb pairs and microbial
249 abundance, we ran linear mixed effects models for each host group (grass and forb) and each
250 differentially abundance microbial family with plant biomass from grass-forb pairs as the
251 response variable and regularized log abundance of the focal family (calculated using the rld
252 function in the DESeq2 package) as the predictor variable. Regularized log abundance (hereafter
253 referred to as normalized abundance) is on the log2 scale and is normalized to library size to
254 allow for direct comparison of microbial abundance across samples. We also included plant
255 species as a random intercept.

256 Finally, we calculated the proportion of each differentially abundant family in the joint
257 microbiome predicted to originate from either the individual forb or grass microbiomes by
258 grouping ASVs into taxonomic families and then summarizing the fraction of ASVs within each
259 family attributed to each possible source using SourceTracker.

260 **Results**

261 *Bacterial microbiomes differ between invasive grasses and native forbs*

262 Overall, the rhizosphere bacterial microbiome varied between invasive grasses and native
263 forbs, but the fungal microbiome did not. The structure of forb bacterial microbiomes was
264 significantly different from the structure of grass bacterial microbiomes ($p < 0.001$; Fig. 2).

265 Further, bacterial Shannon diversity (Fig. 3) was significantly higher in forb microbiomes than in
266 grass microbiomes ($p = 0.035$). Conversely, fungal communities showed marginally
267 nonsignificant differences in structure ($p = 0.07$; Fig. S1) and no significant differences in
268 Shannon diversity of fungal communities among treatments ($p = 0.85$; Fig. S2). Community
269 structure was significantly different between background soil and all treatment groups for both
270 the bacterial ($p < 0.001$, Fig. S3a) and fungal communities ($p < 0.001$, Fig. S3b). There were few
271 species-level differences, with more variation between groups than between species within
272 groups (Figs. S4, S5).

273 *Bacterial microbiomes of grass-forb pairs differ from the microbiomes of grasses and forbs*

274 Grass-forb pairs displayed significantly different joint bacterial microbiomes from
275 microbiomes of both groups grown alone (forb: $p < 0.001$; grass: $p < 0.001$; Fig. 2). Grass-forb
276 pair microbiomes also had marginally higher Shannon diversity than grass microbiomes ($p =$
277 0.059), but did not significantly differ from forb microbiomes ($p = 0.292$). As reported above,
278 there were no significant differences found between treatments in the fungal microbiome, so we
279 did not conduct post-hoc analyses to assess pairwise contrasts between treatment groups.

280 *Microbiomes of grass-forb pairs are sourced equally from invasive grasses and native forbs*

281 For bacteria, SourceTracker identified similar fractions of ASVs in grass-forb pairs
282 predicted to have originated from grasses and from forbs ($p = 0.341$, grass mean ± se: 40.34 ±
283 1.33%, forb: 42.13 ± 1.14%). Only relatively low proportions of the community came from
284 background soil or unknown sources (background: 8.57 ± 0.48%, unknown: 8.96 ± 0.60%). For
285 fungi, the fraction of the community predicted to originate from grasses was marginally higher
286 than that from forbs ($p = 0.066$, grass: 38.88 ± 2.26%, forb: 32.65 ± 2.01%). Additionally, the
287 fraction of the community originating from forbs was significantly lower than the fraction of the

288 bacterial community originating from forbs ($p < 0.001$). A small proportion of the fungal
289 community was predicted to derive from background soil (5.16 ± 0.67%). A much larger
290 proportion of the fungal community was estimated to be from unknown sources (23.31 ± 2.11%),
291 which was significantly higher than the contribution of unknown sources to the bacterial
292 community ($p < 0.001$).

293 *Six bacterial and one fungal family displayed differential abundances between forbs,*
294 *grasses, and pairs*

295 Differential abundance analysis (DESeq2) revealed 9 bacterial families (out of a total of
296 302 bacterial families) (Fig. S6a) that differed in abundance between individual grass and forb
297 microbiomes, with six of these families remaining differentially between joint grass-forb pair
298 microbiomes and individual microbiomes: Methylophilaceae, Fibrobacteraceae,
299 Clostridiaceae_1, Burkholderiaceae, Rhodocyclaceae, and Veillonellaceae (Fig. 4). Of these six
300 bacterial families, only Burkholderiaceae had an untransformed mean relative abundance of
301 greater than one percent (11.69 ± 0.39%; Table S1). There were an additional 10 bacterial
302 families that varied between pairs and individuals, but were not found to be differentially
303 abundant between grasses and forbs grown alone (Fig. S7).

304 Methylophilaceae was the only family with higher normalized abundance in forbs than in
305 grasses and their normalized abundance declined in pairs relative to forbs. Fibrobacteraceae,
306 Clostridiaceae_1 and Veillonellaceae were all higher in normalized abundance in grasses than in
307 forbs and increased in grass-forb pairs relative to forbs. Both Burkholderiaceae and
308 Rhodocyclaceae were higher in grasses than in forbs, but decreased in grass-forb pairs relative to
309 grasses.

310 Using DESeq2, we found only one fungal family (out of 231) that was differentially
311 abundant between grasses and forbs, and between pairs and individuals (Tubeufiaceae) (Fig.
312 S6b, Fig. S8). This family had higher normalized abundance in forbs than in grasses and
313 subsequently lower normalized abundance in grass-forb pairs than in forbs. Two other families
314 that did not vary between grasses and forbs showed variation between pairs and either grasses or
315 forbs.

316 *Normalized abundances of key microbial families are correlated with grass dominance over*
317 *forbs*

318 When forbs and grasses were grown alone, grass and forb biomass did not significantly
319 differ ($p = 0.435$), however in grass-forb pairs, grass biomass was significantly higher than forb
320 biomass ($p < 0.001$), indicating that despite no inherent differences in biomass between groups at
321 this stage, grasses outperformed forbs when grown together. We found no significant
322 relationship between forb biomass and grass biomass in grass-forb pairs (est = -0.153, se =
323 0.102, $p = 0.138$), suggesting that decreases in forb biomass were not solely due to increases in
324 grass biomass. Instead, individual plant performance in pairs was closely related to microbial
325 abundance. Of the six families that differentially varied between grasses and forbs grown alone,
326 and remained differentially abundant in pairs, all but Rhodocyclaceae displayed relationships
327 with either forb or grass biomass in paired pots (Fig. 5, Table S2). Moreover, the four families
328 that displayed negative correlations with forb biomass and/or positive correlations with grass
329 biomass (Burkholderiaceae, Fibrobacteraceae, Clostridiaceae_1, and Veillonellaceae) had higher
330 predicted proportions originating from grass microbiomes according to SourceTracker, while the
331 one family that displayed a positive correlation with forb biomass and a negative correlation with

332 grass biomass (Methylophilaceae) had a higher predicted proportion originating from forb
333 microbiomes (Fig. 6).
334 The normalized abundance of Methylophilaceae was positively correlated with forb
335 biomass in pairs, and negatively correlated with grass biomass in pairs, indicating that as the
336 normalized abundance of this family decreased, grass performance increased and forb
337 performance decreased. The normalized abundance of Fibrobacteraceae was negatively
338 correlated with forb biomass in pairs, but was not correlated with grass biomass, indicating that
339 as the normalized abundance of this family increased, forb performance decreased despite no
340 change in grass performance. The normalized abundance of Clostridiaceae_1 was positively
341 correlated with grass biomass, and marginally negatively correlated with grass biomass. The
342 normalized abundance of Burkholderiaceae was positively correlated with grass biomass and
343 marginally negatively correlated with forb biomass. Finally, the normalized abundance of
344 Veillonellaceae was marginally positively correlated with grass biomass.
345 In the remaining 13 differentially abundant bacterial families, only Weeksellaceae, which
346 did not vary between grasses and forbs, showed a significant relationship with biomass (Fig. S9,
347 S10; Table S2). Similarly, only the fungal family Ceratobasidiaceae , which again did not vary
348 between forbs and grasses, showed a significant relationship with biomass (Fig. S11, S12; Table
349 S2).
350 **Discussion**
351 The rhizosphere bacterial community of native forbs is structurally distinct from those of
352 invasive grasses and has higher alpha diversity. Interactions between grasses and forbs here
353 correlated with shifts in the bacterial rhizosphere towards communities dissimilar to both
354 individual forb and grass microbiomes, with marginally higher diversity than grasses alone.

355 Studies in similar annual grasslands have found little change in bacterial structure with increased
356 invasive grass abundance [61–63] but invader dominance has been linked to decreased bacterial
357 diversity in other systems [33,64]. While we expected the microbiomes of grass-forb pairs to be
358 a mixed community sourced from both forbs and grasses, we also expected the joint rhizosphere
359 to be dominated by the microbes of the dominant competitor as in Hortal et al. [18] and Lozano
360 et al. [19]. In partial support of this, we found that four out of the six families that varied both
361 between forbs and grasses and between individuals and pairs came predominantly from grasses
362 and increased in abundance relative to forbs, while the one family that was majority sourced
363 from forbs saw a decrease in abundance. At the community level, however, we found that the
364 novel assemblage of bacteria in pairs was sourced nearly equally from both groups. These results
365 suggest that the invasions of grasses into native forb habitats may be associated with microbiome
366 shifts in both groups; however, these changes may have more negative consequences for native
367 forbs than invasive grasses.

368 Consistent with invasive annual grasses aiding in native forb declines across California
369 annual grasslands, we found that grasses outperformed forbs in paired pots despite no differences
370 when grown alone. Plants were well-watered and harvested before shading could become
371 problematic, therefore differences in performance likely resulted from below-ground
372 interactions. Given the large role played by microbes in below-ground interactions, the lack of a
373 relationship between grass and forb performance in pairs, and the relationship between microbial
374 abundance and plant performance in pairs, we speculate that invasive grass dominance over
375 native forbs here is partially mediated by microbes. Invaded grassland soils tend to be depleted in
376 plant available nitrogen due to the nutrient-demanding nature of invasive grasses [32,62,63], but
377 with increased microbial activity and faster nitrogen cycling [24,31,63]. These microbe-driven

378 changes may selectively benefit fast-growing grasses over natives, especially in nutrient-poor
379 serpentine soils where added nitrogen has been found to preferentially aid invasives [51,65].
380 Invasive grasses may further benefit from decreased microbial diversity, which has been linked
381 to both decreased forb nitrogen uptake and increased grass nitrogen uptake [66]. Beyond
382 competition for nutrients, grasses and forbs may also indirectly compete through recruitment of
383 microbes harmful to natives [67] and/or helpful to invasives [36]. Other studies in serpentine
384 grasslands have shown that soil primed with invasive grass negatively affected native forb
385 growth due to changes in the microbial community [68,69]. Although future work should be
386 conducted to tease apart the role of plant density on microbial shifts and to understand the degree
387 to which microbial shifts drive competitive outcomes in this system, our results add to a body of
388 evidence that the invasion of grasses alters the microbiome and suggest that their dominance may
389 be linked to these shifts.

390 Plant performance in pairs could not be explained by the performance of their neighbor.
391 Instead, plant performance in pairs was tied to the abundance of key bacterial families. We found
392 links between bacterial normalized abundance and plant biomass in grass-forb pairs for five of
393 the six main differentially abundant families: three that were correlated with decreased forb
394 performance: Burkholderiacae (marginal), Clostridacaeae_1 (marginal), and Fibrobacteraceae,
395 three that were correlated with increased grass performance: Burkholderiaceae, Clostridiaceae_1
396 (marginal), and Veillonellaceae (marginal), and one that was correlated with both decreased
397 grass performance and increased forb performance: Methylophilaceae.

398 Methylophilaceae have been found in soils with high heavy metal content and include
399 microbes critical for heavy metal attenuation and immobilization [70,71]. Members of
400 Methylophilaceae also form beneficial symbioses with plants [72–74]. This family may include

401 important taxa for serpentine-adapted species with roles in heavy metal attenuation and plant
402 growth [73]. Forbs contributed a higher percentage of ASVs from this family to the joint grass-
403 forb rhizosphere microbiome, further supporting close associations between native forbs and this
404 group. If interactions with invasive grasses are driving declines in this family as our results
405 suggest, grass dominance in this system may be partially due to a decrease in locally adapted
406 microbes similar to Cavalieri et al. [30].

407 Grasses were likely driving higher normalized abundances of Fibrobacteraceae,
408 Veillonellaceae, and Clostridiaceae_1, as they contributed a higher percentage of ASVs from
409 these families to the joint rhizosphere microbiome in pairs. Although Fibrobacteraceae is
410 typically lower in abundance in soils with high heavy metal content [71], this family is also
411 positively associated with invasive grass dominance [75]. Further, all three families are known
412 for their cellulose-degrading properties [76–78], and therefore may contain important taxa for
413 organic matter degradation [79]. Both Fibrobacteraceae and Clostridiaceae_1 contain known
414 nitrogen-fixers [80–82]. If taxa in these families are increasing nutrient availability, they could
415 be disproportionately helping fast-growing invasive annual grasses [51,65].

416 Burkholderiaceae are considered keystone members in grasslands as endophytes or
417 pathogens [83–86] and are known for their antimicrobial properties [87–89], nitrogen-fixing
418 abilities [80,90], and competitive dominance, especially in N-limited environments [91]. Some
419 members are even known to suppress fungal pathogens [92,93]. It is possible that the
420 Burkholderiacae observed in pairs here are either providing positive functional benefits to
421 grasses, or are less harmful to grasses than forbs as pathogens.

422 In contrast to our bacterial findings, we found no significant differences in fungal
423 communities across treatments, either in terms of structure or diversity. This suggests loose

424 fungal associations with plant hosts, possibly due to functional redundancy of fungi across large
425 geographic scales [94]. Within annual grasslands, however, there is evidence of invader-driven
426 fungal shifts [61] and increases in fungal relative abundance [95]. Our limited fungal findings
427 may indicate that fungi are generally less important than bacteria for competitive outcomes
428 between plants [96]. Alternatively, fungi may be important, but complex fungal-fungal
429 interactions at the community level may cancel out fitness effects on plants by beneficial and
430 detrimental fungi [97]. In addition, experimental constraints such as unaccounted fungal regional
431 source pools (such as airborne local spores or local watersheds [94]), plant age and development
432 at time of sampling [16], and methodology (e.g. sampling rhizosphere here instead of root
433 endophytes (e.g. Emam et al. [98]) or fungi in the rhizoplane (e.g. Edwards et al. [1])), may have
434 prevented the observation of interactions between the fungal community and plant competition.

435 *Conclusions*

436 Native annual forbs are host to a community of microbes distinct from, and more diverse
437 than, those of their invasive competitors, but these close associations may be disrupted by
438 invasive grasses. The joint bacterial rhizosphere of invasive grasses and native forbs differed
439 from those of plants grown alone, with grasses contributing more to the abundance of ASVs
440 from families that were linked to decreases in forb performance and/or increased in grass
441 performance. Moreover, three of these families were higher in abundance in joint microbiomes
442 relative to those of forbs alone. Forbs on the other hand contributed more to the abundance of
443 ASVs from only one family that was associated with increased forb performance and decreased
444 grass performance, but this family declined in abundance in the joint rhizosphere relative to
445 forbs. Given the correlative nature of our study, more research is needed to understand the role of
446 the microbiome in invasive grass dominance over native forbs and to clarify whether the

observed changes in plant performance were indeed driven by these candidate bacterial families. Regardless, our study highlights the importance of considering the microbiome in ecosystems facing dominance by invasive species.

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Figure 1. (a) Native annual forb species (from left to right, top to bottom): *Clarkia purpurea, Calycadenia pauciflora, Lasthenia californica, Agoseris heterophylla, Plantago erecta,* and *Hemizonia congesta.* (b) Invasive annual grasses (from left to right): *Bromus hordeaceus, Taeniatherum caput-medusae,* and *Avena fatua.* (c) Shade-house set-up. Photos of *C. purpurea*, *P. erecta, H. congesta*, *C. pauciflora*, *B. hordeaceus*, *T. caput-medusae*, and *A. fatua* by Paul Aigner. Remaining photos by authors.

Figure 2. Bacterial microbiome structure differs between grasses and forbs, and shifts during competition. Principal coordinates analysis (PCoA) visualization of weighted UniFrac distances of bacterial communities associated with the rhizosphere. Points in the ordination are colored and represented by shapes based on the rhizosphere of forbs grown alone (purple circles), grasses grown alone (green triangles) and grass-forb pairs, i.e. competition (yellow squares). Ellipses represent the 95% confidence interval around the centroid of each group.

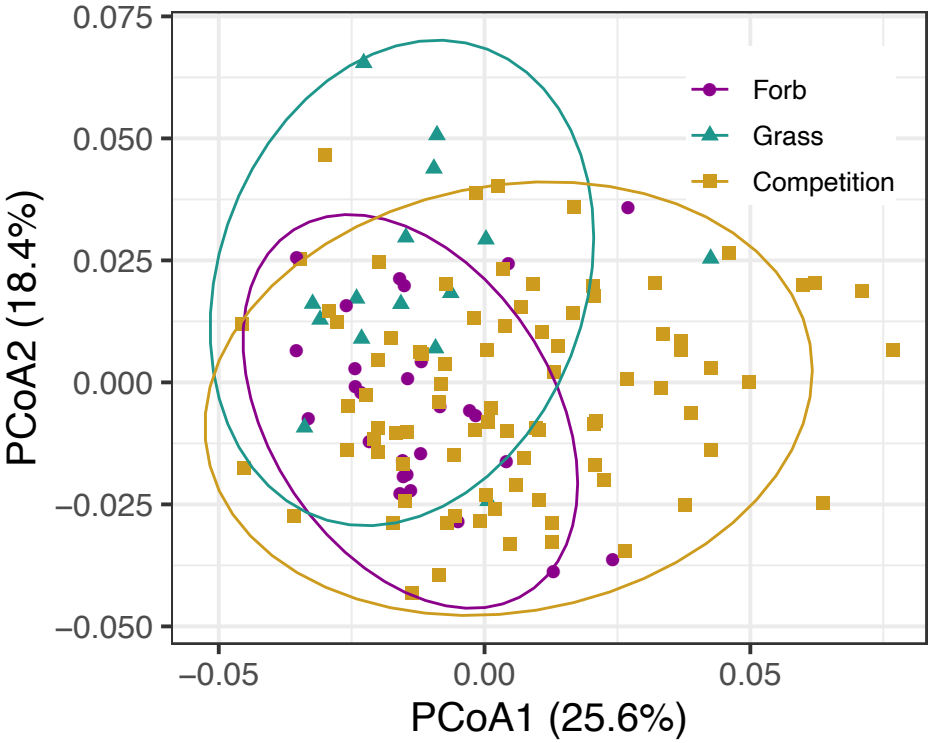


Figure 3. Shannon diversity of bacterial microbiomes is highest in forbs. Shannon diversity was used to assess alpha diversity for bacterial microbiomes for each treatment (forbs grown alone, grasses grown alone, competition). Comparisons that are significantly different from each other are notated by different letters (e.g. a vs. b).

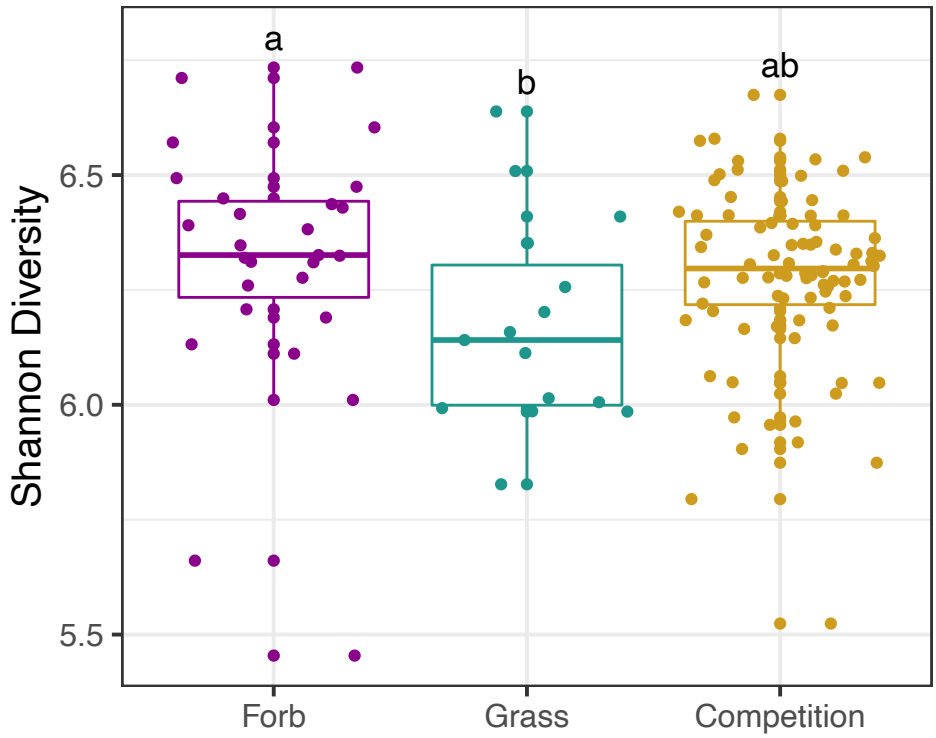
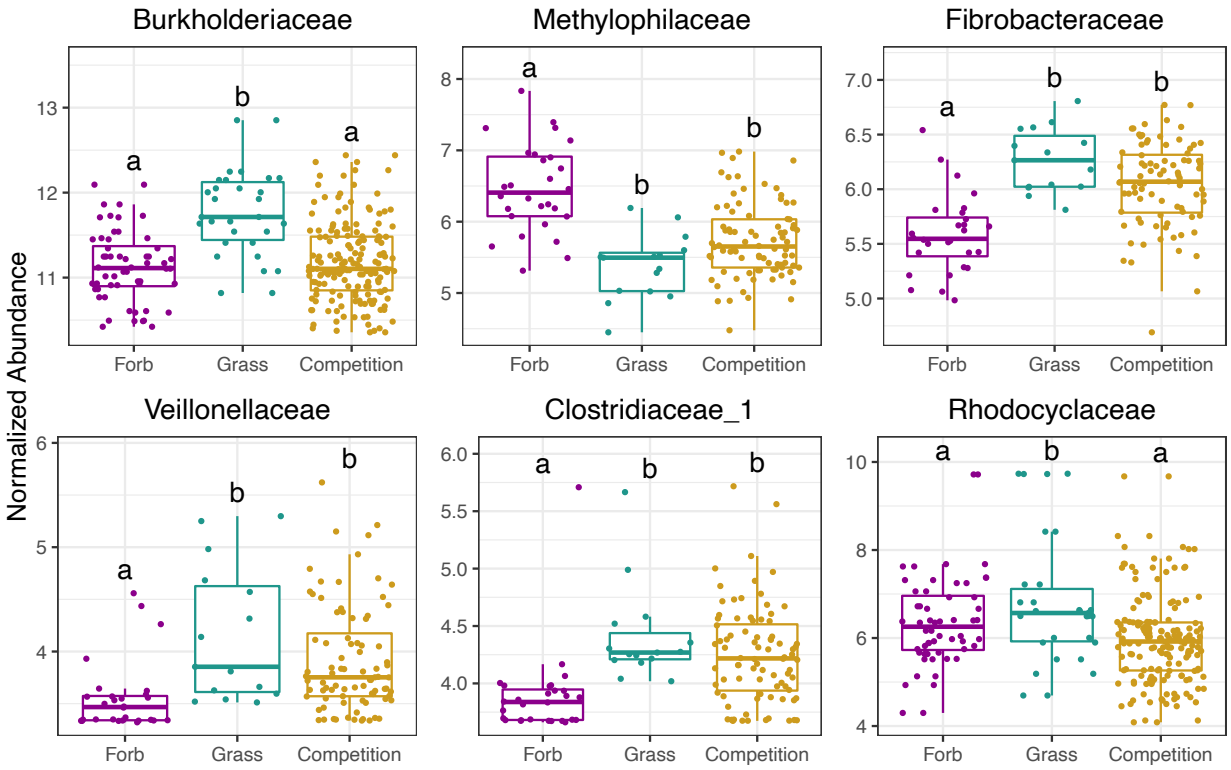


Figure 4. Bacterial families that are differentially abundant between grass and forb microbiomes, and between grass-forb pairs and either grass or forb microbiomes. Using DESeq2, bacterial families were identified whose abundance differed significantly between treatments (forbs grown alone, grasses grown alone, grown together in competition). Each plot shows the normalized abundance of families for each treatment and comparisons that are significant from each other are notated by different letters (e.g. a vs. b).



762 **Figure 5.** Bacterial family abundances correlated with forb (purple) and grass (green) biomass in

763 pairs. Solid line indicates significance ($p < 0.05$), dashed line indicates marginal significance (p

764 < 0.10). For regression results see Table S2.

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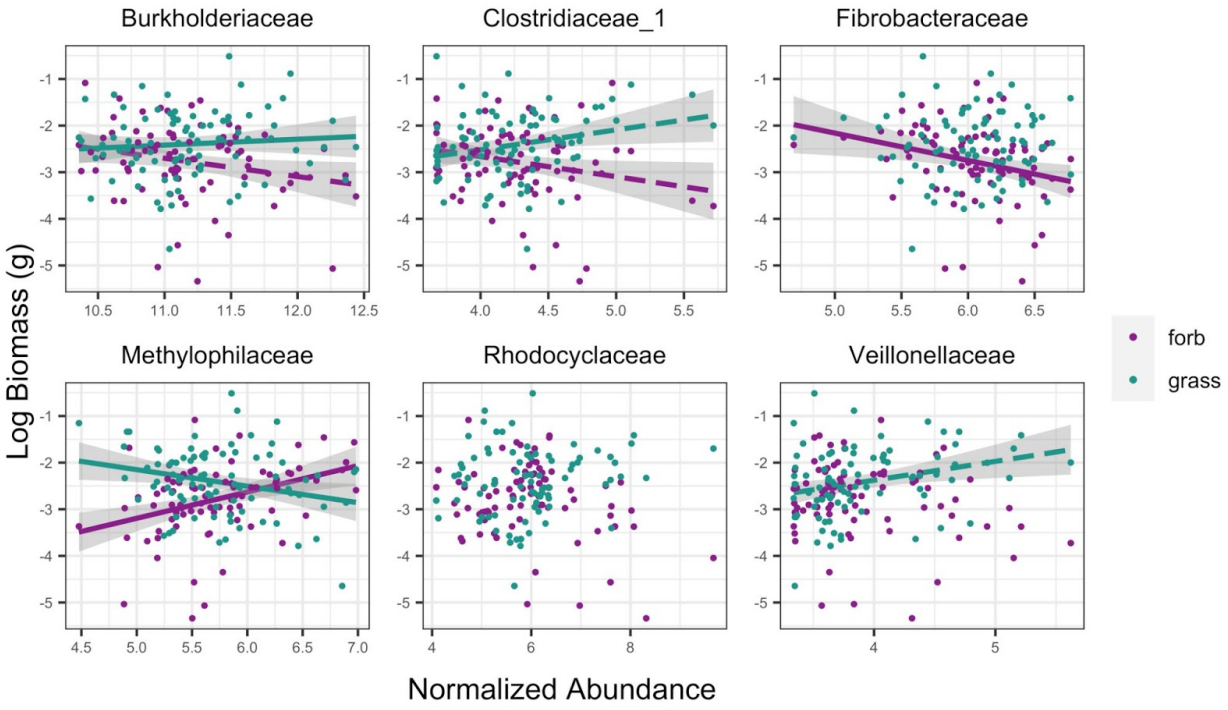

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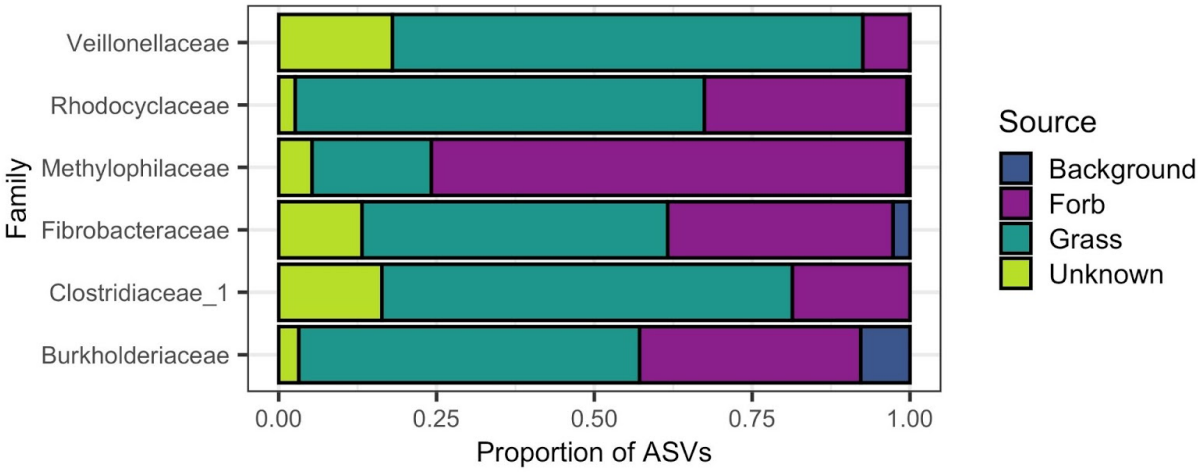
Figure 6. Proportion of ASVs predicted to colonize from each source for each of the six differentially abundant families. SourceTracker was used to predict whether ASVs in competition treatments originated from grasses (dark green), forbs (purple), the background soil mix used in the experiment (blue), or unknown habitats (light green) and then the results were summarized by grouping ASVs by taxonomic family. Families that were negatively correlated with forb performance and/or positively correlated with grass performance were predominantly sourced from grass. Methylophilaceae, the one family that showed a positive relationship with forb biomass under competition and a negative relationship with grass biomass under competition, was predominantly sourced from forbs.