Title: Soil type shapes unique pathogen communities on nearby populations of a California native bunchgrass

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Abstract

The role of infectious disease in regulating host populations is increasingly recognized, but the environmental conditions that facilitate versus hinder pathogen-mediated population regulation remain poorly understood. We compared the relative contributions of soil type and pathogen community composition to foliar disease burden in a perennial bunchgrass species found on two distinct soil types that support distinct plant communities in a California grassland. We hypothesized that populations on different soil types would have significantly different disease burdens caused by unique pathogen communities. To test this hypothesis, we compared foliar disease burden and foliar fungal pathogen communities in nearby populations of *Stipa pulchra* found in nonserpentine greenstone soil that is dominated by invasive Mediterranean grasses, and in serpentine soil, a harsh soil high in heavy metals and low in essential nutrients that supports a diverse community of native plant species. We analyzed the chemical makeup of serpentine and nonserpentine plant tissue to understand potential impacts of soil chemistry on plant health and pathogen community composition. We found that serpentine and nonserpentine *S. pulchra* experienced consistent, low disease pressure caused by distinct communities of foliar pathogens, and that serpentine plants, like the soil in which they grew, had elevated Ni and Mg

content and decreased C, N, Ca, and P content compared to nonserpentine plants. The results imply that pathogens are unlikely to regulate the population dynamics of this native plant, and that pathogen communities are structured either by plant community composition or tissue chemistry. Local variation in soil type and annually variable conditions associated with high species turnover in pathogen communities may create a refuge from disease outbreaks for *S. pulchra*, contributing to the low disease burden observed on this and other Mediterranean grassland species.

Introduction

How environmental heterogeneity affects species interactions, and ultimately population growth, remains a fundamental ecological question. Infectious disease, which arises from interactions between hosts, parasites, and the environment, is particularly likely to vary across populations in differing environments. Both biotic factors, such as the phylogenetic structure of the host community, and abiotic factors, such as elevation, have been associated with the presence of specific diseases and the severity of their impacts on their hosts (Abbate and Antonovics 2014, Parker et al. 2015). Additionally, mounting evidence suggests that specific characteristics of pathogen community structures, including the identities and abundances of the diseases present in a host population, mediate the outcomes of host – pathogen interactions (Seabloom et al. 2015, Borer et al. 2016). Together, these observations highlight the importance of understanding interactions between environmental variation, pathogen community composition, and pathogen pressure on host populations to assess the potential for disease to regulate host population dynamics. For plant hosts, such assessments are important both in conservation and agricultural contexts because identifying populations at high risk for severe disease outbreaks and applying preventative measures may prevent local species extinctions and improve crop yields, respectively. While studies have frequently explored the interactions of

single host – pathogen pairs under differing abiotic conditions (e.g., Abbate and Antonovics 2014) or the effect of varying pathogen community composition on disease burden (e.g., Telfer et al. 2010), relationships between abiotic variables and pathogen community structures remain largely unexplored in natural systems (but see Spear 2017).

California grasslands are an ideal model system in which to study interactions between environmental variation, pathogen community composition, and disease burden because these plant communities are widespread, exist across a variety of environmental gradients, and include distinct plant community subtypes (Eviner 2016). Here, we take advantage of the natural occurrence of the native perennial bunchgrass *Stipa pulchra* on distinct soil types—serpentine, hosting diverse, native-dominated grassland communities and a nonserpentine greenstone (hereafter, nonserpentine), dominated by exotic plants—to understand the extent to which soil type and time influence the landscape of disease encountered by a single plant species, including foliar fungal pathogen community composition and disease severity.

Differences in plant community composition and soil chemistry suggest that *S. pulchra* populations growing in distinct soil types are likely to host distinct fungal pathogen communities and/or experience differing disease burdens, particularly if the phenotype of *S. pulchra* is influenced by the soil in which it grows. *S. pulchra* naturally occurs in both nonserpentine soils and in relatively rare serpentine soils that host grassland plant communities. These two soil types differ in their chemistry and host distinct plant communities, resulting in a suite of environmental differences that may influence pathogen infection, reproduction, and persistence (Huenneke et al. 1990, Harrison and Viers 2007). In contrast to the majority of California grasslands, which are heavily invaded by exotic species, serpentine grasslands have a diverse array of native species adapted to chemically and physically harsh soils: low in essential plant nutrients (N, P, K, Ca), high in heavy metals that are potentially toxic to plants (Co, Ni, Cr, Fe), and rocky and shallow,

with poor moisture retention (Proctor and Woodell 1975, Oze et al. 2004, 2008, McNaughton 1968, Harrison and Viers 2007, Eviner 2016).

Soil properties determine a variety of factors likely to influence the occurrence and outcomes of plant infections, including soil moisture levels and plant chemistry and physiology (Cunningham et al. 1999, McElrone et al. 2005, Smith 2007). Spores of different fungal species vary in both their ability to survive in dry and moist environments and their tolerance of heavy metals (Coley-Smith and Cooke 1971, Iram et al. 2009). Moreover, plants of the same species grown in drier and lower-nutrient soils can have thicker epidermises and higher concentrations of phenolic compounds, many of which have antifungal properties (Osbourne 1996, Cunningham et al. 1999). Finally, soil chemistry may influence pathogen infection either through hyperaccumulation of heavy metals, which can bolster chemical defenses against natural enemies, or by limiting common mechanisms of plant defense (Springer et al. 2006, Rascio and Navari-Izzo 2011).

In this study, we ask: (1) how does disease burden differ on plants growing in different soil types? (2) how does the species composition of pathogen communities differ on plants growing in different soil types? and (3) by what mechanisms—including host community composition and plant tissue chemistry—does soil type affect disease burden and pathogen composition? We hypothesize that both disease burden and pathogen species composition differ on *S. pulchra* individuals growing in serpentine versus nonserpentine soils, either because of differences in the composition of the surrounding plant community, differences in plant physiological responses, or both. We further hypothesize that the soil type *S. pulchra* grows in alters foliar tissue chemistry in ways that may affect disease burden, with higher heavy metal content and/or lower C, N, and P content in serpentine plants. If so, these foliar chemistry differences may translate into either (a) higher disease in serpentine plants relative to

nonserpentine plants, facilitated by negative impacts of limited plant resource availability on plant health (as in Springer 2006), or (b) lower disease in serpentine plants due to heightened chemical and/or physiological plant defenses in response to serpentine soil properties. The results of this study will aid in assessing disease risk for plant populations in natural and agricultural contexts by providing information about the spatial and temporal scales over which soil type and other environmental factors can affect the spread of outbreaks.

Methods

All surveys and sample collection took place at Jasper Ridge Biological Preserve (JRBP), a 485-hectare site in San Mateo County, CA managed by Stanford University (37.4° N, 122.2° W). JRBP has a Mediterranean climate with cool winters (mean 9.2°C), warm summers (mean 20.1°C), and annually variable precipitation that predominantly occurs in the winter and spring (averaging 622.5 mm per year) (Ackerly et al. 2002). The growing season occurs during the rainy season and plants senesce in the early summer. This study was conducted during the growing seasons from mid-April to mid-May in 2015, 2016, and 2017. There was highly variable annual precipitation across the three years (491 mm, 604 mm, and 985mm in 2015, 2016, and 2017, respectively; Weather Underground).

Substantial populations of *S. pulchra* occur in both serpentine and nonserpentine grasslands at JRBP (McNaughton 1968). The serpentine plant communities at JRBP are more diverse than the nearby nonserpentine plant communities and include many native grasses and forbs, in contrast to the invasive-dominated nonserpentine grasslands (McNaughton 1968). Plant species commonly found in serpentine grasslands at JRBP include *Stipa pulchra*, *Elymus glaucus*, *Elymus multisetus, Eschscholzia californica*, and the invasive grass *Bromus hordeaceus* (McNaughton 1968, Hobbs and Mooney 1985). The nonnative annual species that dominate the nonserpentine grasslands but are absent from serpentine grasslands include *Avena barbata,*

Avena fatua, *Bromus diandrus,* and *Erodium botrys* (McNaughton 1968, Hobbs and Mooney 1985).

Quantification of disease

To assess plant disease burden, we quantified the percentage of living leaf area exhibiting symptoms of fungal disease in serpentine and nonserpentine populations of *S. pulchra*. We selected three serpentine grassland sites and three nonserpentine grassland sites such that each serpentine site was paired with a nearby (~170m away) nonserpentine site. Individual sites within each soil type were >300m apart. Each year, we randomly placed four 5-meter transects at each site and assessed the infection status of the *S. pulchra* individual nearest each meter mark, resulting in five individual plants per transect surveyed each year (N = 60 plants per soil type per year). We visually estimated the percentage of living foliar tissue damaged by pathogens for six arbitrarily-selected leaves per plant. All survey sites were located along the ridgetop at JRBP, where serpentine and nonserpentine (greenstone) soils are present in discrete adjacent bands (Figure 1a, Oze et al. 2004). The relative flatness of the ridgetop ensured that all survey sites were at roughly the same elevation and had similar slopes, aspects, and water availability (Oze et al. 2008).

We compared the mean percentage of diseased leaf area and proportion of surveyed leaves with disease in serpentine and nonserpentine *S. pulchra* using Welch's two-tailed t-tests for the data over all years. We used analysis of variance (ANOVA) to test for the effects of year on disease levels in serpentine and nonserpentine *S. pulchra*. When ANOVA results were statistically significant, we used pairwise t-tests with Bonferroni adjustments of p-values to account for multiple comparisons to identify the interactions contributing to the significant result.

143 *Isolation and molecular identification of foliar fungal pathogens*

144 To identify the fungal foliar pathogens infecting serpentine and nonserpentine *S. pulchra,*
145 we harvested one segment of symptomatic leaf tissue for culturing and identification of the
146 causative fungal pathogen(s) from each surveyed individual with disease. We excised, surface
147 sterilized, and plated a 2mm^2 square of tissue from each sample at the leading edge of infection.
148 We observed the plates for growth for eight weeks and isolated morphologically distinct hyphae
149 into axenic culture. We extracted and sequenced genomic DNA from the ITS region for each
150 isolate. Fungal isolation and sequencing methods followed Spear and Mordecai (2018). We
151 estimated taxonomic placement of fungal isolates by grouping sequences into operational
152 taxonomic units with 97% sequence similarity (OTUs), a proxy for species delineation based on
153 the range of intraspecific ITS divergence (O'Brien et al. 2005), and then comparing assembled
154 DNA sequences to the entries in the UNITE fungal database (see Supplemental Information for
155 additional details).

156 *Analyses of fungal community composition*

157 To characterize pathogen community composition in serpentine and nonserpentine *S.*
158 *pulchra* populations, we compared the diversity and assessed the similarity of communities of
159 fungi cultured from the diseased tissue of surveyed plants. To describe fungal community
160 diversity, we (1) calculated observed taxa richness and Fisher's alpha, a widely used measure of
161 species richness that is robust to uneven sample sizes (Fisher et al. 1943, Magurran 1988, Hansen
162 and Coleman 1998); (2) generated taxa accumulation curves to understand sampling efficacy;
163 and (3) counted the number of fungal isolates in each genus, family, order, and class from each
164 grassland type. We compared fungal community composition across grassland types and across
165 years visually, using non-metric multidimensional scaling (NMDS), and statistically, using

166 permutational multivariate analyses of variance (PERMANOVA) with the Adonis and
167 pairwise.perm.manova functions (Anderson 2001, Oksanen et al. 2016, Hervé 2017). To account
168 for low sample size in some transects, we considered the combined isolates from two transects at
169 each site in each year to be a distinct community (for a total of two communities per site per
170 year) for these analyses. For the NMDS and PERMANOVA analyses, we used the function
171 vegdist with the abundance-based Chao method, accounting for unsampled species, to create a
172 matrix of pairwise community dissimilarities (Chao et al. 2016, Oksanen et al. 2016). Finally, we
173 used the Morisita-Horn index, which is independent of sample size and diversity, to make
174 pairwise comparisons of similarity within and between the serpentine and nonserpentine fungal
175 communities from each year (N = 265 isolates, 200 bootstrap replicates) (Wolda 1981, Jost et al.
176 2011).

177 *Chemical analyses of leaf tissue*

178 We analyzed C, N, Ca, Cr, Mg, Ni, and P content of the foliar tissue of *S. pulchra* grown
179 in serpentine and nonserpentine soils *in situ* and in pots. In 2016, we collected the youngest fully
180 expanded and entirely asymptomatic leaf of each individual surveyed in the field (N = 58
181 nonserpentine, 60 serpentine). We dried the samples at room temperature for three months, then
182 at 60°C for 48 hours, ground them to a powder, and then dried them again at 60°C for 24 hours.
183 We measured C and N content of these samples with a Carlo Erba NA-1500 elemental analyzer
184 using atropine standards.

185 To compare Ca, Cr, Mg, Ni, and P content, which requires more tissue biomass than
186 could be collected from plants in the field, we used a set of plants grown in pots in the
187 greenhouse and then moved to JRBP for a separate experiment (see Supplement for additional
188 information). In total, we grew 480 plants in serpentine soil sourced from our survey sites at

189 JRBP and 480 plants in nonserpentine greenhouse soil (PROMIX HP Mycorrhizae TM High
190 Porosity Professional Growing Medium, Premier Horticulture Inc.). To ensure that serpentine-
191 and nonserpentine-grown plants encountered similar soil biota, we triple autoclave-sterilized
192 serpentine soil (40-minute cycles at 120°C) and then immediately inoculated it with greenhouse
193 soil in a ratio of 93:7 by weight (serpentine soil treatment; Heinze et al. 2015) and mixed
194 sterilized serpentine soil into greenhouse soil in the same ratio (nonserpentine soil treatment, to
195 account for possibility of microorganisms surviving autoclaving). Autoclave sterilization of soil
196 has been shown to alter physical and chemical properties and thus has the potential to affect our
197 results. However, the documented instances of autoclaving-induced changes to extractable
198 levels of the elements we measured, including increased extractable Ca:Mg and decreased
199 extractable Ni, suggest that this procedure is more likely to increase than decrease the similarity
200 of nutrient availability in serpentine and nonserpentine soils (Tadros 1957, Wolf et al. 1989,
201 Abou-Shanab et al. 2003). Therefore, any significant differences observed between the foliar
202 chemistry of *S. pulchra* grown in autoclaved serpentine and unsterilized nonserpentine soils that
203 indicate plant tissue chemistry matching soil chemistry should not be due to autoclave
204 sterilization effects.

205 Elemental content of these plants was measured via inductively coupled plasma optical
206 emission spectrometry (ICP-OES). Because the minimum amount of leaf tissue for which some
207 heavy metals can be detected via ICP-OES is 0.5g and most of our *S. pulchra* had a total
208 aboveground biomass of less than 0.5g, each measurement represents the combined elemental
209 content of equal weights of oven-dried foliar tissue from two plants. Plants were randomly
210 selected for analysis from among all individuals with an aboveground biomass of at least 0.29g.
211 All asymptomatic leaves were removed from each plant's base and processed as described
212 above. A 0.5g portion of each pooled sample was digested in 10ml of 70% trace metal quality

213 nitric acid with microwave assistance. Digested samples were diluted to 16.7% acid
214 concentration with DI water and the concentrations of Ca, Cr, Mg, Ni, and P were measured in
215 parts per billion by a Thermo Scientific iCAP 6300 spectrometer (Thermo Scientific, Waltham,
216 MA, USA) (Cantarelli et al. 2010).

217 To assess the potential for soil chemistry to mediate plant-pathogen interactions, we
218 related soil type to leaf tissue chemistry of *in situ* and potted *S. pulchra*, and chemistry of *in situ*
219 plants to disease burden. We compared the mean mol/kg dried weight of leaf tissue for Ca, Cr,
220 Mg, and Ni for potted serpentine and nonserpentine plants with Wilcoxon rank sum tests. We
221 also compared mean C:N and Ca:Mg ratios that are indicative of plant health (Ciompi et al.
222 1996, Brady et al. 2005). We used Pearson's product-moment correlation to test for significant
223 monotonic relationships between the amount of diseased tissue observed on individual plants in
224 the field and their foliar C and N content.

225 All statistical analyses were done in R version 3.4.1. We used the packages ggplot2
226 (Wickham 2016), vegan (Oksanen et al. 2016), SpadeR (Chao et al. 2016), bipartite (Dormann et
227 al. 2008), fossil (Vavrek 2011), BiodiversityR (Kindt and Coe 2005), rich (Rossi 2011), and
228 RVAideMemoire (Hervé 2017).

229 **Results**

230 *Pathogen damage*

231 Pathogen damage was similarly ubiquitous and low-burden in serpentine and
232 nonserpentine soils. Every plant surveyed exhibited evidence of foliar fungal-caused disease.
233 The mean percentage of diseased leaf area observed across all years was 1.66 for nonserpentine
234 and 1.63 for serpentine *S. pulchra,* respectively ($t = 0.11$, $df = 356.2$, p-value = 0.91; Figure 1b),
235 and the mean proportion of pathogen-damaged leaves was 0.83 for both soil types. Although the

236 percentage of diseased tissue on serpentine plants increased slightly each year from 2015 to 2017
237 (F-value = 4.49, p-value = 0.035), the Bonferroni-adjusted p-values for t-tests for each pair of
238 years were not statistically significant (p-values = 0.16, 0.45, 1.00). There was no statistically
239 significant effect of year on nonserpentine pathogen damage (F-value = 1.35, p-value=0.246).
240

241 *Fungal pathogen community*

242 We isolated 267 unique fungal isolates from 258 symptomatic tissue pieces with fungal
243 growth (144 nonserpentine, 114 serpentine from 36 nonserpentine and 34 serpentine transects,
244 out of 360 total tissue pieces). Of the 267 unique fungal isolates, we successfully sequenced 256
245 isolates. The sequenced isolates clustered into 30 operational taxonomic units (OTUs) based on
246 97% sequence similarity, representing 23 genera, 15 families, 8 orders, and 5 fungal classes. The
247 largest OTU contained 48 isolates and the smallest OTUs consisted of one isolate each. OTUs
248 are hereafter referred to as "species" (O'Brien et al. 2005).

249 The serpentine fungal community was more diverse than the nonserpentine community at
250 every taxonomic level (Tables S-1 to S-6). Twenty-two fungal species were isolated from
251 nonserpentine (Fisher's alpha = 7.261, 95% CI = 3.597, 10.925) and 24 from serpentine *S.*
252 *pulchra* (Fisher's alpha = 9.324, 95% CI = 4.664, 13.983); 16 of these species (53%) were shared
253 (Figure 2, Tables 1 and S-1). Novel genera were isolated for both community types in every year
254 of surveying (Table S-6). Species accumulation curves did not approach horizontal asymptotes
255 for either soil type, and the serpentine curve had a steeper slope than the nonserpentine curve
256 (Figure S-1). These curves suggest that neither fungal community was fully described, and that
257 there were more unsampled fungal species associated with the plants growing in serpentine soil
258 than those in nonserpentine.

Both fungal communities were dominated by a few abundant species (isolated >10 times), but the numerically dominant species differed between nonserpentine and serpentine soils: *Alternaria* sp. 2 and *Ramularia* sp., respectively (Figure S-2, Table S-1). The nonserpentine community included five abundant species, representing 62% of the isolates, while the serpentine community included two abundant species, representing 51% of serpentine isolates (Figure S-2, Table S-1). The serpentine community had a higher proportion of rare species (<3 isolates) than the nonserpentine community, 63% versus 36%, respectively. One *Drechslera* species was abundant in the nonserpentine community and rare in the serpentine (Table S-1).

Soil type significantly affected pathogen community composition, based on pairwise PERMANOVA analysis of two-transect fungal communities and NMDS visualization of community similarity ($F = 4.585$, $R^2 = 0.07567$, $p = 0.001$; Figure 3). Serpentine fungal communities were generally more similar to one another than they were to nonserpentine fungal communities, and vice versa (Figure 3, Table 1).

Serpentine fungal community composition was more consistent across years than nonserpentine fungal community composition (Table 1). While nonserpentine fungal community similarity was unpredictable between years, with some very dissimilar communities (Morisita-Horn overlap = 0.295) and some very similar ones (Morisita-Horn overlap = 0.882), serpentine fungal communities were always relatively similar between years, with Morisita-Horn overlap ranging from 0.707 to 0.786. Morisita-Horn overlap for nonserpentine and serpentine fungal communities in each year was also variable, ranging from 0.535 to 0.725.

Leaf tissue chemistry and disease burden

As expected from soil chemistry differences, leaf chemistry in plants grown on serpentine versus nonserpentine soil differed in mean C, N, Ca, Mg, Ni, and P content, and in Ca:Mg ratio.

Serpentine plants had significantly lower mean Ca, C, N, and P content and Ca:Mg ratio than nonserpentine plants and significantly higher Ni and Mg (Table S-7, Figure 4). Cr content and C:N ratio were similar for both soil types. Nitrogen content in asymptomatic foliar tissue from plants surveyed in the field was positively related to the percentage of diseased tissue observed for nonserpentine, but not serpentine plants, based on Pearson's product-moment correlation (for N: nonserpentine cor. = 0.328, p-value = 0.012, serpentine cor. = -0.19, p-value = 0.14; for C: nonserpentine cor. = 0.08, p-value = 0.53, serpentine cor. = -0.04, p-value = 0.76) (Figure 5).

Discussion

Here we show that a diverse suite of pathogens caused ubiquitous, low-grade damage on host individuals across growing seasons and environmental conditions, and that plant populations and communities appeared to shape the diversity and composition of fungal pathogen communities in this system. The consistent presence of low-grade foliar disease in *S. pulchra* and evidence from recent work suggesting that these symptoms inflict only minimal plant fitness costs (Spear and Mordecai 2018, Uricchio et al. 2018) imply a coevolutionary relationship between *S. pulchra* and its foliar pathogens that has led to plant-fungal interactions nearer the commensal than the parasitic end of the symbiotic spectrum. This finding contrasts with prevalent hypotheses that disease plays a major role in structuring plant communities by regulating host population dynamics through mechanisms including enemy escape effects, density- or distance-dependent Janzen-Connell effects, and epidemic-driven community shifts (Janzen 1970, Connell J.H. 1971, Day and Monk 1974, Keane and Crawley 2002, Mitchell and Power 2003, Petermann et al. 2008, Mordecai 2011). While evidence from tropical climates supports these hypotheses (Mangan et al. 2010, Bagchi et al. 2014, Spear et al. 2015), this study adds to a small body of literature from Mediterranean and semi-arid grasslands that indicates little to no role for such mechanisms (Mordecai 2013, Spear and Mordecai 2018, Uricchio et al.

307 2018). However, we did not address pathogens with potential for more direct impacts on plant
308 survival and fecundity, such as flower, seed, and seedling pathogens (Gilbert 2002).

309 As predicted, the chemical composition of *S. pulchra* reflected the chemistry of the soil in
310 which it grew (except for Cr levels) (Oze et al. 2008) (Figure 4, Table S-7). Contrary to our
311 hypothesis, differences in soil chemistry that extended to plant chemistry did not affect disease
312 burden (Figures 1 and 4, Table S-6). Elevated heavy metal content in serpentine-grown plants
313 was far below "hyperaccumulation" levels that have been shown to inhibit pathogenic infection
314 in plants (e.g., 1.92×10^{-2} mol Cr/kg required for hyperaccumulation versus 3.30×10^{-4} mol
315 Cr/kg observed in serpentine *S. pulchra*) (Jaffre et al. 1976, Brooks et al. 1977, Boyd et al. 1994,
316 Martens and Boyd 1994, Table S-7), but the consistently low disease burden observed in both
317 soil types suggests that *S. pulchra* is able to effectively resist or suppress damage by foliar
318 pathogens such that heightened defenses via opportunistic hyperaccumulation would provide
319 little to no fitness benefit.

320 This study provides some of the first evidence that local growing conditions influence
321 structure of the pathogen communities of a single host species even over relatively small spatial
322 scales of a few hundred meters (Figure 1a, Figure 3). The close proximity of the sampled
323 nonserpentine and serpentine plants suggests that environmental filtering, rather than dispersal
324 limitation, plays a key role in fungal community assembly. We hypothesize that either tissue
325 chemistry, plant community composition, or both influence the composition of fungal
326 communities and their variation across environments.

327 Differing heavy metal content in the leaves of nonserpentine and serpentine *S. pulchra*
328 may influence fungal community assembly through a variety of mechanisms. Soil Ca content is
329 known to influence the outcomes of plant-pathogen interactions through its roles in cellular and

330 biochemical pathways in both the plant immune system (Lamb et al. 1989, Levine et al. 1996,
331 Blumwald et al. 1998, Romeis et al. 2001) and fungal pathogen virulence, growth, and
332 reproduction (Magalhães et al. 1991, Warwar and Dickman 1996, Lee and Lee 1998, Sebghati et
333 al. 2000, Shaw and Hoch 2000, Uhm et al. 2003, Brandhorst et al. 2005). Fungal pathogen
334 interspecific variation in reliance on Ca for successful infection and/or fungal response to Ca-
335 dependent plant immune defenses could therefore explain differences in nonserpentine and
336 serpentine fungal community composition. Previous studies showed that *Hesperolinon* sp.
337 infection frequency and severity by the fungal rust *Melampsora lini* in California grasslands
338 depended on plant tissue Ca levels linked to serpentine soil chemistry (Springer et al. 2006,
339 Springer 2009). Divergent tolerances of heavy metals other than Ca among fungi may act as
340 additional filters on the species present in nonserpentine and serpentine communities (Iram et al.
341 2009).

342 Differences in nonserpentine and serpentine fungal communities may also result from
343 differences in the plant community. Serpentine *S. pulchra* pathogen communities were more
344 diverse than nonserpentine communities at the species, genus, family, order, and class levels
345 (Tables S-1 through S-6). Results from previous studies that demonstrate a positive relationship
346 between host diversity and pathogen diversity indicate that higher plant species diversity in
347 serpentine grasslands might contribute to higher fungal diversity if native forbs and bunchgrasses
348 that specialize on serpentine soils share foliar fungal pathogens with *S. pulchra* (Hechinger and
349 Lafferty 2005, Kamiya et al. 2014, Spear 2017). Research on five common invasive and native
350 grasses (including *S. pulchra*) at JRBP shows that multi-host foliar fungal pathogens dominate
351 this system, supporting this hypothesis (Spear and Mordecai 2018).

352 Alternatively, serpentine and nonserpentine fungal communities could differ due to
353 genetic divergence between the *S. pulchra* populations found in these soils. The gene-for-gene

354 model of the evolution of virulence hypothesizes that a given pathogen can infect a given host
355 only if the pathogen does not possess an avirulence gene recognized by a matching resistance
356 gene in the host (Flor 1956, Parker and Gilbert 2004). To date, there have been few studies of
357 genetic differences between serpentine and nonserpentine populations of the same plant species.
358 However, genetic and chemical analyses of serpentine and nonserpentine populations of the
359 native forb *Lasthenia californica* at JRBP show that these populations represent two
360 phylogenetically distinct species and that each species both accumulates ions in significantly
361 different concentrations and has a unique flavonoid profile (Desrochers and Bohm 1993,
362 Rajakaruna and Bohm 1999, Chan et al. 2002, Rajakaruna et al. 2003). Since many flavonoids
363 have antifungal properties, serpentine and nonserpentine *S. pulchra* could also contain different
364 genotypes with different potential for resistance to any given fungus due to divergent resistance
365 gene and/or flavonoid profiles (Parker and Gilbert 2004, Treutter 2006).

366 Species turnover between years contributed substantially to fungal diversity (Table 1,
367 Table S-6, Figure 3), and species accumulation curves suggest that many more fungal species
368 remain to be discovered in this system (Figure S-1). Between-year variation in these
369 communities may be due to highly variable annual precipitation (2015 rainfall = 491mm; 2016 =
370 604mm, 2017 = 985mm) (Weather Underground). Timing and amount of precipitation might
371 alter infection dynamics by washing away fungal spores, transmitting spores to new hosts, and/or
372 influencing plant community composition (Hobbs and Mooney 1991, Madden 1997).The year-
373 to-year dissimilarity of fungal pathogen communities hosted by *S. pulchra* in both serpentine and
374 nonserpentine soils highlights the necessity of long-term data collection to accurately
375 characterize plant-disease interaction networks. This temporal variability also points to the
376 potential importance of the storage effect, in which temporally variable environmental conditions
377 act as ecological niches that facilitate species diversity, in maintaining diverse fungal

378 communities in California grasslands (Warner and Chesson 1985).

379 The variation in fungal community composition we observed on a single host species
380 with soil chemistry and over time suggests possible mechanisms preventing foliar disease from
381 regulating plant host populations in California grasslands. Transitions between distinct soil types
382 over spatial scales within the range of fungal spore dispersal may prevent disease outbreaks by
383 hampering the spread of pathogens that are more or less virulent depending on leaf tissue
384 chemistry. Variable climatic conditions favoring successful infection by different fungal species
385 each growing season (Table S-6), combined with plant senescence during the dry season each
386 year, suggest temporal barriers to pathogens evolving high virulence on both nonserpentine and
387 serpentine plants. The processes that limit disease burden and impact in *S. pulchra* suggest that
388 climate and growth form may explain differences with previous studies that reported larger
389 pathogen impacts. For example, seasonal senescence and local environmental barriers to
390 transmission in semi-arid and Mediterranean systems may limit the severity of individual
391 pathogen outbreaks, in contrast to year-round suitable climates and potentially more homogenous
392 biotic and abiotic environments in tropical systems. Life histories that promote the accumulation
393 of large disease burdens, along with relatively high and constant humidity, may be important
394 determinants of the potential for fungal pathogens to structure plant communities.

395 Clarifying the specific mechanisms that drive differences in serpentine and nonserpentine
396 fungal communities will require inoculation experiments to test how plant susceptibility to
397 infection by different fungal species changes with plant tissue chemistry and plant genetics.
398 Additionally, characterization of comprehensive plant-pathogen networks including all plant
399 species co-occurring with *S. pulchra* in serpentine and nonserpentine grasslands will improve our
400 understanding of the role of the surrounding plant community in structuring fungal pathogen
401 communities.

402 *Conclusions*

403 Fungi associated with foliar disease in the native bunchgrass *S. pulchra* are relatively
404 benign and are unlikely to maintain the diversity of grassland plants, but contribute substantially
405 to the overall biodiversity of California grasslands. The high taxonomic diversity and rarity of
406 serpentine fungi demonstrates that this community is potentially at risk of species loss via host-
407 parasite coextinction as annual grasses continue to expand their ranges at the cost of native
408 plants, and human activities including development and increased C and N deposition contribute
409 to the loss of serpentine grasslands that already make up <2% of the California's surface area
410 (Huenneke et al. 1990, Vallano et al. 2012). This work demonstrates that, in addition to >200
411 endemic plant species, California's serpentine grasslands support unique communities of
412 symbiotic fungi, providing additional motivation for active conservation of native grassland
413 communities based on the far-reaching loss of biodiversity across trophic levels associated with
414 their disappearance (Sprent 1992, Dobson et al. 2008, Dunn et al. 2009, Lafferty 2012).

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Figure legends

Figure 1. Pathogen damage was consistently low across three years and both grassland types. Panel (a) maps the locations of surveyed nonserpentine sites (blue) and serpentine sites (green) along the ridgetop at JRBP. Panel (b) shows mean percentage (bars) and standard error (error bars) of diseased leaf area for surveyed serpentine and nonserpentine *S. pulchra,* by year.

Figure 2. Pathogens isolated from *Stipa pulchra* growing in nonserpentine and serpentine grasslands. Bipartite network showing species interactions between *S. pulchra* plants (left) growing in serpentine grasslands (green) and nonserpentine grasslands (blue) and fungal species cultured from symptomatic leaf tissue (white bars on the right). The length of each white bar

correlates with the number of isolates in the operational species unit (based on 97% sequence similarity). The thickness of lines connecting left and right bars represents the number of times a particular fungal species was isolated from *S. pulchra* in a particular grassland type. The nonserpentine fungi came from 15 genera, 8 families, 4 orders, and 3 classes; the serpentine fungi came from 20 genera, 14 families, 7 orders, and 5 classes (Tables S-1 to S-6).

Figure 3. Distinct fungal communities isolated from *Stipa pulchra* growing in nearby serpentine and nonserpentine sites across years. Non-metric multidimensional scaling visualization of serpentine and nonserpentine fungal community similarity. Each point corresponds to the combined fungal community of two transects at the same site in the same year. Serpentine communities are shown in green and nonserpentine communities are shown in blue. Circles, triangles, and squares represent fungal communities sampled in 2015, 2016, and 2017, respectively. Ellipses enclose 95% confidence intervals for ordination of serpentine and nonserpentine communities. Community dissimilarity increases with the distance between points. PERMANOVA analysis showed a significant effect of soil type on fungal community composition ($F = 3.623$, $R^2 = 0.0618$, $p = 0.001$).

Figure 4. Plants grown in nonserpentine soil had higher C, N, P, Ca, and Ca:Mg and lower Mg and Ni than those grown in serpentine soil. Box plots comparing elemental content of dried foliar tissue from nonserpentine- and serpentine-grown *S. pulchra*. In each plot, the thick black bar represents the median, the height of the box represents the interquartile range (IQR), the whiskers represent the maximum and minimum points within 1.5*IQR of the 25th and 75th percentiles, and the circles represent points outside this range. An asterisk above a panel indicates a statistically significant difference between serpentine- and nonserpentine-grown plants ($p < 0.05$) indicated by a Wilcoxon ranked-sum test. Panel (a) shows percent C of dried foliar tissue ($W = 2713$, p-value = 1.65×10^{-7}); panel (b), percent N ($W = 2346$, p-value =

0.0011); panel (c), C:N ratio (W = 1398, p-value = 0.066); panel (d), moles P per kilogram dried leaf tissue (W = 390, p-value = 2.83×10^{-9}); panel (e), moles Ca per kilogram dried leaf tissue (W = 400, p-value = 1.45×10^{-11}); panel (f), Ca:Mg ratio (W = 400, p-value = 1.451×10^{-11}); panel (g), moles Cr per kilogram dried leaf tissue (W = 192.5, p-value = 0.8498); panel (h), moles Mg per kilogram dried leaf tissue (W = 1, p-value = 2.90×10^{-11}); and panel (i), moles Ni per kilogram dried leaf tissue (W = 0, p-value = 6.79×10^{-8}).

Figure 5. C, N, and C:N predict the mean foliar pathogen damage with opposite trends in serpentine versus nonserpentine grasslands. Foliar percent C (Panels (a) and (b)), percent N (Panels (c) and (d)) and C:N ratio (Panels (e) and (f)) versus mean percentage of diseased leaf area for serpentine and nonserpentine *S. pulchra* plants, with linear regression (lines) and 95% confidence intervals (grey bands). An asterisk following a p-value indicates a statistically significant result ($p<0.05$).

Figures

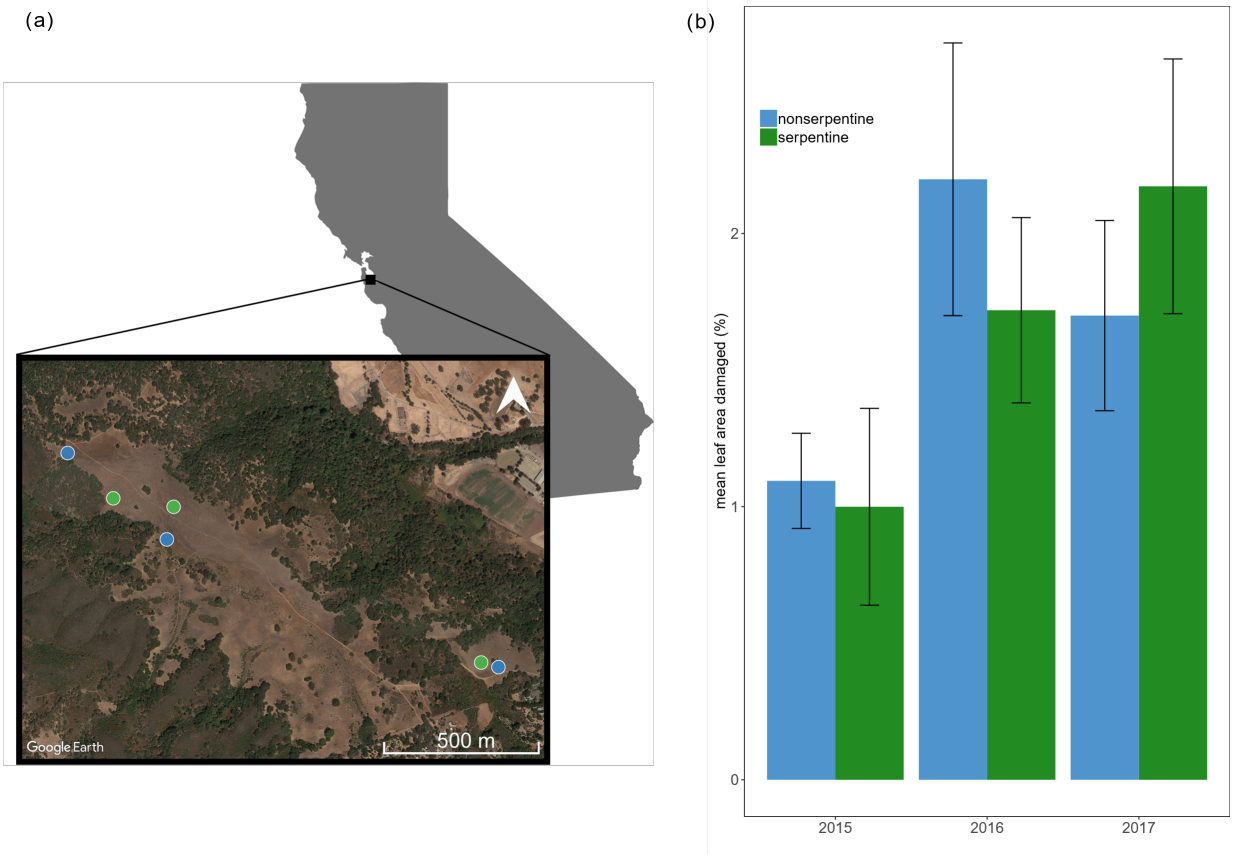
(a)
Google Earth
500 m
(b)
nonserpentine
serpentine
mean leaf area damaged (%)
0
1
2
2015
2016
2017

Figure 1.

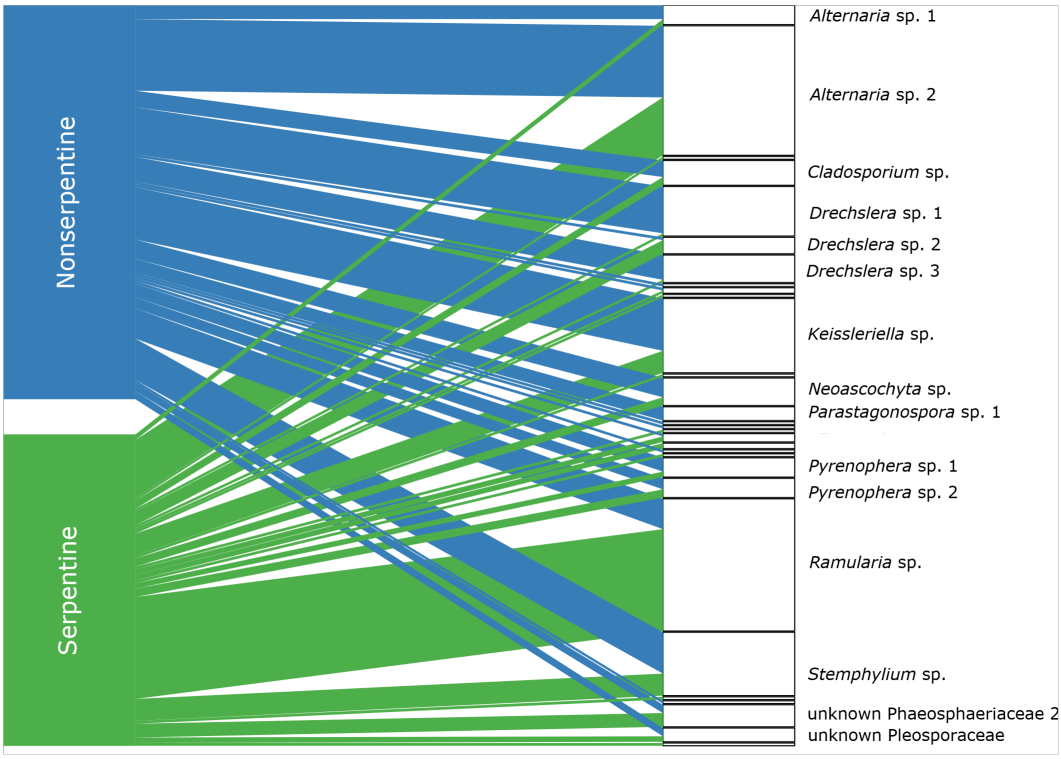
Nonserpentine
Serpentine
Alternaria sp. 1
Alternaria sp. 2
Cladosporium sp.
Drechslera sp. 1
Drechslera sp. 2
Drechslera sp. 3
Keissleriella sp.
Neoascochyta sp.
Parastagonospora sp. 1
Pyrenophera sp. 1
Pyrenophera sp. 2
Ramularia sp.
Stemphylium sp.
unknown Phaeosphaeriaceae 2
unknown Pleosporaceae

Figure 2.

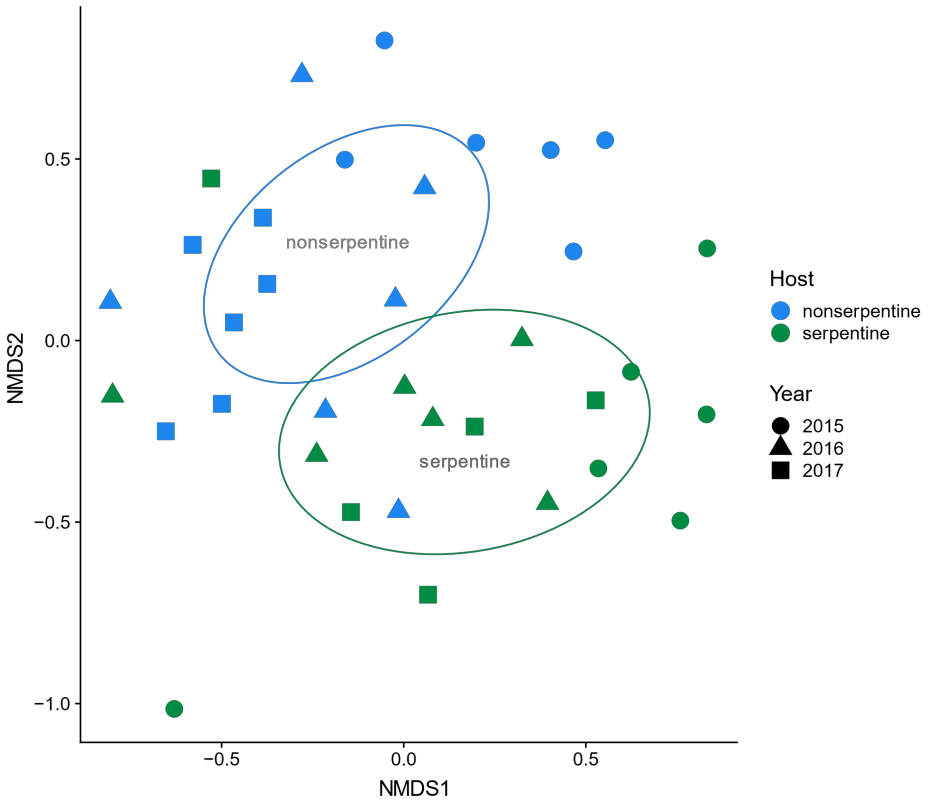
0.5
0.0
−0.5
−1.0
NMDS2
−0.5
0.0
0.5
NMDS1
nonserpentine
serpentine
Host
nonserpentine
serpentine
Year
2015
2016
2017

Figure 3.

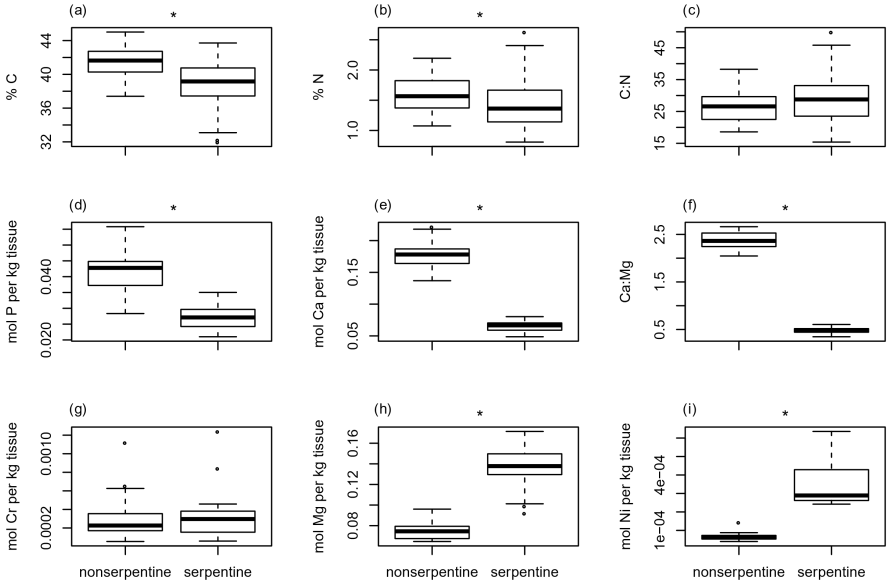
(a)
% C
32
36
40
44
*
(b)
% N
1.0
2.0
*
(c)
C:N
15
25
35
45
(d)
mol P per kg tissue
0.020
0.040
*
(e)
mol Ca per kg tissue
0.05
0.15
*
(f)
Ca:Mg
0.5
1.5
2.5
*
(g)
mol Cr per kg tissue
0.0002
0.0010
(h)
mol Mg per kg tissue
0.08
0.12
0.16
*
(i)
mol Ni per kg tissue
1e−04
4e−04
*
nonserpentine
serpentine

Figure 4.

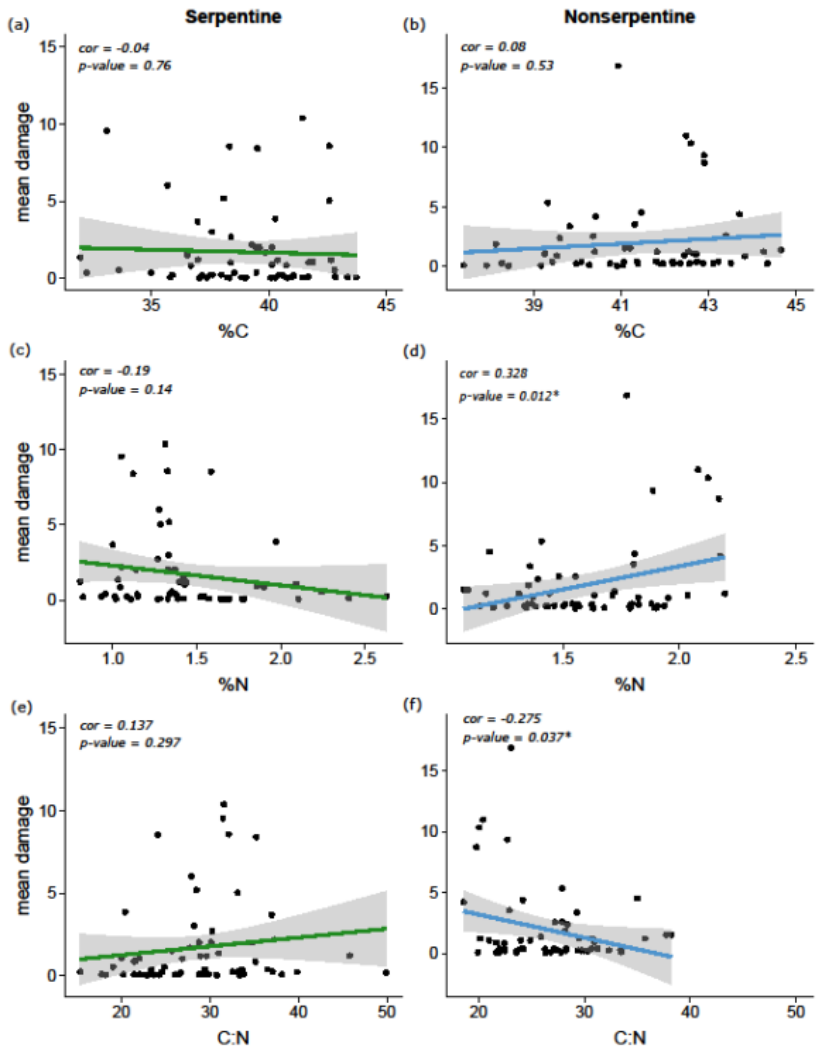
(a)
Serpentine
cor = -0.04
p-value = 0.76
mean damage
%C
(b)
Nonserpentine
cor = 0.08
p-value = 0.53
%C
(c)
cor = -0.19
p-value = 0.14
mean damage
%N
(d)
cor = 0.328
p-value = 0.012*
%N
(e)
cor = 0.137
p-value = 0.297
mean damage
C:N
(f)
cor = -0.275
p-value = 0.037*
C:N

Figure 5.

716 **Tables**

717 **Table 1. Fungal community diversity was higher and communities were distinct in *S.***
718 ***pulchra* plants growing in serpentine versus nonserpentine grasslands, across all years.**
719 Fungal community similarity by soil type and year. The leftmost column lists the fungal
720 communities considered, with the number of species in each community in parentheses. The
721 second column lists Fisher's alpha for each community. The third column shows the number of
722 shared species and, in parentheses, the percentage of total observed species that were shared for
723 each pair of communities. The rightmost column lists the estimated Morisita-Horn community
724 overlap value, based on absolute species abundances, for each pair of communities. The
725 Morisita-Horn index ranges from 0 to 1, with 1 indicating complete overlap.

Fungal community pair	Fisher's alpha	Shared species	Morisita-Horn Similarity Index
All years serpentine (24)	9.324	16	0.675
All years nonserpentine (22)	7.261	(53.0%)	
2015 serpentine (11)	7.955	5	0.535
2015 nonserpentine (12)	5.205	(27.8%)	
2016 serpentine (16)	7.897	11	0.766
2016 nonserpentine (13)	5.861	(61.1%)	
2017 serpentine (12)	6.784	8	0.725
2017 nonserpentine (12)	5.136	(50.0%)	
2015 serpentine (11)	7.955	7	0.707
2016 serpentine (16)	7.897	(35.0%)	
2015 serpentine (11)	7.955	5	0.721
2017 serpentine (12)	6.784	(27.8%)	
2016 serpentine (16)	7.897	7	0.786
2017 serpentine (12)	6.784	(33.3%)	
2015 nonserpentine (12)	5.205	6	0.668
2016 nonserpentine (13)	5.861	(31.5%)	
2015 nonserpentine (12)	5.205	5	0.295
2017 nonserpentine (12)	5.136	(26.3%)	
2016 nonserpentine (13)	5.861	7	0.882
2017 nonserpentine (12)	5.136	(38.9%)	

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