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

3 **Authors:**

4 Johannah E. Farner[1], Erin R. Spear[1,2], Erin A. Mordecai[1,3]

5 [1]Biology Department, Stanford University, Stanford, California 94305 USA

6 [2]Biology Department, Regis University, Denver, Colorado 80221 USA

7 [3]E-mail: emordeca@stanford.edu

8 **Abstract**

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

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

31 **Introduction**

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

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48 single host – pathogen pairs under differing abiotic conditions (e.g., Abbate and Antonovics
49 2014) or the effect of varying pathogen community composition on disease burden (e.g., Telfer
50 et al. 2010), relationships between abiotic variables and pathogen community structures remain
51 largely unexplored in natural systems (but see Spear 2017).

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

61 Differences in plant community composition and soil chemistry suggest that *S. pulchra*
62 populations growing in distinct soil types are likely to host distinct fungal pathogen communities
63 and/or experience differing disease burdens, particularly if the phenotype of *S. pulchra* is
64 influenced by the soil in which it grows. *S. pulchra* naturally occurs in both nonserpentine soils
65 and in relatively rare serpentine soils that host grassland plant communities. These two soil types
66 differ in their chemistry and host distinct plant communities, resulting in a suite of environmental
67 differences that may influence pathogen infection, reproduction, and persistence (Huenneke et al.
68 1990, Harrison and Viers 2007). In contrast to the majority of California grasslands, which are
69 heavily invaded by exotic species, serpentine grasslands have a diverse array of native species
70 adapted to chemically and physically harsh soils: low in essential plant nutrients (N, P, K, Ca),
71 high in heavy metals that are potentially toxic to plants (Co, Ni, Cr, Fe), and rocky and shallow,

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72 with poor moisture retention (Proctor and Woodell 1975, Oze et al. 2004, 2008, McNaughton
73 1968, Harrison and Viers 2007, Eviner 2016).

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

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

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

102 **Methods**

103 All surveys and sample collection took place at Jasper Ridge Biological Preserve (JRBP),
104 a 485-hectare site in San Mateo County, CA managed by Stanford University (37.4° N, 122.2°
105 W). JRBP has a Mediterranean climate with cool winters (mean 9.2°C), warm summers (mean
106 20.1°C), and annually variable precipitation that predominantly occurs in the winter and spring
107 (averaging 622.5 mm per year) (Ackerly et al. 2002). The growing season occurs during the
108 rainy season and plants senesce in the early summer. This study was conducted during the
109 growing seasons from mid-April to mid-May in 2015, 2016, and 2017. There was highly variable
110 annual precipitation across the three years (491 mm, 604 mm, and 985mm in 2015, 2016, and
111 2017, respectively; Weather Underground).

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

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120 *Avena fatua*, *Bromus diandrus,* and *Erodium botrys* (McNaughton 1968, Hobbs and Mooney
121 1985).

122 *Quantification of disease*

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

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

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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

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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

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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

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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

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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.

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

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

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

280 *Leaf tissue chemistry and disease burden*

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

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

290 **Discussion**

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

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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

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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

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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

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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.

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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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421

422 **References**

423 Abbate, J. L., and J. Antonovics. 2014. Elevational disease distribution in a natural plant–
424 pathogen system: insights from changes across host populations and climate. Oikos
425 123:1126–1136.

426 Abou-Shanab, R. A., J. S. Angle, T. A. Delorme, R. L. Chaney, P. Van Berkum, H. Moawad, K.
427 Ghanem, and H. A. Ghozlan. 2003. Rhizobacterial effects on nickel extraction from soil and
428 uptake by Alyssum murale. New Phytologist 158:219–224.

429 Ackerly, D. D., C. A. Knight, S. B. Weiss, K. Barton, and K. P. Starmer. 2002. Leaf size,
430 specific leaf area and microhabitat distribution of chaparral woody plants: Contrasting
431 patterns in species level and community level analyses. Oecologia 130:449–457.

432 Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance.
433 Austral Ecology 26:32–46.

434 Bagchi, R., R. E. Gallery, S. Gripenberg, S. J. Gurr, L. Narayan, C. E. Addis, R. P. Freckleton,
435 and O. T. Lewis. 2014. Pathogens and insect herbivores drive rainforest plant diversity and
436 composition. Nature 506:85–88.

437 Blumwald, E., G. S. Aharon, and B. C-H. Lam. 1998. Early signal transduction pathways in
438 plant-pathogen interactions. Trends in Plant Science 3:342–346.

439 Borer, E. T., A. Laine, and E. W. Seabloom. 2016. A multiscale approach to plant disease using
440 the metacommunity concept. Annual Review of Phytopathology 54:397–418.

441 Boyd, R. S., J. J. Shaw, and S. N. Martens. 1994. Nickel hyperaccumulation defends
442 Streptanthus polygaloides (Brassicaceae) against pathogens. American Journal of Botany

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443 81:294–300.

444 Brady, K. U., A. R. Kruckeberg, and H. D. Bradshaw Jr. 2005. Evolutionary ecology of plant
445 adaptation to serpentine soils. Annual Review of Ecology, Evolution, and Systematics
446 36:243–266.

447 Brandhorst, T. T., G. M. Gauthier, R. A. Stein, and B. S. Klein. 2005. Calcium binding by the
448 essential virulence factor BAD-1 of Blastomyces dermatitidis. Journal of Biological
449 Chemistry 280:42156–42163.

450 Brooks, R. R., J. Lee, R. D. Reeves, and T. Jaffre. 1977. Detection of nickeliferous rocks by
451 analysis of herbarium specimens of indicator plants. Journal of Geochemical Exploration
452 7:49–57.

453 Cantarelli, M. A., R. G. Pellerano, L. A. Del Vitto, E. J. Marchevsky, and J. M. Camiña. 2010.
454 Characterisation of two South American food and medicinal plants by chemometric
455 methods based on their multielemental composition. Phytochemical Analysis 21:550–555.

456 Chan, R., B. G. Baldwin, and R. Ornduff. 2002. Cryptic goldfields: A molecular phylogenetic
457 reinvestigation of Lasthenia californica sensu lato and close relatives (Compositae:
458 Heliantheae sensu lato). American Journal of Botany 89:1103–1112.

459 Chao, A., K. H. Ma, T. C. Hsieh, and C.-H. Chiu. 2016. SpadeR: Species prediction and
460 diversity estimation with R.

461 Ciompi, S., E. Gentili, L. Guidi, and G. F. Soldatini. 1996. The effect of nitrogen deficiency on
462 leaf gas exchange and chlorophyll fluorescence parameters in sunflower. Plant Science
463 118:177–184.

464 Coley-Smith, J. R., and R. C. Cooke. 1971. Survival and Germination of Fungal Sclerotia.

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465 Annual Review of Phytopathology:65–92.

466 Connell J.H. 1971. On the role of natural enemies in preventing competitive exclusion in some
467 marine animals and in rain forest trees. Dynamics of numbers in populations. Proceedings
468 of the Advanced Study Institute. Centre for Agricultural Publication and Documentation,
469 Wageningen, The Netherlands:298–312.

470 Cunningham, S. A., B. Summerhayes, and M. Westoby. 1999. Evolutionary divergences in leaf
471 structure and chemistry, comparing rainfall and soil nutrient gradients. Ecology 69:569–
472 588.

473 Day, F. P., and C. D. Monk. 1974. Vegetation patterns on a southern Appalachian watershed.
474 Ecology 55:1064–1074.

475 Desrochers, A. M., and B. A. Bohm. 1993. Flavonoid variation in Lasthenia california
476 (Asteraceae). Biochemical Systematics and Ecology 21:449–453.

477 Dobson, A., K. D. Lafferty, A. M. Kuris, R. F. Hechinger, and W. Jetz. 2008. Homage to
478 Linnaeus: how many parasites? how many hosts? Proceedings of the National Academy of
479 Sciences 105:11482–11489.

480 Dormann, C. F., B. Gruber, and J. Fruend. 2008. Introducing the bipartite package: analysing
481 ecological networks. R news 8:8–11.

482 Dunn, R. R., N. C. Harris, R. K. Colwell, L. P. Koh, and N. S. Sodhi. 2009. The sixth mass
483 coextinction: are most endangered species parasites and mutualists? Proceedings of the
484 Royal Society B: Biological Sciences 276:3037–3045.

485 Eviner, V. T. 2016. Grasslands. Pages 449–477 Ecosystems of California. University of
486 California Press.

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487 Fisher, R. A., A. S. Corbet, and C. B. Williams. 1943. The relation between the number of
488 species and the number of individuals in a random sample of an animal population. Source
489 Journal of Animal Ecology 12:42–58.

490 Flor, H. H. 1956. The complementary genic systems in flax and flax rust. Advances in Genetics
491 8:29–54.

492 Gilbert, G. S. 2002. Evolutionary ecology of plant diseases in natural ecosystems. Annual
493 Review of Phytopathology 40:13–43.

494 Hansen, R. A., and D. C. Coleman. 1998. Litter complexity and composition are determinants of
495 the diversity and species composition of oribatid mites (Acari: Oribatida) in litterbags.
496 Applied Soil Ecology 9:17–23.

497 Harrison, S. P., and J. H. Viers. 2007. Serpentine Grasslands. Pages 145–155 California
498 Grasslands: Ecology and Management. University of California Press.

499 Hechinger, R. F., and K. D. Lafferty. 2005. Host diversity begets parasite diversity: bird final
500 hosts and trematodes in snail intermediate hosts. Proceedings of the Royal Society B:
501 Biological Sciences 272:1059–1066.

502 Heinze, J., J. Bergmann, M. C. Rillig, and J. Joshi. 2015. Negative biotic soil-effects enhance
503 biodiversity by restricting potentially dominant plant species in grasslands. Perspectives in
504 Plant Ecology, Evolution and Systematics 17:227–235.

505 Hervé, M. "RVAideMemoire: testing and plotting procedures for biostatistics. . R. package
506 version 0. 9–6. (2017). 2017. RVAideMemoire: testing and plotting procedures for
507 biostatistics.

508 Hobbs, R. J., and H. A. Mooney. 1985. Community and population dynamics of serpentine

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509 annual grassland in relation to gopher disturbances. Oecologia 67:342–351.

510 Hobbs, R. J., and H. A. Mooney. 1991. Effects of rainfall variability and gopher disturbance on
511 serpentine annual grassland dynamics. Ecology 72:59–68.

512 Huenneke, L. F., S. P. Hamburg, R. Koide, H. A. Mooney, P. M. Vitousek, L. F. Huenneke, S. P.
513 Hamburg, R. Koide, H. A. Mooney, and P. M. Vitousek. 1990. Effects of soil resources on
514 plant invasion and community structure in Californian serpentine grassland. Ecology
515 71:478–491.

516 Iram, S., I. Ahmad, B. Javed, S. Yaqoob, K. Akhtar, M. R. Kazmi, and Badar-uz-Zaman. 2009.
517 Fungal tolerance to heavy metals. Pakistan Journal of Botany 41:2583–2594.

518 Jaffre, T., R. R. Brooks, J. Lee, and R. D. Reeves. 1976. Sebertia acuminata: a hyperaccumulator
519 of nickel from New Caledonia. Science 193:579–580.

520 Janzen, D. H. 1970. Herbivores and the number of tree species in tropical forests. The American
521 Naturalist 104:501–528.

522 Jost, L., A. Chao, and R. L. Chazdon. 2011. Compositional similarity and beta diversity. Page
523 345 *in* A. E. Magurran and B. J. McGill, editors. Biological diversity: frontiers in
524 measurement and assessment. Oxford University Press, Oxford, England.

525 Kamiya, T., K. O'Dwyer, S. Nakagawa, and R. Poulin. 2014. Host diversity drives parasite
526 diversity: meta-analytical insights into patterns and causal mechanisms. Ecography 37:689–
527 697.

528 Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis.
529 Trends in Ecology and Evolution 17:164–170.

530 Kindt, R., and R. Coe. 2005. BiodiversityR. Tree diversity analysis. A manual and software for

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531 common statistical methods for ecological and biodiversity studies. World Agroforestry
532 Centre (ICRAF), Nairobi.

533 Lafferty, K. D. 2012. Biodiversity loss decreases parasite diversity: Theory and patterns.
534 Philosophical Transactions of the Royal Society B: Biological Sciences 367:2814–2827.

535 Lamb, C. J., M. A. Lawton, M. Dron, and R. A. Dixon. 1989. Signals and transduction
536 mechanisms for activation of plant defenses against microbial attack. Cell 56:215–224.

537 Lee, S. C., and Y. H. Lee. 1998. Calcium/calmodulin-dependent signaling for appressorium
538 formation in the plant pathogenic fungus Magnaporthe grisea. Molecules & Cells 8:698–
539 704.

540 Levine, A., R. I. Pennell, M. E. Alvarez, R. Palmer, and C. Lamb. 1996. Calcium-mediated
541 apoptosis in a plant hypersensitive disease resistance response. Current Biology 6:427–437.

542 Madden, L. V. 1997. No effects of rain on splash dispersal of fungal pathogens. Canadian
543 Journal of Plant Pathology 19:225–230.

544 Magalhães, B. P., R. Wayne, R. A. Humber, E. J. Shields, and D. W. Roberts. 1991. Calcium-
545 regulated appressorium formation of the entomopathogenic fungus Zoophthora radicans.
546 Protoplasma 160:77–88.

547 Magurran, A. E. 1988. Ecological diversity and its measurement. Chapman & Hall.

548 Mangan, S. A., S. A. Schnitzer, E. A. Herre, K. M. L. MacK, M. C. Valencia, E. I. Sanchez, and
549 J. D. Bever. 2010. Negative plant-soil feedback predicts tree-species relative abundance in a
550 tropical forest. Nature 466:752–755.

551 Martens, S. N., and R. S. Boyd. 1994. The ecological significance of nickel hyperaccumulation:
552 a plant chemical defense. Oecologia 98:379–384.

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553 McElrone, A. J., C. D. Reid, K. A. Hoye, E. Hart, and R. B. Jackson. 2005. Elevated CO2
554 reduces disease incidence and severity of a red maple fungal pathogen via changes in host
555 physiology and leaf chemistry. Global Change Biology 11:1828–1836.

556 McNaughton, S. J. 1968. Structure and function in California grasslands. Ecology 49:962–972.

557 Mitchell, C. E., and A. O. Power. 2003. Release of invasive plants from fungal and viral
558 pathogens. Nature 421:625–627.

559 Mordecai, E. A. 2011. Pathogen impacts on plant communities: unifying theory, concepts, and
560 empirical work. Ecological Monographs 81:429–441.

561 Mordecai, E. A. 2013. Consequences of pathogen spillover for cheatgrass-invaded grasslands:
562 coexistence, competitive exclusion, or priority effects. The American Naturalist 181:737–
563 47.

564 O'Brien, H. E., J. L. Parrent, J. A. Jackson, J. Moncalvo, and R. Vilgalys. 2005. Fungal
565 community analysis by large-scale sequencing of environmental samples. Applied and
566 Environmental Microbiology 71:5544–5550.

567 Oksanen, J., R. Kindt, P. Legendre, and B. O'Hara. 2016. Vegan: community ecology package.

568 Osbourne, A. E. 1996. Preformed antimicrobial compounds and plant defense against fungal
569 attack. The Plant Cell 8:1821–1831.

570 Oze, C., S. Fendorf, D. K. Bird, and R. G. Coleman. 2004. Chromium geochemistry in
571 serpentinized ultramafic rocks and serpentine soils from the Franciscan complex of
572 California. American Journal of Science 304:67–101.

573 Oze, C., C. Skinner, A. W. Schroth, and R. G. Coleman. 2008. Growing up green on serpentine
574 soils: biogeochemistry of serpentine vegetation in the Central Coast Range of California.

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575 Applied Geochemistry 23:3391–3403.

576 Parker, I. M., and G. S. Gilbert. 2004. The evolutionary ecology of novel plant-pathogen
577 interactions. Annual Review of Ecology, Evolution, and Systematics 35:675–700.

578 Parker, I. M., M. Saunders, M. Bontrager, A. P. Weitz, R. Hendricks, R. Magarey, K. Suiter, and
579 G. S. Gilbert. 2015. Phylogenetic structure and host abundance drive disease pressure in
580 communities. Nature 520:542–544.

581 Petermann, J. S., A. J. F. Fergus, L. A. Turnbull, and B. Schmid. 2008. Janzen-Connell effects
582 are widespread and strong enough to maintain diversity in grasslands. Ecology 89:2399–
583 2406.

584 Proctor, J., and S. R. J. Woodell. 1975. The ecology of serpentine soils. Advances in Ecological
585 Research 9:255–366.

586 Rajakaruna, N., B. G. Baldwin, R. Chan, A. M. Desrochers, B. A. Bohm, and J. Whitton. 2003.
587 Edaphic races and phylogenetic taxa in the Lasthenia californica complex (Asteraceae:
588 Heliantheae): an hypothesis of parallel evolution. Molecular Ecology 12:1675–1679.

589 Rajakaruna, N., and B. A. Bohm. 1999. The edaphic factor and patterns of variation in Lasthenia
590 californica (Asteraceae). American Journal of Botany 86:1576–1596.

591 Rascio, N., and F. Navari-Izzo. 2011. Heavy metal hyperaccumulating plants: how and why do
592 they do it? and what makes them so interesting? Plant Science 180:169–181.

593 Romeis, T., A. A. Ludwig, R. Martin, and J. D. G. Jones. 2001. Calcium-dependent protein
594 kinases play an essential role in a plant defence response. EMBO Journal 20:5556–5567.

595 Rossi, J.-P. 2011. rich: an R package to analyse species richness. Diversity 3:112–120.

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596 Seabloom, E. W., E. T. Borer, K. Gross, A. E. Kendig, C. Lacroix, C. E. Mitchell, E. A.
597 Mordecai, and A. G. Power. 2015. The community ecology of pathogens: coinfection,
598 coexistence and community composition. Ecology Letters 18:401–415.

599 Sebghati, T. S., J. T. Engle, and W. E. Goldman. 2000. Intracellular parasitism by Histoplasma
600 capsulatum: fungal virulence and calcium dependence. Science 290:1368–1372.

601 Shaw, B. D., and H. C. Hoch. 2000. Ca2+regulation of Phyllosticta ampelicida pycnidiospore
602 germination and appressorium formation. Fungal Genetics and Biology 31:43–53.

603 Smith, V. 2007. Host resource supplies influence the dynamics and outcome of infectious
604 disease. Integrative and Comparative Biology 47:310–316.

605 Spear, E. R. 2017. Phylogenetic relationships and spatial distributions of putative fungal
606 pathogens of seedlings across a rainfall gradient in Panama. Fungal Ecology 26:65–73.

607 Spear, E. R., P. D. Coley, and T. A. Kursar. 2015. Do pathogens limit the distributions of tropical
608 trees across a rainfall gradient? Journal of Ecology 103:165–174.

609 Spear, E. R., and E. A. Mordecai. (n.d.). Foliar pathogens do not stabilize coexistence of
610 competing species in a California grassland. Ecology.

611 Spear, E. R., and E. A. Mordecai. 2018. Foliar pathogens do not stabilize coexistence of
612 competing species in a California grassland. Ecology 0:1–10.

613 Sprent, J. F. A. 1992. Parasites lost? International Journal for Parasitology 22:139–151.

614 Springer, Y. P. 2009. Do extreme environments provide a refuge from pathogens? A
615 phylogenetic test using serpentine flax. American Journal of Botany 96:2010–2021.

616 Springer, Y. P., B. A. Hardcastle, and G. S. Gilbert. 2006. Soil calcium and plant disease in

bioRxiv preprint doi: https://doi.org/10.1101/545632; this version posted February 10, 2019. 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-ND 4.0 International license.

617 serpentine ecosystems: A test of the pathogen refuge hypothesis. Oecologia 151:10–21.

618 Tadros, T. M. 1957. Evidence of the presence of an edapho-biotic factor in the problem of
619 serpentine tolerance. Ecology 38:14–23.

620 Telfer, S., X. Lambin, R. Birtles, P. Beldomenico, S. Burthe, S. Paterson, and M. Begon. 2010.
621 Species interactions in a parasite community drive infection risk in a wildlife population.
622 Science 330:243–247.

623 Treutter, D. 2006. Significance of flavonoids in plant resistance: a review. Environmental
624 Chemistry Letters 4:147–157.

625 Uhm, K.-H., I.-P. Ahn, S. Kim, and Y.-H. Lee. 2003. Calcium/calmodulin-dependent signaling
626 for prepenetration development in Colletotrichum gloeosporioides. Phytopathology 93:82–
627 87.

628 Uricchio, L. H., S. C. Daws, E. R. Spear, and E. A. Mordecai. 2018. Exotic species dominate due
629 to niche overlap in a complex grassland community. in review:1–35.

630 Vallano, D. M., P. C. Selmants, and E. S. Zavaleta. 2012. Simulated nitrogen deposition
631 enhances the performance of an exotic grass relative to native serpentine grassland
632 competitors. Plant Ecology 213:1015–1026.

633 Vavrek, M. J. 2011. Fossil: palaeoecological and palaeogeographical analysis tools.
634 Palaeontologia Electronica 14.

635 Warner, R. R., and P. L. Chesson. 1985. Coexistence mediated by recruitment fluctuations: a
636 field guide to the storage effect. The American Naturalist 125:769–787.

637 Warwar, V., and M. B. Dickman. 1996. Effects of calcium and calmodulin on spore germination
638 and appressorium development in Colletotrichum trifolii. Applied and Environmental

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639 Microbiology 62:74–79.

640 Weather Underground. (n.d.). Jasper Ridge Biological Preserve KCAWOODS19.
641 https://www.wunderground.com/personal-weather-station/dashboard?ID=KCAWOODS19.

642 Wickham, H. 2016. Ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.

643 Wolda, H. 1981. Similarity indices, sample size and diversity. Oecologia 50:296–302.

644 Wolf, D. C., T. H. Dao, H. D. Scott, and T. L. Lavy. 1989. Influence of sterilization methods on
645 selected soil microbiological, physical, and chemical properties. Journal of Environment
646 Quality 18:39.

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651 **Figure legends**

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

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

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

665 **Figure 3. Distinct fungal communities isolated from *Stipa pulchra* growing in nearby**
666 **serpentine and nonserpentine sites across years.** Non-metric multidimensional scaling
667 visualization of serpentine and nonserpentine fungal community similarity. Each point
668 corresponds to the combined fungal community of two transects at the same site in the same
669 year. Serpentine communities are shown in green and nonserpentine communities are shown in
670 blue. Circles, triangles, and squares represent fungal communities sampled in 2015, 2016, and
671 2017, respectively. Ellipses enclose 95% confidence intervals for ordination of serpentine and
672 nonserpentine communities. Community dissimilarity increases with the distance between
673 points. PERMANOVA analysis showed a significant effect of soil type on fungal community
674 composition ($F = 3.623$, $R^2 = 0.0618$, $p = 0.001$).

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

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684 0.0011); panel (c), C:N ratio (W = 1398, p-value = 0.066); panel (d), moles P per kilogram dried
685 leaf tissue (W = 390, p-value = 2.83×10^{-9}); panel (e), moles Ca per kilogram dried leaf tissue
686 (W = 400, p-value = 1.45×10^{-11}); panel (f), Ca:Mg ratio (W = 400, p-value = 1.451×10^{-11});
687 panel (g), moles Cr per kilogram dried leaf tissue (W = 192.5, p-value = 0.8498); panel (h),
688 moles Mg per kilogram dried leaf tissue (W = 1, p-value = 2.90×10^{-11}); and panel (i), moles Ni
689 per kilogram dried leaf tissue (W = 0, p-value = 6.79×10^{-8}).

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

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697 **Figures**

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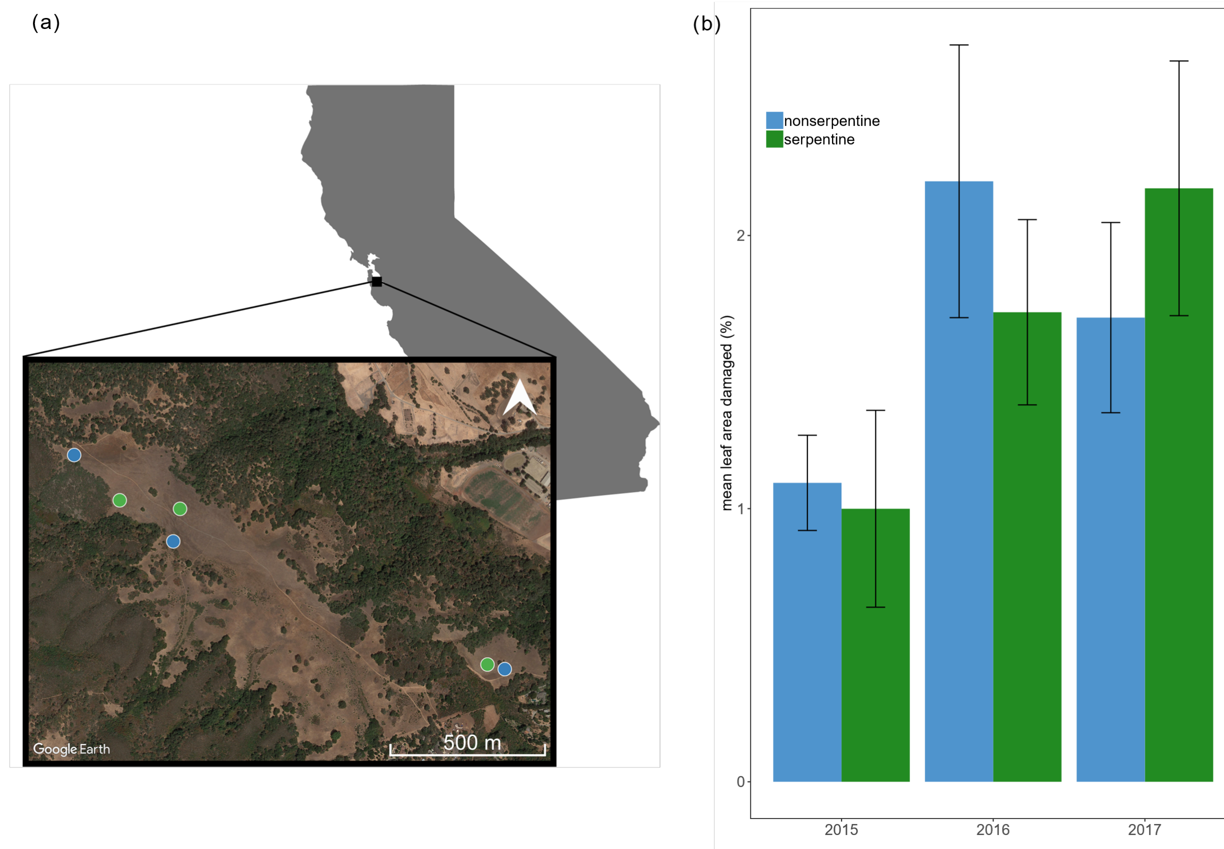

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699 **Figure 1.**

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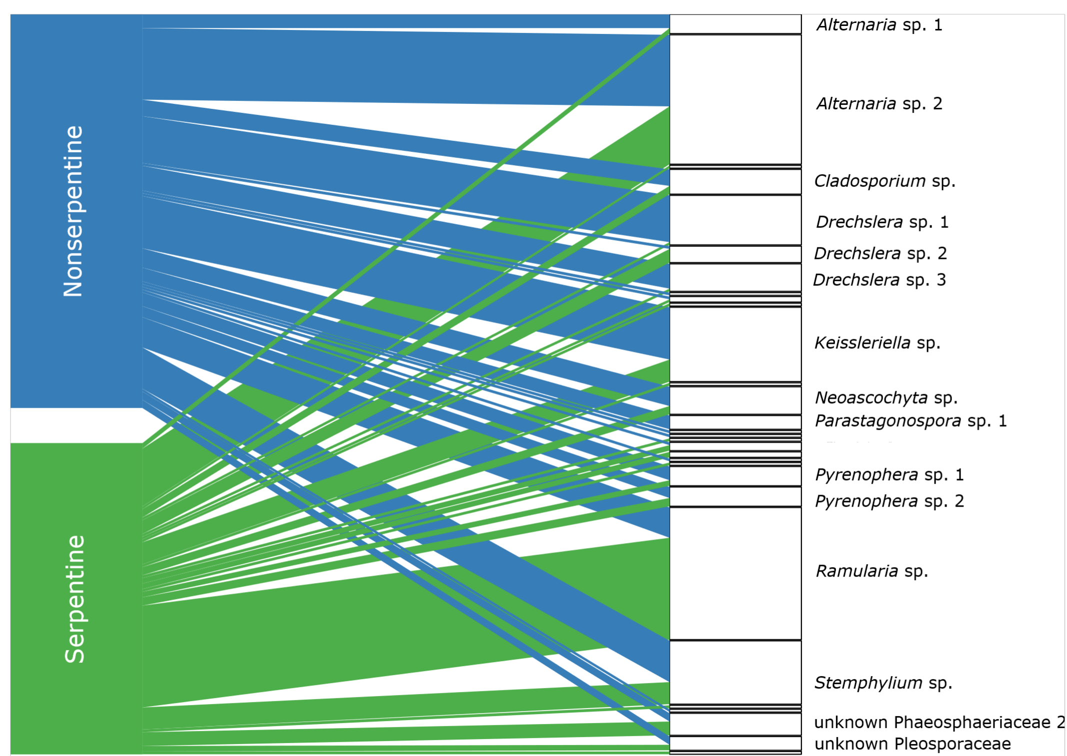

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701 **Figure 2.**

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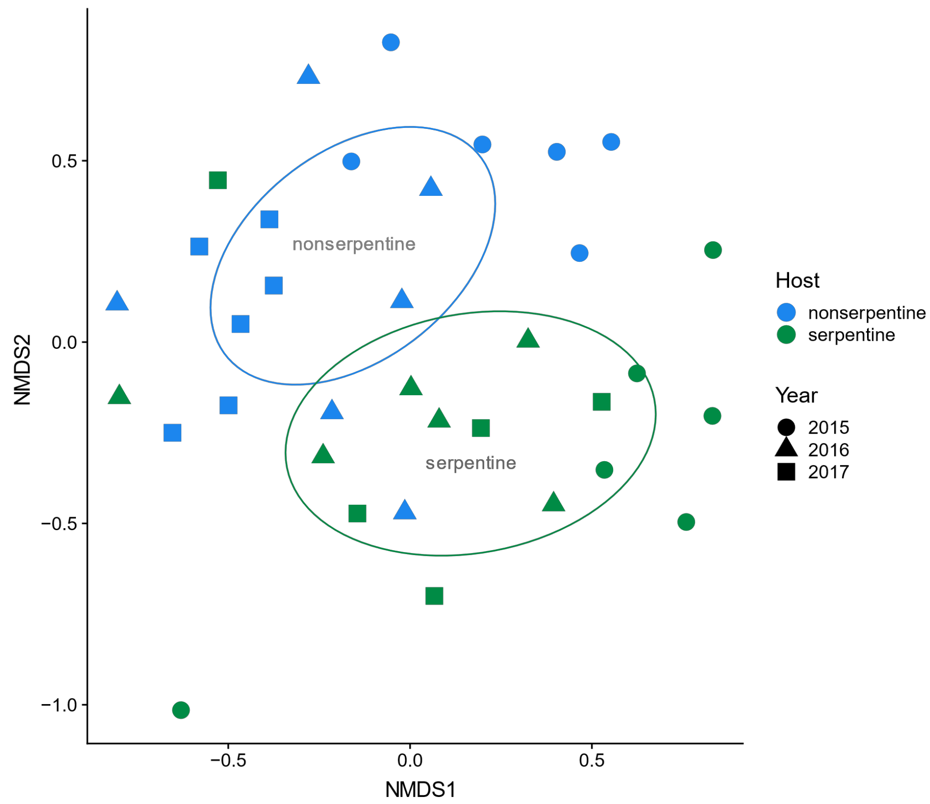

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703 **Figure 3.**

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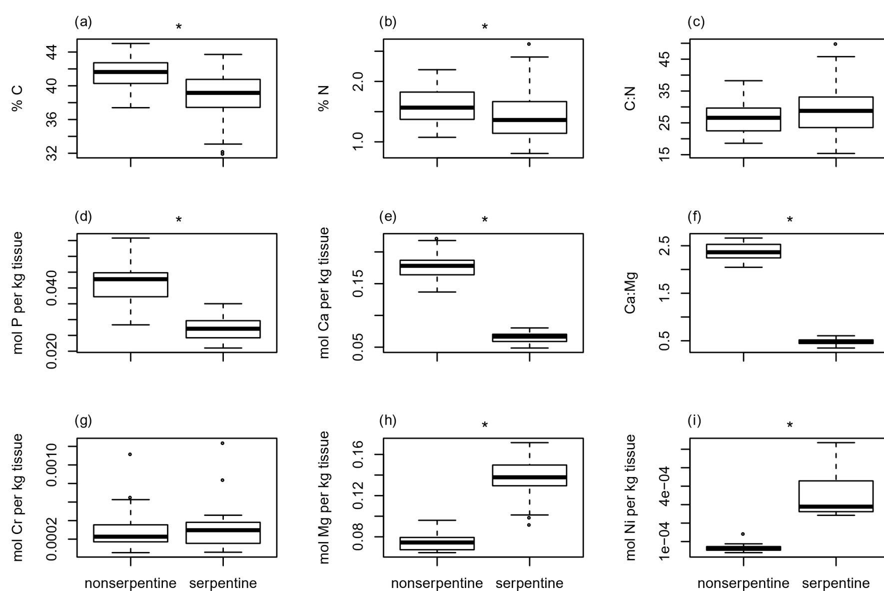

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707 **Figure 4.**

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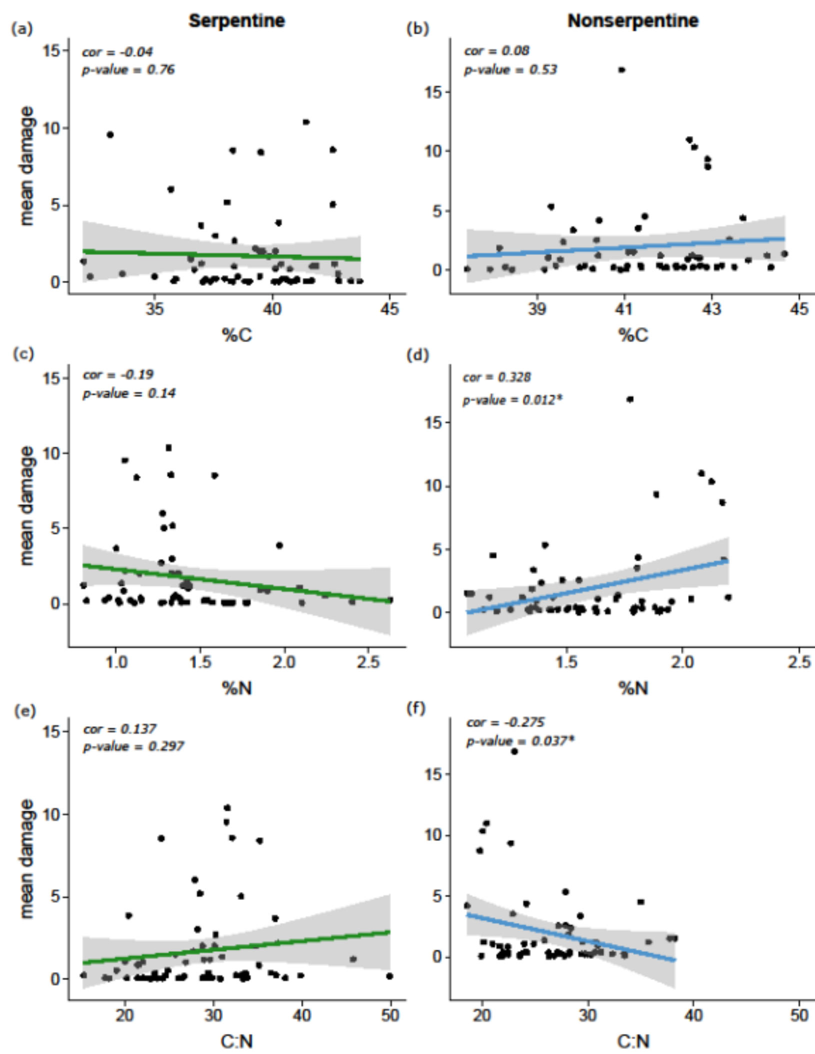

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709 **Figure 5.**

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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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