Habitat type and interannual variation shape unique fungal pathogen communities on a California native bunchgrass

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Abstract

The role of infectious disease in regulating host populations is increasingly recognized, but how environmental conditions affect pathogen communities and infection levels remains poorly understood. Over three years, we compared foliar disease burden, fungal pathogen community composition, and plant tissue chemistry in the perennial bunchgrass *Stipa pulchra* occurring in adjacent serpentine and nonserpentine grassland habitats with distinct soil types and plant communities. We found that serpentine and nonserpentine *S. pulchra* experienced consistent, low disease pressure associated with distinct fungal pathogen communities with high interannual species turnover, and that plant chemistry differed with habitat type. The results indicate that this species experiences minimal foliar disease associated with diverse fungal pathogen communities that are structured across landscapes by spatially and temporally variable conditions. Highly variable conditions associated with fungal species turnover may create a refuge from disease outbreaks for *S. pulchra*, contributing to the low disease burden observed on this and other Mediterranean grassland species.

Key words: California grasslands, leaf spot pathogen, plant fungal pathogens, serpentine soil, Stipa pulchra

53 **Introduction**

54 How environmental heterogeneity affects species interactions, and ultimately population
55 growth rates and structures, remains a fundamental ecological question. Infectious disease, which
56 arises from interactions between hosts, parasites, and the environment, is particularly likely to
57 vary across populations in differing environments. Biotic factors, such as the phylogenetic
58 structure of the host community, and abiotic factors, such as elevation, have been associated with
59 the presence of specific diseases and the severity of their impacts on their hosts (Abbate and
60 Antonovics 2014; Parker *et al.* 2015). Additionally, mounting evidence suggests that specific
61 characteristics of pathogen community structures, including the identities and abundances of the
62 diseases present in a host population, mediate the outcomes of host – pathogen interactions
63 (Seabloom *et al.* 2015; Borer *et al.* 2016).

64 Together, these observations highlight the importance of understanding interactions
65 between environmental variation, pathogen community composition, and pathogen pressure on
66 host populations to assess the potential for disease to regulate host population dynamics.
67 For plant hosts, such assessments are important both in conservation and agricultural contexts
68 because identifying populations at high risk for severe disease outbreaks and applying
69 preventative measures may prevent local species extinctions and improve crop yields. While
70 studies have frequently explored the interactions of single host – pathogen pairs under differing
71 abiotic conditions (e.g., Abbate and Antonovics 2014), the effect of varying pathogen
72 community composition on disease burden and relationships between habitat characteristics and
73 pathogen community structures remain largely unexplored in natural systems (but see Rottstock
74 *et al.* 2014 and Spear 2017).

75 Fungal pathogens are particularly important to study in this context because they cause
76 the majority of known plant diseases, have an increasing rate of emergence, and pose a
77 substantial threat to plant health and population persistence in natural and agricultural settings
78 (Agrios 2005; Fisher *et al.* 2012). Dramatic diebacks of North American chestnut trees and oaks
79 caused by fungal pathogens (Anagnostakis 1987; Rizzo and Garbelotto 2003) and economically
80 devastating crop losses caused by fungal rusts shared among wild and domesticated plants
81 (Loehrer and Schaffrath 2011; Nazareno *et al*. 2018) demonstrate this threat. These examples
82 indicate that understanding the communities of fungal pathogens that infect wild plants is
83 important for developing effective protection strategies for both natural biodiversity and human

84 food supplies. However, we lack basic information about the identities, diversity, and effects of
85 the fungal pathogens that infect most plant species (Burdon and Laine 2019). Therefore, there is
86 a pressing need for systematic surveys of these pathogen communities and their variation across
87 natural habitats.

88 Evidence that abiotic and biotic factors drive both natural fungal endophyte community
89 assembly in healthy leaves and fungal foliar disease dynamics in agricultural systems suggests
90 that different habitats are likely to affect fungal foliar pathogen community composition and
91 disease burden. For example, the repeated finding that foliar endophyte community structures
92 change with temperature, humidity, and rainfall (Cordier *et al.* 2012, Zimmerman and Vitousek
93 2012, Bálint *et al.*, 2015, Millberg *et al.* 2015, Giauque and Hawkes 2016, Barge *et al.*, 2019)
94 suggests that climate is likely to also shape fungal pathogen communities. Evidence from the
95 plant pathology literature supports this expectation: for foliar fungal pathogens of wheat, barley,
96 and lettuce, among others, infection dynamics are closely linked to temperature and moisture
97 availability (Shaw 1986, Moss *et al.* 1987, Eversmyer *et al.* 1988, Huber and Gillespie 1992,
98 Magarey et al. 2004, Bernard *et al.* 2013, Clarkson *et al.* 2014, Velásquez *et al.* 2018).

99 Additionally, local habitat characteristics, such as soil and plant physical and chemical
100 properties, plant community composition, and plant genetics, can determine the availability and
101 quality of susceptible and competent hosts for different fungal pathogen species (Snoeijers *et al.*
102 2000, Mitchell *et al.* 2002, Solomon *et al.* 2003, Bolton *et al.* 2008, Rottstock et al. 2014,
103 Velásquez *et al.* 2018). Differences in soil nutrient content, plant nutrient content, and plant
104 genetic backgrounds are known to influence the identities and abundances of fungal endophytes
105 that inhabit the leaves of individual host species (Eschen *et al.* 2010, Busby *et al.* 2014). The
106 likely relevance of these same factors to fungal pathogens is supported by extensive work in
107 agricultural systems wherein nitrogen fertilization and plant genetic diversity are both associated
108 with disease prevalence and severity (Zhu *et al.* 2000, Solomon *et al.* 2003, Verosoglou *et al.*
109 2013).

110 Notably, general understanding of the factors that shape foliar endophyte communities is
111 hampered by the short lengths of most observation periods, which frequently result in annual or
112 seasonal snapshots of fungal community composition that cover only one or two years (but see
113 Giauque and Hawkes, 2016). Extended observation periods can provide the context necessary to

114 determine whether such snapshots are broadly representative versus exceptional, a key step for
115 assessing the overall strength of different predictors for infection patterns.

116 Here, we examine the effects of habitat type on the burden and species composition of
117 natural plant pathogens infecting a single host species over a span of three years. Specifically, we
118 take advantage of the natural occurrence of the California native perennial bunchgrass *Stipa*
119 *pulchra* in two unique, adjacent grassland types to understand the extent to which habitat type
120 and time influence the landscape of disease encountered by a single plant species, including
121 foliar fungal pathogen community composition and disease severity. Stark differences in soil
122 properties and plant community structures between serpentine and nonserpentine greenstone
123 (hereafter, nonserpentine) grassland habitats result in a suite of environmental differences that
124 may influence fungal pathogen infection, reproduction, and persistence (Harrison and Viers
125 2007; Burdon and Laine 2019). Additionally, high interannual variation in climate conditions
126 and plant community composition in California grasslands may lead to temporal variation in
127 infection dynamics through impacts on fungal growth and survival (Hobbs and Mooney 1991,
128 Fernandez-Going *et al.* 2012). Therefore, in this study we ask: (1) how does disease burden
129 differ on plants growing in different habitat types and over time? (2) how does the species
130 composition of fungal pathogen communities differ on plants growing in different habitat types
131 and over time? and (3) are there differences in plant tissue chemistry between habitat types that
132 correspond to differences in disease burden?

133 Because of differences between serpentine and nonserpentine plant community
134 composition, plant physiology, and plant genetics, we hypothesize that both disease burden and
135 pathogen species composition differ on *S. pulchra* individuals growing in these two habitats.
136 Moreover, because abiotic and biotic conditions vary substantially between growing seasons in
137 California grasslands, we hypothesize that disease burden and pathogen species composition
138 differ interannually on *S. pulchra.* Finally, we hypothesize that habitat type influences *S. pulchra*
139 foliar tissue chemistry in ways that may affect disease burden, with lower C and N content in
140 serpentine plants growing in nutrient-poor soils. If so, these foliar chemistry differences may
141 translate into either (a) higher disease burden in serpentine plants relative to nonserpentine
142 plants, facilitated by negative impacts of limited plant resource availability on plant health (as in
143 Springer *et al.,* 2006), or (b) lower disease burden in serpentine plants due to lower plant nutrient
144 content limiting resources available for fungal growth.

By contributing novel information about previously uncharacterized plant fungal pathogen communities in California grasslands, the results of this study will provide new insight into the diversity and natural history of the fungi that infect the leaves of wild grasses in temperate climates. Further, 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 environmental heterogeneity can affect the species composition and severity of foliar pathogen infection.

Materials and Methods

Study system

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 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 985 mm in 2015, 2016, and 2017, respectively; Weather Underground).

S. pulchra occurs at substantial densities in both serpentine and nonserpentine grasslands at JRBP (McNaughton 1968). The extent of genetic differentiation between serpentine and nonserpentine *S. pulchra* is unknown. While there are no physical barriers at this site to prevent gene flow between individuals in different habitat types, there is high genetic divergence between serpentine and nonserpentine populations of the forb *Lasthenia californica* occurring in the same grasslands at JRBP (Chan *et al.* 2002). The individuals studied here may thus represent either a single continuous population or multiple populations that are locally adapted to different habitat types.

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; Huenneke *et al.* 1990; Field *et al.* 1996). Plant species commonly found in serpentine grasslands at JRBP include native *Stipa*

175 *pulchra*, *Elymus glaucus*, *Elymus multisetus, Eschscholzia californica*, and the invasive grass
176 *Bromus hordeaceus* (McNaughton 1968; Hobbs and Mooney 1985). The nonnative annual
177 species that dominate the nonserpentine grasslands but are absent from serpentine grasslands
178 include *Avena barbata, Avena fatua*, *Bromus diandrus,* and *Erodium botrys* (McNaughton 1968;
179 Hobbs and Mooney 1985; Field *et al.* 1996). The serpentine and nonserpentine grasslands at
180 JRBP share a land use history of livestock grazing that ceased in 1960, when conservation of
181 these areas became a management priority (Bocek and Reese 1992).

182 All survey sites were located along the ridgetop at JRBP, where serpentine and
183 nonserpentine (greenstone) soils are present in discrete adjacent bands (Fig. 1a) (Oze *et al.*
184 2004). Chemical analyses of the soils on this ridgetop show consistent, significant differences in
185 serpentine and nonserpentine soil chemistry, with the serpentine soils enriched in heavy metals
186 and trace elements (Fe, Cr, Ni, Mn, and Co), and impoverished in essential plant nutrients (Streit
187 *et al.* 1993, Oze *et al.* 2004, 2008). The relative flatness of the ridgetop ensured that all survey
188 sites were at roughly the same elevation and had similar slopes, aspects, and water availability
189 (Oze *et al.* 2008).

190 *Quantification of disease*

191 To assess plant disease burden, we quantified the percentage of living leaf area exhibiting
192 symptoms of fungal disease in serpentine and nonserpentine populations of *S. pulchra*. We
193 selected three serpentine grassland sites and three nonserpentine grassland sites such that each
194 serpentine site was paired with a nearby (~170m away) nonserpentine site (Fig. 1a). Individual
195 sites within each habitat type were >300m apart and the maximum distance between any two
196 sites was 1600m (Fig. 1a). Each year, we randomly placed four 5-meter transects at each 40m x
197 40m site and assessed the infection status of the *S. pulchra* individual nearest each meter mark,
198 resulting in five individual plants per transect surveyed each year (N = 60 plants per habitat type
199 per year). The infection status of individual plants was not tracked across years, although some
200 plants could have been sampled repeatedly by chance. Following the protocol for estimation of
201 foliar infection severity on grasses described in Mitchell *et al.* 2002 and Spear and Mordecai
202 2018, a single surveyor visually estimated the percentage of living foliar tissue damaged by
203 pathogens for six arbitrarily-selected leaves per plant. We used printed field reference guides
204 showing known amounts of damage on leaves to standardize estimates. Damage was indicated

205 by spots and lesions which ranged in color from dark brown to pale yellow (Fig. 1b). Senesced
206 tissue and herbivore damage (commonly in the form of scrapes and holes in the leaf surface) was
207 excluded from the analyzed leaf area. For damage estimates of 5% or greater, values were
208 determined to the nearest 5%. Damage levels below 5% were assigned values of 1% or 0.1%.
209 We used analysis of variance (ANOVA), with individual plants nested within sites, to test
210 for effects of habitat type, year, habitat type x year, and site on log-transformed mean percent
211 disease damage on serpentine and nonserpentine *S. pulchra* (N = 36 serpentine and 36
212 nonserpentine transects). When ANOVA results were statistically significant, we used t-tests for
213 every possible pairwise combination, with Bonferroni adjustments of p-values to account for
214 multiple comparisons, to identify the interactions contributing to the significant result.

215 *Isolation and molecular identification of foliar fungal pathogens*

216 To identify the fungal foliar pathogens infecting serpentine and nonserpentine *S. pulchra*
217 over all years, we harvested one segment of symptomatic leaf tissue for culturing and
218 identification of the putatively causal fungal pathogen(s) from each individual included in the
219 damage survey with symptoms of disease. This tissue segment came from the first surveyed leaf
220 with disease symptoms on each plant. We excised, surface sterilized, and plated a $4mm^2$ square
221 of tissue from each sample at the leading edge of infection, to improve the chances of isolating
222 the causal fungal pathogen, on malt extract agar with 2% chloramphenicol to prevent bacterial
223 growth. While it is possible that the growing medium used may have led to selective isolation
224 and sequencing of fungi other than those causing symptoms, our use of small segments of leaf
225 tissue from the advancing margin of disease was intended to prevent this outcome under the
226 assumption that symptom-causing organisms should be dominant in visibly diseased tissue and
227 particularly at its expanding margin. We observed the plates for growth for eight weeks and
228 isolated morphologically distinct hyphae into axenic culture. Six segments of surface sterilized,
229 asymptomatic tissue were plated as a control and showed no growth. We extracted and
230 sequenced genomic DNA from the ITS region for each isolate. Fungal isolation and sequencing
231 methods with ITS1F and ITS4 primers followed Spear and Mordecai 2018.

232 We estimated taxonomic placement of fungal isolates by grouping sequences into
233 operational taxonomic units with 97% sequence similarity (OTUs), a proxy for species
234 delineation based on the range of intraspecific ITS divergence (O'Brien *et al.* 2005), and then

235 comparing assembled DNA sequences to the entries in the UNITE fungal database. To prepare
236 sequences for taxonomic placement, we first trimmed forward and reverse Sanger sequencing
237 reads to a maximum error rate of 5% and then automatically assembled bidirectional reads into
238 consensus sequences with a minimum of 20% overlap and 85% sequence similarity in Geneious
239 (Kearse *et al.* 2012), discarding consensus sequences less than 450 bp in length and/or less than
240 60% in read quality. We grouped the remaining internal transcribed spacer (ITS) sequences into
241 OTUs using USEARCH 10.0.240 (Edgar 2010) and estimated the taxonomic placement of each
242 OTU in mothur v.1.40.5 (http://mothur.org/; Schloss 2009) using the naïve Bayes classification
243 approach (classify.seqs with options: method='wang', cutoff=0, iters=200). We used a
244 bootstrapping confidence score of at least 80% to assign a species name and at least 60% to
245 assign taxonomy at higher ranks.

246 *Analyses of fungal community composition*

247 To characterize foliar fungal community composition in serpentine and nonserpentine *S.*
248 *pulchra* populations, we compared the diversity and assessed the similarity of communities of
249 fungi cultured from the diseased tissue of surveyed plants. To describe fungal community
250 diversity, we calculated observed taxa richness and Fisher's alpha, a widely used measure of
251 species richness that is robust to uneven sample sizes (Fisher *et al.* 1943; Magurran 2013);
252 generated taxa accumulation curves to understand sampling efficacy (Oksanen *et al.* 2016); and
253 counted the number of fungal isolates in each genus, family, order, and class from each grassland
254 type. We compared fungal community composition across grassland types and across years
255 visually, using non-metric multidimensional scaling (NMDS), and statistically, using
256 permutational multivariate analyses of variance (PERMANOVA) with pairwise Adonis
257 functions for non-normalized OTU read counts in the R package vegan (Anderson 2001; Arbizu
258 2016; Oksanen *et al.* 2016). To assess spatial turnover in fungal communities, we included a site
259 distance factor in the statistical analyses, with comparisons between sites <600m apart
260 categorized as "near" and comparisons between sites >600m apart categorized as "far" (Fig. 1a).
261 To account for low culturing success in some transects, we considered the combined isolates
262 from two nearby transects at each site in each year (N = 18 serpentine and 18 nonserpentine
263 communities, generated from 36 total transects per habitat type) to be distinct communities for
264 NMDS visualization.

265 For ANOVA and PERMANOVA analyses we considered the combined isolates from
266 each site in each year to be distinct communities (N = 9 serpentine and 9 nonerspentine
267 communities). When PERMANOVA results were statistically significant, we used pairwise
268 PERMANOVAs with Bonferroni adjustments to p-values to clarify which fungal communities
269 were significantly different from one another (Arbizu 2016). For the NMDS and PERMANOVA
270 analyses, we used the function vegdist with the abundance-based Chao method, accounting for
271 unsampled species, to create a matrix of pairwise community dissimilarities (Chao *et al.* 2016;
272 Oksanen *et al.* 2016). We also used the Morisita-Horn index, which is independent of sample
273 size and diversity, to make pairwise comparisons of similarity within and between the serpentine
274 and nonserpentine fungal communities from each year (N = 265 isolates, 200 bootstrap
275 replicates), and within the entire fungal community between years (Wolda 1981; Jost *et al.*
276 2011). Finally, we used ANOVA to test for effects of habitat type, year, site distance, year x
277 habitat type, and year x site distance interactions on fungal diversity (Fisher's alpha) and
278 richness at the site level.

279 To perform these fungal community analyses, we used the packages BiodiversityR (Kindt
280 and Coe 2005), bipartite (Dormann *et al.* 2008), fossil (Vavrek 2011), rich (Rossi 2011), SpadeR
281 (Chao *et al*. 2016), and vegan (Oksanen *et al.* 2016) with custom commands (Gardener 2014) in
282 R version 3.4.1.

283 *Chemical analyses of leaf tissue*

284 In 2016, we analyzed C and N content of the foliar tissue of the *S. pulchra* plants
285 surveyed in serpentine and nonserpentine grasslands. Because both leaf age and infection status
286 might influence leaf chemistry, we collected the youngest fully expanded and entirely
287 asymptomatic leaf of each individual surveyed in the field (N = 60 serpentine, 58 nonserpentine).
288 We dried the samples at room temperature for three months, then at 60°C for 48 hours, ground
289 them to a powder, and then dried them again at 60°C for 24 hours. We measured C and N
290 content of these samples with a Carlo Erba NA-1500 elemental analyzer using atropine
291 standards.

292 To assess the potential for habitat type to mediate plant-pathogen interactions through its
293 influence on plant tissue chemistry, we related habitat type to leaf tissue chemistry of *in situ S.*
294 *pulchra*, and chemistry of these plants to disease burden. We compared the mean percentage of

295 C and N per dried weight of leaf tissue for surveyed serpentine and nonserpentine plants with
296 Wilcoxon rank sum tests. We also compared mean C : N ratio, which is indicative of plant health
297 (Ciompi *et al.* 1996; Brady *et al.* 2005). We used Pearson's product-moment correlation to test
298 for significant monotonic relationships between the log-transformed mean amount of diseased
299 tissue observed on individual plants in the field and their foliar C and N content.

300 All statistical analyses were done in R version 3.4.1. In addition to the R packages
301 specified in the fungal community analyses methods, we used the packages cowplot (Wilke
302 2019), data.table (Dowle and Srinivasan 2019), RColorBrewer (Neuwirth 2014), reshape2
303 (Wickham 2007), tidyverse (Wickham 2017), and xtable (Dahl *et al*. 2019) to manipulate, plot,
304 and export data and results.

305 **Results**

306 *Pathogen damage*

307 Pathogen damage was similarly ubiquitous yet low-burden in serpentine and
308 nonserpentine grassland habitats. Every plant surveyed exhibited evidence of foliar fungus-
309 caused disease. The mean percentage of diseased leaf area observed across all years was 1.63 for
310 serpentine and 1.66 for nonserpentine *S. pulchra,* respectively (Fig. 1c), and the mean proportion
311 of surveyed leaves with pathogen damage was 0.83 for serpentine and 0.85 for nonserpentine
312 plants. Year significantly affected log-transformed mean percent damage (ANOVA: df = 2, SS =
313 9.41, F-value = 5.375, p-value = 7.04×10^{-3}) (Table 1). There were significant differences in
314 percent damage between was between 2015 and 2016 and between 2015 and 2017 (2015 mean
315 percent damage = 1.05, 2016 = 1.96, 2017 = 1.94, t-values = -2.66, -3.70, Bonferonni-adjusted p-
316 values = 2.5×10^{-3}, 8.1×10^{-4}).

317 *Fungal pathogen community*

318 We isolated 267 unique fungal isolates from 258 plated symptomatic tissue pieces with
319 fungal growth (144 nonserpentine and 114 serpentine tissue pieces from 36 nonserpentine and 34
320 serpentine transects, out of 360 total tissue pieces). Of the 267 unique fungal isolates, we
321 successfully sequenced 256 isolates. The sequenced isolates clustered into 30 operational
322 taxonomic units (OTUs) based on 97% sequence similarity, representing 23 genera, 15 families,
323 8 orders, and 5 fungal classes. We were unable to identify 18 OTUs to the fungal species level,

324 and four of these to the genus level due to a lack of close sequence matches in the UNITE
325 database and/or a lack of taxonomic information for close sequence matches (Table 2). The
326 taxonomic results indicate that many of the OTUs represent close relatives of known plant
327 pathogens including *Alternaria, Cladosporium, Drechslera, Phaeosphaeria, Pyrenophora,*
328 *Stemphylium, Ramularia,* and *Wojnowiciella* spp. (Table 2) (Kirk *et al.* 2008; Stukenbrock and
329 McDonald 2008; Havis *et al.* 2015; Marin-Felix *et al.* 2017, 2019). The most common OTU was
330 represented by 48 isolates and the least common OTUs were represented by one isolate each.
331 OTUs are hereafter referred to as "species" (O'Brien et al. 2005).

332 Fungal community diversity was similar between serpentine and nonserpentine habitats
333 and between years (Table 2). Although fungal richness was higher at every taxonomic level in
334 the serpentine relative to the nonserpentine, these differences were not statistically significant
335 (Tables 2, S1-S5). Twenty-four fungal species were isolated from serpentine *S. pulchra* (Fisher's
336 alpha = 9.324, 95% CI = 4.664, 13.983) and 22 from nonserpentine (Fisher's alpha = 7.261, 95%
337 CI = 3.597, 10.925); 16 of these species (53%) were shared (Fig. 2; Tables 1, 2). Habitat type,
338 year, and year x habitat type were not significantly related to fungal richness or Fisher's alpha at
339 the site level (ANOVA: Tables S6, S7). Novel genera were isolated for both community types in
340 every year of surveying, with only seven of 30 (23%) observed species and six of 23 genera
341 (26%) isolated in all three years (Table S5). Species accumulation curves did not approach
342 horizontal asymptotes for either habitat type, suggesting that neither fungal community was fully
343 described (Fig. S1).

344 Both fungal communities were dominated by a few abundant species (isolated >10
345 times), but the most abundant species differed between nonserpentine and serpentine habitats:
346 these were *Alternaria* cf. *infectoria* and *Ramularia* cf. *proteae*, respectively (Table 2; Fig. S2).
347 The nonserpentine community included five abundant species, representing 62% of the isolates,
348 while the serpentine community included two abundant species, representing 51% of serpentine
349 isolates (Table 2; Fig S2). The serpentine community had a higher proportion of rare species (<3
350 isolates) than the nonserpentine community, 63% versus 36%, respectively. One *Drechslera*
351 species was abundant in the nonserpentine community and rare in the serpentine (Table 2; Fig.
352 S2).

353 Habitat type and year significantly affected fungal community composition, based on

354 two-way PERMANOVA analysis of site-level fungal communities and NMDS visualization of
355 community similarity (habitat type: F-value = 5.112, R^2 = 0.163, p = 0.003; year: F-value =
356 5.655, R^2 = 0.360, p = 0.001; Fig. 3; Table 4). Serpentine fungal communities were generally
357 more similar to one another than they were to nonserpentine fungal communities, and vice versa
358 (Fig. 3; Tables 2, 3). Community composition differed significantly between 2015 and 2017, and
359 2016 and 2017, but not between 2015 and 2016 (Table S8). Differences in fungal community
360 composition between years did not correspond to differences in disease severity between years
361 (Figure 1c, Table S8).

362 Interpretation of the significance of variation in values of Morisita-Horn community
363 overlap that fall between zero (no overlap) and one (complete overlap) is a longstanding
364 challenge in community ecology (Horn 1966, Rodrigues and Vieira 2010). Qualitatively,
365 estimates of the Morisita-Horn similarity index for communities within habitat types suggest that
366 the amount of interannual species turnover is more consistent within serpentine than
367 nonserpentine communities (Fig. 3, Tables 2, 3). While serpentine community overlap between
368 years ranged from 0.707 to 0.786, nonserpentine community overlap ranged between 0.295 and
369 0.882 (Table 3). Within-year variation in species turnover between habitat types was
370 comparatively moderate, with Morisita-Horn overlap ranging from 0.535 to 0.725 (Table 3).

371 *Leaf tissue chemistry and disease burden*

372 As expected from differences in soil type between habitats, leaf chemistry in plants
373 occurring on serpentine versus nonserpentine soils differed in mean C and N content. Serpentine
374 plants had significantly lower mean C and N content than nonserpentine plants (Fig. 4; Table
375 S9). C:N ratio was similar for plants in both habitat types. Log-transformed mean percent
376 symptomatic tissue was not significantly associated with percent C, percent N, or C:N ratio in
377 asymptomatic foliar tissue for serpentine or nonserpentine plants, based on Pearson's product-
378 moment correlation tests (Fig. 4).

379 **Discussion**

380 Here we show that a diverse suite of foliar fungi was associated with ubiquitous, low-
381 burden damage on host individuals across growing seasons and habitat types. Habitat type
382 appeared to shape fungal community composition in this system, but not foliar disease burden. In
383 contrast, time appeared to shape both fungal community composition and disease burden.

384 However, interannual variation in pathogen community composition did not correspond to yearly
385 differences in disease burden (Figs. 1c, 3, Table S8). While C and N content measured in young,
386 healthy leaves differed between habitat types, variation in leaf chemistry did not explain
387 variation in infection severity. The results provide some of the first evidence that local growing
388 conditions structure the pathogen communities infecting a single host species even over
389 relatively small areas of a few hundred meters, where hosts and pathogens experience similar
390 climate conditions (Figs. 1a, 3; Table 3). Overall, the findings imply that environmental
391 heterogeneity within landscapes may both generate barriers to fungal disease spread in wild plant
392 populations and bolster fungal diversity.

393 *Fungal community composition*

394 The close proximity of the sampled nonserpentine and serpentine plants and the finding
395 that habitat type, but not distance between sites, predicted fungal community composition
396 suggest that habitat filtering, rather than dispersal limitation, plays a key role in fungal
397 community assembly (Table 4). Fungal communities varied between habitats in their
398 composition, and the stability of these communities over time varied between habitats (Figs. 1c,
399 2, 3, S2; Tables 1-4). The results imply that differences in environmental conditions—both biotic
400 and abiotic—beyond climate averages can lead to differences in plant-fungal interactions in
401 distinct habitat types. In this system, influential habitat characteristics might include differences
402 in pathogen sharing among hosts in native-dominated serpentine grasslands versus nonnative-
403 dominated nonserpentine grasslands, and in plant genotypes occurring in different habitats.
404 Differences in plant nutrient content between habitat types suggest that soil influence on plant
405 phenotype may also contribute to differences between fungal communities (Fig. 4). In addition to
406 C and N differences, potted *S. pulchra* plants grown from seed and serpentine soils collected in
407 these sites had significantly lower Ca and P content and higher Ni and Mg content than similar
408 plants grown in greenhouse soils, potentially suggesting additional phenotypic differences
409 between serpentine and nonserpentine plants that may affect pathogen community composition
410 (Farner *et al.*, unpublished data). Finally, habitat type might impact disease dynamics if differing
411 water availability between serpentine and nonserpentine soils alters leaf physiology in ways that
412 affect infection processes. We hypothesize that some or all of these factors influence the
413 composition of fungal communities and their variation across environments.

414 Differences in nonserpentine and serpentine fungal communities may result from
415 differences in the plant communities found on these soils. The presence of additional fungal
416 taxonomic diversity and the relatively constrained amount of species turnover between years in
417 the serpentine align with patterns in serpentine versus nonserpentine grassland plant community
418 diversity and temporal stability (Fig. S2; Tables 1, 3, S1-S5) (Fernandez-Going *et al.* 2012).
419 Results from previous studies that demonstrate a positive relationship between host diversity and
420 pathogen diversity indicate that higher plant species diversity in serpentine grasslands might
421 contribute to higher fungal diversity if native forbs and bunchgrasses that specialize on
422 serpentine soils share foliar fungal pathogens with *S. pulchra* (Hechinger and Lafferty 2005;
423 Spear 2017). Research on five common invasive and native grasses (including *S. pulchra*) at
424 JRBP shows that multi-host foliar fungal pathogens dominate this system, supporting this
425 hypothesis (Spear and Mordecai 2018).

426 Differing chemistry between the leaves of nonserpentine and serpentine *S. pulchra* may
427 influence fungal community assembly through a variety of mechanisms. Fertilization with
428 nitrogen, which was elevated in nonserpentine compared to serpentine leaf tissue (Fig. 4b),
429 differentially impacts foliar disease severity depending on host and fungal pathogen identities
430 (Dordas 2008, Verosoglou *et al.* 2013). The mechanisms by which nitrogen mediates plant-
431 fungal pathogen interaction outcomes are unknown, but might involve impacts on plant immune
432 pathways and/or fungal pathogen virulence, growth, and reproduction. Eschen *et al.* propose
433 fungal interspecific variation in nutritional requirements as an explanation for correlations
434 between soil and plant nitrogen content and fungal endophyte community composition in
435 *Cirsium arvense* (Eschen *et al.* 2010). While our results are qualitatively similar to those of
436 Eschen *et al.*, greenhouse inoculation experiments with different fungal pathogens and plants of
437 known tissue chemistries are needed to test this hypothesis.

438 Importantly, our study design limits our mechanistic understanding of how variation in
439 plant chemistry and fungal communities between habitats may be related. Measurements of leaf
440 chemistry taken from young, healthy leaves may not be representative of the chemistry of the
441 older, larger leaves that both fungi and surveyors may be more likely to encounter. Further, the
442 low number of sampled fungal communities for which we measured plant chemistry prevents
443 statistical analysis of relationships between fungal community composition and plant nutrient
444 content. However, our hypothesis that differences in plant tissue chemistry between serpentine

445 and nonserpentine habitats shape fungal pathogen presence is supported by previous studies that
446 showed *Hesperolinon* sp. infection frequency and severity by the fungal rust *Melampsora lini* in
447 California grasslands depended on plant tissue Ca levels linked to serpentine soil chemistry
448 (Springer *et al.* 2006; Springer 2009).

449 Serpentine and nonserpentine fungal communities may also differ due to genetic
450 divergence between the *S. pulchra* populations found in these soils. The gene-for-gene model of
451 the evolution of virulence hypothesizes that a given pathogen can infect a given host only if the
452 pathogen does not possess an avirulence gene recognized by a matching resistance gene in the
453 host (Flor 1956; Parker and Gilbert 2004). To date, there have been few studies of genetic
454 differences between serpentine and nonserpentine populations of the same plant species.
455 However, genetic and chemical analyses of serpentine and nonserpentine populations of the
456 native forb *Lasthenia californica* at JRBP show that these populations represent two
457 phylogenetically distinct species, each of which accumulates ions in significantly different
458 concentrations and has a unique flavonoid profile (Desrochers and Bohm 1993; Chan *et al.* 2002;
459 Rajakaruna *et al.* 2003). Since many flavonoids have antifungal properties, serpentine and
460 nonserpentine *S. pulchra* could also contain different genotypes with different potential for
461 resistance to any given fungus due to divergent resistance gene and/or flavonoid profiles (Parker
462 and Gilbert 2004; Treutter 2006).

463 In addition to fungal species turnover between habitat types, interannual turnover
464 contributed substantially to fungal diversity in this system, with only seven of 30 total species
465 isolated in all three years (Fig. 3; Tables 1, S5). Interannual variation in these communities may
466 be driven by a variety of mechanisms, including highly variable yearly precipitation (2015
467 rainfall = 491 mm; 2016 = 604 mm, 2017 = 985 mm) (Weather Underground). Timing and
468 amount of precipitation might alter infection dynamics by washing away fungal spores and/or
469 transmitting spores to new hosts (Madden 1997). Additionally, yearly variation in plant
470 population sizes, occurrences of different plant genotypes, and plant community composition
471 may also drive temporal variation in fungal communities (Hobbs and Mooney 1991). Although
472 few studies track interannual variation in fungal communities on plants, the year-to-year
473 dissimilarity of those hosted by *S. pulchra* is similar to that observed across a diverse taxonomic
474 range of plants including ferns, legumes, and shrubs (Olmo-Ruiz and Arnold 2014; Cotton *et al.*
475 2015; Zhang *et al.* 2016). In this context, our results are consistent with a growing body of

476 evidence for the general importance of storage effects—in which temporally variable conditions
477 act as ecological niches that facilitate species diversity—in plant-associated fungal communities
478 (Warner and Chesson 1985). This finding highlights the necessity of long-term data collection to
479 accurately characterize plant-fungal interaction networks.

480 *Foliar disease burden*

481 Time, but not habitat type, shaped disease burden, but mean levels of foliar infection
482 observed were low even at their peak of 1.9% symptomatic leaf area in 2017. This level of
483 damage is similar to that observed on other grass species in nonserpentine grasslands at JRBP
484 (Spear and Mordecai 2018), and on taxonomically diverse tropical forest plants in Mexico and
485 Hawaii (García-Guzmán and Dirzo 2001, Funk and Throop 2010). Recent work in the JRBP
486 grassland system shows that symptoms in the amounts observed inflict only minimal plant
487 fitness costs, suggesting that *S. pulchra* is able to effectively resist or suppress damage by foliar
488 pathogens in both serpentine and nonserpentine grasslands (Spear and Mordecai 2018; Uricchio
489 *et al.* 2019). These expectations align with assessments in wheat-fungal pathogen systems that
490 indicate yield reductions of ≤ 1% for each percent of symptomatic leaf area (Milus 1993, Khan *et*
491 *al.* 1997). The finding that between-habitat differences in foliar C and N content do not
492 correspond to differences in infection severity aligns with the finding that infection severity did
493 not differ between habitats. Additionally, similar C:N ratios suggesting that serpentine and
494 nonserpentine are equally healthy correspond to the consistently low damage observed across
495 habitats. However, the limitations described above to interpreting observed relationships between
496 plant chemistry and fungal community composition apply equally to observed relationships
497 between plant chemistry and disease severity.

498 The variation in fungal community composition we observed on a single host species
499 with habitat type and over time suggests possible mechanisms preventing severe outbreaks of
500 foliar disease in California grasslands. Transitions between distinct soil types over distances
501 within the range of fungal spore dispersal may prevent the spread of pathogens that are sensitive
502 to leaf tissue chemistry. Interannually variable conditions favoring successful infection by
503 different fungal species each growing season (Table S5), combined with plant senescence during
504 the dry season each year, suggest temporal barriers to pathogens evolving high virulence on both
505 nonserpentine and serpentine plants.

506 Clarifying the specific mechanisms that drive differences in serpentine and nonserpentine
507 fungal communities will require inoculation experiments to test how plant susceptibility to
508 infection by different fungal species changes with plant tissue chemistry and plant genetics.
509 Additionally, characterization of comprehensive plant-pathogen networks including all plant
510 species co-occurring with *S. pulchra* in serpentine and nonserpentine grasslands will improve our
511 understanding of the role of the surrounding plant community in structuring fungal pathogen
512 communities.

513 *Fungal diversity*

514 Compared to other plant species' interactions with both pathogenic and non-pathogenic
515 fungi, *S. pulchra* appears to host an unusually species-rich community of culturable fungi within
516 its diseased foliar tissue. Where we isolated 30 fungal species, past studies that described fungal
517 pathogen diversity for more than 100 different plant hosts consistently found fewer than 20
518 fungal pathogens per species, and almost all hosts had fewer than ten pathogens (Mitchell and
519 Power 2003; Hantsch *et al.* 2013; Rottstock *et al.* 2014). The level of fungal diversity we
520 documented is also high compared to that of culturable fungal endophyte communities associated
521 with asymptomatic plant tissues: of 35 plant species analyzed by Arnold and colleagues, only
522 five were found to host 30 or more endopyhtes (Arnold and Lutzoni 2007; Del Olmo-Ruiz and
523 Arnold 2014). Notably, studies that utilize high-throughput sequencing techniques to observe
524 both culturable and unculturable endophytic fungi frequently report hundreds of fungal OTUS
525 colonizing single host species (e.g., Jumpponen and Jones 2009, Zimmerman and Vitousek
526 2012). However, such results are difficult to compare to our own given the mismatch in the
527 detection capabilities of the methods used. Nonetheless, the exceptional diversity of the
528 culturable foliar fungi that inhabit *S. pulchra* is further indicated both by species accumulation
529 curves that suggest many more species in this community remain to be discovered (Fig. S1) and
530 by the lack of close sequence matches in genetic databases for some of the species isolated
531 (Table 2). These findings suggest that the characteristics that make the California Floristic
532 Province a biodiversity hotspot may extend to the fungal diversity associated with this flora
533 (Myers *et al.* 2000).

534 *Conclusions*

535 Fungi associated with foliar disease in the native bunchgrass *S. pulchra* are relatively

benign, likely cause minimal fitness impacts (Spear and Mordecai 2018), and contribute substantially to the overall biodiversity of California grasslands. Variation between habitats appears to play an important role in supporting the fungal diversity observed here, and may also reduce disease risk for *S. pulchra*. The high taxonomic diversity and rarity of serpentine fungi demonstrates that this community is potentially at risk of species loss via host-parasite coextinction as nonnative annual grasses continue to expand their ranges at the cost of native plants, and human activities including development and increased C and N deposition contribute to the loss of serpentine grasslands that already make up <2% of California's surface area (Huenneke *et al.* 1990; Vallano *et al.* 2012). This work demonstrates that, in addition to >200 endemic plant species, California's serpentine grasslands support unique communities of leaf-infecting fungi, providing additional motivation for active conservation of native grassland communities based on the far-reaching loss of biodiversity across trophic levels associated with their disappearance (Sprent 1992; Dunn *et al.* 2009).

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845 **Figure legends**

846 **Figure 1. Pathogen damage on *Stipa pulchra* was consistently low across three years and**
847 **both grassland types.** Panel **(a)** maps the locations of surveyed nonserpentine sites (blue) and
848 serpentine sites (green) along the ridgetop at JRBP. Panel **(b)** shows an example of fungal-
849 caused pathogen foliar damage on *S. pulchra.* Panel **(c)** shows mean percentage (bars) and two
850 standard errors (error bars) of diseased leaf area for surveyed serpentine and nonserpentine *S.*
851 *pulchra,* by year.

852 **Figure 2. Pathogens isolated from *Stipa pulchra* growing in nonserpentine and serpentine**
853 **grasslands.** Bipartite network showing species interactions between *S. pulchra* plants (left)
854 growing in serpentine grasslands (green) and nonserpentine grasslands (blue) and fungal species
855 cultured from symptomatic leaf tissue (white bars on the right). The length of each white bar
856 correlates with the number of isolates in the operational species unit (based on 97% sequence
857 similarity). The thickness of lines connecting left and right bars represents the number of times a
858 particular fungal species was isolated from *S. pulchra* in a particular grassland type. The
859 nonserpentine fungi came from 15 genera, 8 families, 4 orders, and 3 classes; the serpentine
860 fungi came from 20 genera, 14 families, 7 orders, and 5 classes (Tables 1, S1-S5).

861 **Figure 3. Distinct fungal communities were isolated from *Stipa pulchra* growing in nearby**
862 **serpentine and nonserpentine sites across years.** Non-metric multidimensional scaling
863 visualization of serpentine and nonserpentine fungal community similarity. Each point
864 corresponds to the combined fungal community of two transects at the same site in the same
865 year. Serpentine communities are shown in green and nonserpentine communities are shown in
866 blue. Circles, triangles, and squares represent fungal communities sampled in 2015, 2016, and
867 2017, respectively. Ellipses enclose 95% confidence intervals for ordination of serpentine and
868 nonserpentine communities. Community dissimilarity increases with the distance between
869 points. PERMANOVA analysis showed a significant effect of soil type on fungal community
870 composition ($F = 3.623$, $R^2 = 0.0618$, $p = 0.001$).

871 **Figure 4. *Stipa pulchra* foliar C and N content differs between habitat types, but does not**
872 **predict mean pathogen damage.** Foliar percent C (Panel **(a)**), percent N (Panel **(b)**, and C:N
873 ratio (Panels **(c)** versus mean percentage of diseased leaf area for serpentine and nonserpentine *S.*
874 *pulchra* plants.

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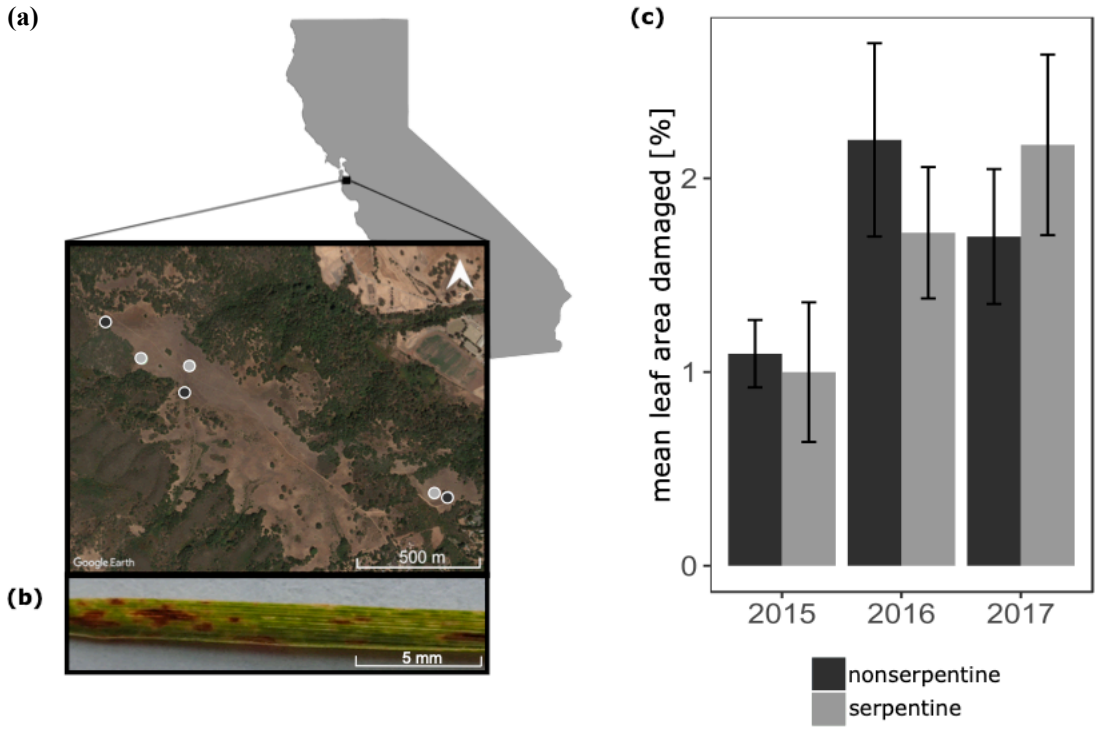
876 **Figures**



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878 **Figure 1.**

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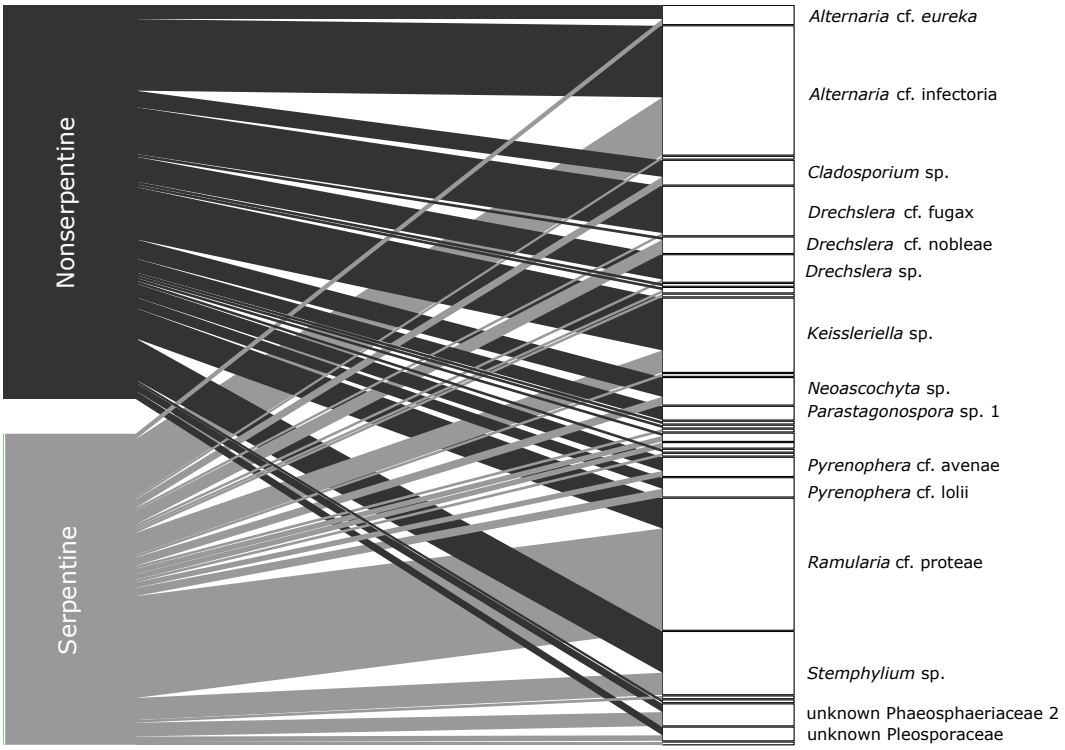
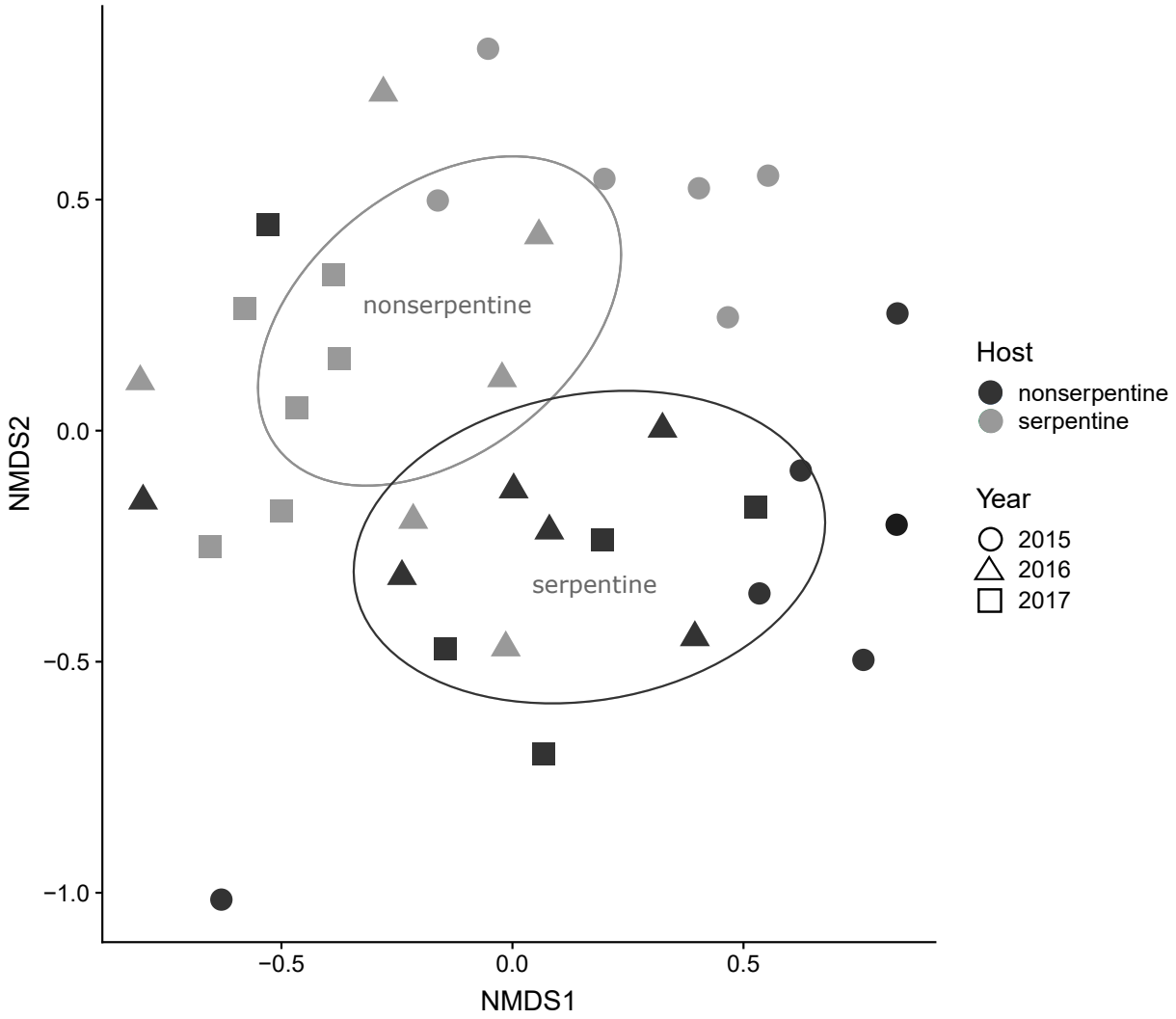

Nonserpentine
Serpentine
Alternaria cf. eureka
Alternaria cf. infectoria
Cladosporium sp.
Drechslera cf. fugax
Drechslera cf. nobleae
Drechslera sp.
Keissleriella sp.
Neoascochyta sp.
Parastagonospora sp. 1
Pyrenophera cf. avenae
Pyrenophera cf. lolii
Ramularia cf. proteae
Stemphylium sp.
unknown Phaeosphaeriaceae 2
unknown Pleosporaceae

Figure 2.


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

Figure 3.

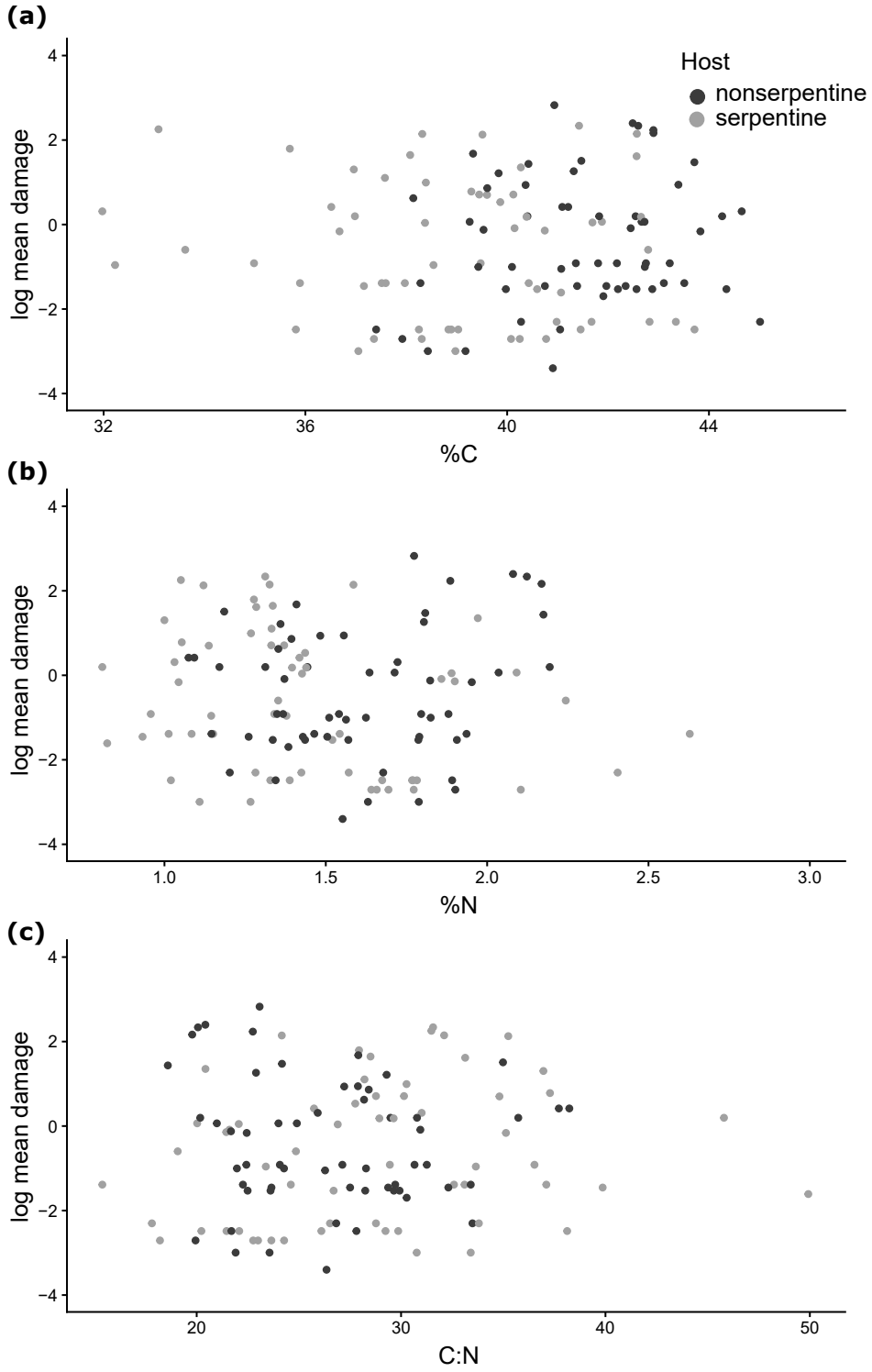
(a)
Host
nonserpentine
serpentine
log mean damage
%C
(b)
log mean damage
%N
(c)
log mean damage
C:N

Figure 4.

904 **Tables**

905 **Table 1. Pathogen damage on *Stipa pulchra* varied significantly by year, but not habitat**
906 **type.**

Variables	df	SS	MSE	F value	p-value
habitat type	1	5.8	5.81	2.63	1.05×10^{-1}
year	2	31.4	15.68	7.10	9.53×10^{-4}*
site	4	20.2	5.05	2.29	5.98×10^{-2}
habitat type x year	2	9.9	4.96	2.25	1.07×10^{-1}

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908 ANOVA results for log-transformed transect-level measurements of percentage of symptomatic
909 living tissue on serpentine and nonserpentine *S. pulchra* (N = 3 sites per habitat type per year,
910 with 12 transects per site per year, for a total of 36 serpentine and 36 nonserpentine transects
911 over all three years). The first column lists the factor(s) tested; the second column degrees of
912 freedom (df); the third column the sum of squares (SS); the fourth column the mean squared
913 error (MSE); the fifth column the F-value; and the 6th column the p-value. P-values < 0.05 are
914 marked with an asterisk.

915 **Table 2. Fungal species isolated from symptomatic *Stipa pulchra* leaves were unevenly**
916 **distributed across habitat types.** Each row represents one of the species isolated. The columns
917 indicate the isolation frequency for each species in each soil type.

Species	Nonserpentine	Serpentine
Alternaria cf. *infectoria*	26	21
Keissleriella sp.	19	8
Drechslera cf. *fugax*	17	1
Stemphylium sp.	15	8
Ramularia cf. *proteae*	11	37
Drechslera sp.	9	1
Neoascochyta sp.	7	3
Cladosporium sp.	6	3
Alternaria cf. *eureka*	5	2
Parastagonospora cf. *avenae*	5	0
Pyrenophera cf. *chaeotmioides*	5	2
Pyrenophera cf. *lolii*	4	3
Phaeosphaerieaceae sp. 2	3	5
Pleosporaceae sp.	3	2
Drechslera cf. *nobleae*	1	5
Epicoccum sp.	1	0
Heyderia sp.	1	1
Parastagonospora cf. *cumpignensis*	1	0
Parastagonospora sp.	1	0
Phaeosphaeria sp.	1	2
Podospora sp.	1	0
Phaeosphaerieaceae sp. 1	1	0
Chalastospora cf. *gossypii*	0	1
Hyphodiscus cf. *brevicollaris*	0	1
Nemania cf. *serpens*	0	1
Phaeophlebiopsis sp.	0	1
Plectania sp.	0	2
Pseudoseptoria sp.	0	1
Helotiales sp.	0	1
Wojnowiciella sp.	0	1

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922 **Table 3. Fungal communities were distinct on *Stipa pulchra* plants growing in serpentine**
923 **versus nonserpentine grasslands, across all years.**

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%)	

924

925 Fungal community similarity by habitat type and year. The leftmost column lists the fungal
926 communities considered, with the number of species in each community in parentheses. The
927 second column lists Fisher's alpha for each community. The third column shows the number of
928 shared species and, in parentheses, the percentage of total observed species that were shared for
929 each pair of communities. The rightmost column lists the estimated Morisita-Horn community
930 overlap value, based on absolute species abundances, for each pair of communities. The
931 Morisita-Horn index ranges from 0 to 1, with 1 indicating complete overlap.

Table 4. PERMANOVA results show significant effects of both habitat type and year on fungal foliar community composition (N = 18 serpentine and 18 nonserpentine sites).

	df	SS	MSE	F-Value	p-value
habitat type	1	0.5700	0.56997	5.1119	0.003*
year	2	1.2611	0.63055	5.6552	0.001*
distance	1	0.0767	0.07667	0.5850	0.675
habitat type x year	2	0.3339	0.16697	1.4975	0.224
habitat type x distance	1	0.0515	0.05147	0.3927	0.815
distance x year	2	0.1697	0.08485	0.6473	0.734

From left to right, columns list factors tested, degrees of freedom (df), sum of squares (SS), mean squared error (MSE), F-value, and p-value. Statistically significant p-values ($p < 0.05$) are marked with an asterisk. The distance factor compares communities at near (<600m) vs. far (>600m) distances from one another.

955

956 **Supporting Information**

957 **Fig. S1** Species accumulation curves for serpentine (green) and nonserpentine (blue) fungal
958 communities isolated from *Stipa pulchra* leaves.

959 **Fig. S2** Fungal species abundance by rank for serpentine (green) and nonserpentine (blue) fungal
960 communities on *Stipa pulchra* leaves.

961 **Table S1** Genus counts for serpentine and nonserpentine fungal communities isolated from *Stipa*
962 *pulchra* leaves.

963 **Table S2** Family counts for nonserpentine and serpentine fungal communities isolated from
964 *Stipa pulchra* leaves.

965 **Table S3** Order counts for nonserpentine and serpentine fungal communities isolated from *Stipa*
966 *pulchra* leaves.

967 **Table S4** Class counts for nonserpentine and serpentine fungal communities isolated from *Stipa*
968 *pulchra* leaves.

969 **Table S5** Presence and absence of genera in nonserpentine and serpentine fungal communities
970 isolated from *Stipa pulchra* leaves, by year.

971 **Table S6** ANOVA table for effects of habitat type, year, and habitat type x year interactions on
972 site-level fungal community diversity in terms of Fisher's alpha.

973 **Table S7** ANOVA table for effects of habitat type, year, and habitat type x year interactions on
974 site fungal species richness.

975 **Table S8** Pairwise PERMANOVA table for effects of year on fungal community composition.

976 **Table S9** Results of chemical analysis of serpentine and nonserpentine *Stipa pulchra* foliar
977 tissue.

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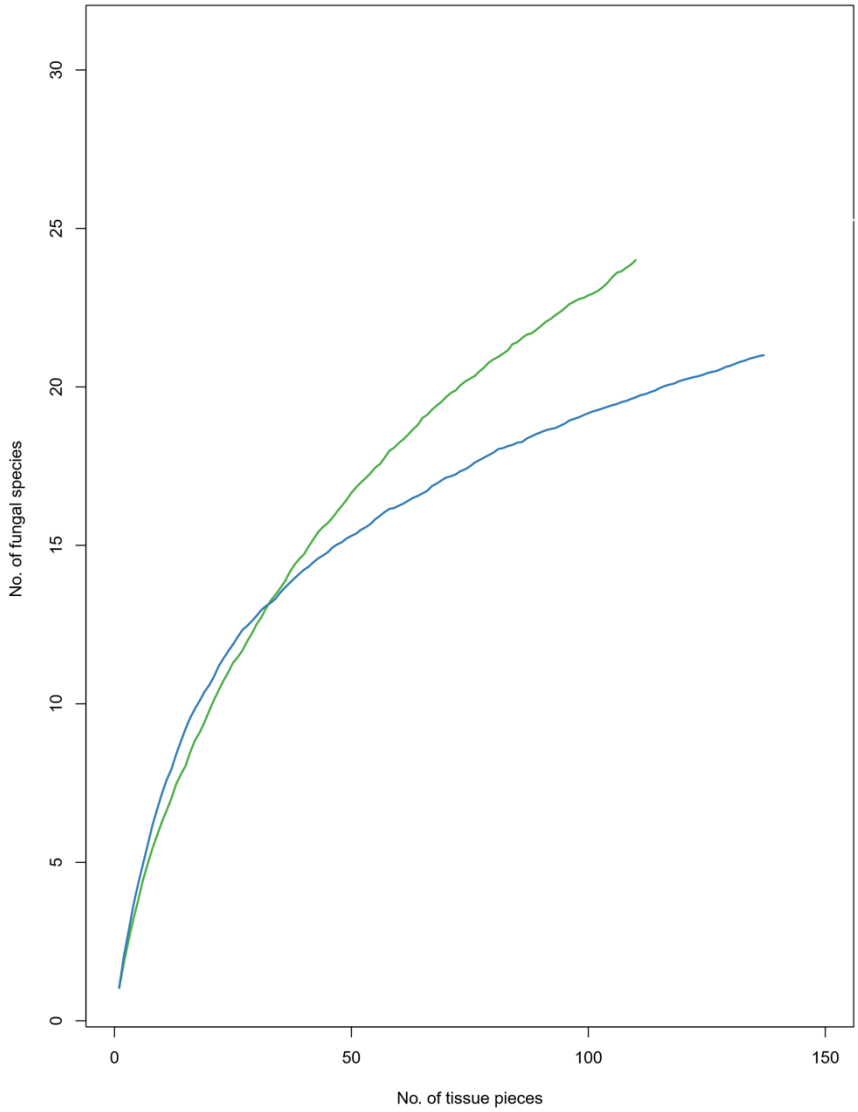
985 **Supporting Figures and Tables**



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987 **Figure S1. Species accumulation curves for serpentine (green) and nonserpentine (blue)**
988 **fungal communities isolated from *Stipa pulchra* leaves.** The cumulative number of unique
989 fungal OTUs (based on 97% sequence similarity; y-axis) is plotted against the number of *S.*
990 *pulchra* leaf tissue pieces sampled (x-axis).

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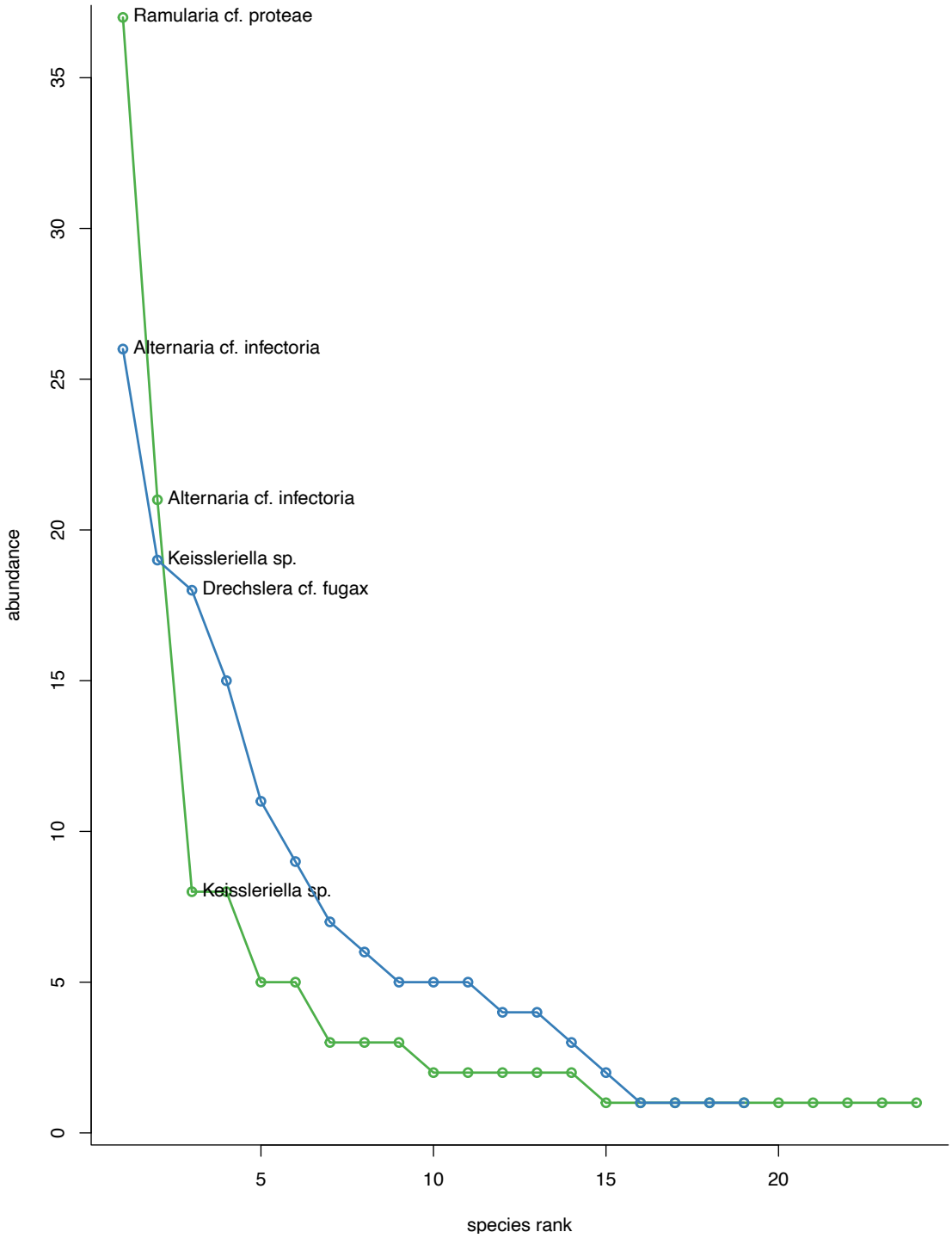

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995 **Figure S2. Fungal species abundance by rank for serpentine (green) and nonserpentine**
996 **(blue) fungal communities on *Stipa pulchra* leaves.** Each point represents the number of times
997 a single species was isolated from *S. pulchra* leaves in each habitat type.
998

999 **Table S1.** Genus counts for serpentine and nonserpentine fungal communities isolated from

1000 *Stipa pulchra* leaves.

Genus	Nonserpentine	Serpentine
Alternaria	31	23
Drechslera	27	7
Keissleriella	19	8
Stemphylium	15	8
Ramularia	11	37
Pyrenophera	9	5
Neoascochyta	7	3
Parastagonospora	7	0
Cladosporium	6	3
Phaeosphaerieaceae	4	5
unknown Pleosporaceae	3	2
Epicoccum	1	0
Heyderia	1	1
Phaeosphaeria	1	2
Podospora	1	0
Chalastospora	0	1
Hyphodiscus	0	1
Nemania	0	1
Phaeophlebiopsis	0	1
Plectania	0	2
Pseudoseptoria	0	1
unkown Helotiales	0	1
Wojnowiciella	0	1

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1002 Numbers in each column represent the number of times a genus was isolated from *S. pulchra*

1003 leaves in each habitat type.

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1005 **Table S2.** Family counts for nonserpentine and serpentine fungal communities isolated from
1006 *Stipa pulchra* leaves.

Family	Nonserpentine	Serpentine
Pleosporaceae	85	45
Lentitheciaceae	19	8
Phaeosphaeriaceae	12	8
Mycosphaerellaceae	11	37
Didymellaceae	8	3
Cladosporiaceae	6	3
Helotiaceae	1	1
Lasiosphaeriaceae	1	0
Dothioraceae	0	1
Hyaloscyphaceae	0	1
Meruliaceae	0	1
Pleosporales *incertae sedis*	0	1
Sacrosomataceae	0	2
unknown Helotiales	0	1
Xylariaceae	0	1

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1008 Numbers in each column represent the number of times a family was isolated from *S. pulchra*
1009 leaves in each habitat type.

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Table S3. Order counts for nonserpentine and serpentine fungal communities isolated from *Stipa pulchra* leaves.

Order	Nonserpentine	Serpentine
Pleosporales	124	65
Capnodiales	17	40
Helotiales	1	3
Sordariales	1	0
Dothideales	0	1
Pezizales	0	2
Polyporales	0	1
Xylariales	0	1

Numbers in each column represent the number of times an order was isolated from *S. pulchra* leaves in each habitat type.

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1041 **Table S4.** Class counts for nonserpentine and serpentine fungal communities isolated from *Stipa*
1042 *pulchra* leaves.

Class	Nonserpentine	Serpentine
Dothideomycetes	141	106
Leotiomycetes	1	3
Sordariomycetes	1	1
Agaricomycetes	0	1
Pezizomycetes	0	2

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1044 Numbers in each column represent the number of times a class was isolated from *S. pulchra*
1045 leaves in each habitat type.

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1062 **Table S5.** Presence (P, shaded) or absence (A, unshaded) of genera in nonserpentine and
1063 serpentine fungal communities isolated from *Stipa pulchra* leaves, by year.

1064

Genus	**Nonserpentine 2015**	**Nonserpentine 2016**	**Nonserpentine 2017**	**Serpentine 2015**	**Serpentine 2016**	**Serpentine 2017**
Alternaria	P	P	P	P	P	P
Chalastospora	A	A	A	P	A	A
Cladosporium	P	A	A	P	A	P
Drechslera	P	P	P	P	P	P
Epicoccum	A	A	P	A	A	A
Heyderia	A	A	P	A	A	P
Hyphodiscus	A	A	A	A	P	A
Keissleriella	P	P	P	P	P	P
Nemania	A	A	A	A	A	P
Neoascochyta	A	P	P	A	P	P
Parastagonospora	P	A	P	A	A	A
Phaeophlebiopsis	A	A	A	A	P	A
Phaeosphaeria	A	P	A	P	P	A
Plectania	A	P	A	A	A	P
Podospora	A	P	A	A	A	A
Pseudoseptoria	A	A	A	A	P	A
Pyrenophera	P	P	P	P	P	P
Ramularia	P	P	A	P	P	P
Stemphylium	A	P	P	P	A	P
unknown Phaeosphaerieaceae	P	P	A	A	P	A
unknown Pleosporaceae	A	P	P	A	P	P
unkown Helotiales	A	A	A	A	P	A
Wojnowiciella	A	A	A	P	A	A

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Table S6. ANOVA table for effects of habitat type, year, and habitat type x year interactions on site-level fungal community diversity in terms of Fisher's alpha.

	df	SS	MSE	F value	p-value
Habitat type	1	0.10	0.10	0.010	0.92
Year	2	3.63	1.82	0.18	0.84
Habitat type x Year	2	17.35	8.68	0.86	0.45

Columns, from left to right, list the variable tested, the degrees of freedom, the sum of squares, the mean squared error, the F value, and the p-value.

Table S7. ANOVA table for effects of habitat type, year, and habitat type x year interactions on site fungal species richness.

	df	SS	MS	F value	p-value
Habitat type	1	6.72	6.72	1.07	0.32
Year	2	3.11	1.56	0.25	0.78
Habitat type x year	2	8.44	4.22	0.67	0.53

Columns, from left to right, list the variable tested, the degrees of freedom, the sum of squares, the mean squared error, the F value, and the p-value.

1122 **Table S8.** Pairwise PERMANOVA table for effects of year on fungal community composition.
1123 From left to right, columns list the pair of years being compared, the F-value, R^2, and the
1124 Bonferroni-adjusted p-value. P-values < 0.05 are marked with an asterisk.

Pair	F-value	R^2	Bonferroni p-value
2015 vs 2016	2.616	0.207	0.114
2015 vs 2017	5.983	0.374	0.009*
2016 vs 2017	4.397	0.304	0.018*

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Table S9. Results of chemical analysis of serpentine and nonserpentine *S. pulchra* foliar tissue. The Serpentine and Nonserpentine columns report elemental content in percentage of leaf tissue by dried weight. The standard error of each mean is listed in parentheses. W, in the third column, is the Wilcoxon-rank sum test statistic comparing serpentine and nonserpentine mean values. Asterisks denote statistically significant p-values ($p < 0.05$).

Element	Serpentine		Nonserpentine		W	p-value	
C	38.97	*(3.44 x 10^{-1})*	41.48	*(2.35 x 10^{-1})*	2713	1.65 x 10^{-7}	*
N	1.44	*(4.96 x 10^{-2})*	1.61	*(3.84 x 10^{-2})*	2346	1.10 x 10^{-3}	*
C:N	28.72	*(8.74 x 10^{-1})*	26.54	*(6.18 x 10^{-1})*	1398	6.62 x 10^{-2}	

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