Soil type and interannual variation shape unique fungal pathogen communities on nearby populations of a California native bunchgrass

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Abstract

The role of infectious disease in regulating host populations is increasingly recognized, but 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* on nearby serpentine and nonserpentine soils that have distinct chemical properties and plant communities. We found that serpentine and nonserpentine *S. pulchra* experienced consistent, low disease pressure associated with distinct communities of pathogens, that interannual fungal species turnover differed with soil type, and that plant chemistry reflected soil chemistry. The results indicate that this species experiences minimal foliar disease associated with diverse fungal pathogen communities that are structured by environmental 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

Introduction

How environmental heterogeneity affects species interactions, and ultimately population growth, remains a fundamental ecological question. Infectious disease, which arises from interactions between hosts, parasites, and the environment, is particularly likely to vary across populations in differing environments. Both biotic factors, such as the phylogenetic structure of the host community, and abiotic factors, such as elevation, have been associated with the presence of specific diseases and the severity of their impacts on their hosts (Abbate and Antonovics 2014; Parker *et al.* 2015). Additionally, mounting evidence suggests that specific characteristics of pathogen community structures, including the identities and abundances of the diseases present in a host population, mediate the outcomes of host – pathogen interactions (Seabloom *et al.* 2015; Borer *et al.* 2016). Together, these observations highlight the importance of understanding interactions between environmental variation, pathogen community composition, and pathogen pressure on host populations to assess the potential for disease to regulate host population dynamics. For plant hosts, such assessments are important both in conservation and agricultural contexts because identifying populations at high risk for severe disease outbreaks and applying preventative measures may prevent local species extinctions and improve crop yields, respectively. While studies have frequently explored the interactions of single host – pathogen pairs under differing abiotic conditions (e.g., Abbate and Antonovics 2014) or the effect of varying pathogen community composition on disease burden (e.g., Telfer *et al.* 2010), relationships between abiotic variables and pathogen community structures remain largely unexplored in natural systems (but see Spear 2017).

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

84 the fungal pathogens that infect most plant species (Burdon and Laine 2019). Therefore, there is
85 a pressing need for systematic surveys of these pathogen communities and their variation across
86 environmental conditions in natural systems.

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

96 Differences in plant community composition and soil chemistry suggest that *S. pulchra*
97 populations growing in distinct soil types are likely to host distinct fungal pathogen communities
98 and/or experience differing disease burdens, particularly if the phenotype of *S. pulchra* is
99 influenced by the soil in which it grows. *S. pulchra* naturally occurs in both nonserpentine soils
100 and in relatively rare serpentine soils that host grassland plant communities. These two soil types
101 differ in their chemistry and host distinct plant communities, resulting in a suite of environmental
102 differences that may influence fungal pathogen infection, reproduction, and persistence (Harrison
103 and Viers 2007; Burdon and Laine 2019). In contrast to the majority of California grasslands,
104 which are heavily invaded by exotic species, serpentine grasslands have a diverse array of native
105 species adapted to chemically and physically harsh soils that are low in essential plant nutrients
106 (N, P, K, Ca), high in heavy metals that are potentially toxic to plants (Co, Ni, Cr, Fe), and rocky
107 and shallow, with poor moisture retention (Harrison and Viers 2007; Oze *et al.* 2008; Eviner,
108 2016).

109 Soil properties determine a variety of factors likely to influence the occurrence and
110 outcomes of plant fungal infections, including soil moisture levels and plant chemistry and
111 physiology (Cunningham *et al.* 1999; McElrone *et al.* 2005; Smith 2007). Spores of different
112 fungal species vary in both their ability to survive in dry and moist environments and their
113 tolerance of heavy metals (Coley-Smith and Cooke 1971; Iram *et al.* 2009). Moreover, plants of

the same species grown in drier and lower-nutrient soils can have thicker epidermises and higher concentrations of phenolic compounds, many of which have antifungal properties (Osbourne 1996; Cunningham *et al.* 1999). Finally, soil chemistry may influence pathogen infection either through hyperaccumulation of heavy metals, which can bolster chemical defenses against natural enemies, or by limiting common mechanisms of plant defense (Springer *et al.* 2006; Rascio and Navari-Izzo 2011).

In this study, we ask: (1) how does disease burden differ on plants growing in different soil types? (2) how does the species composition of fungal pathogen communities differ on plants growing in different soil types? and (3) by what mechanisms—including host community composition and plant tissue chemistry—might soil type affect disease burden and pathogen composition? We hypothesize that both disease burden and pathogen species composition differ on *S. pulchra* individuals growing in serpentine versus nonserpentine soils, either because of differences in the composition of the surrounding plant community, differences in plant physiological responses, or both. We further hypothesize that the soil type *S. pulchra* grows in alters foliar tissue chemistry in ways that may affect disease burden, with higher heavy metal content and/or lower C, N, and P content in serpentine plants. If so, these foliar chemistry differences may translate into either (a) higher disease burden in serpentine plants relative to nonserpentine plants, facilitated by negative impacts of limited plant resource availability on plant health (as in Springer *et al.,* 2006), or (b) lower disease burden in serpentine plants due to heightened chemical and/or physiological plant defenses in response to serpentine soil properties. In addition to contributing novel information about previously uncharacterized plant fungal pathogen communities in California grasslands, the results of this study will aid in assessing disease risk for plant populations in natural and agricultural contexts by providing information about the spatial and temporal scales over which soil type and other environmental factors can affect the spread of outbreaks.

Materials and Methods

All surveys and sample collection took place at Jasper Ridge Biological Preserve (JRBP), a 485-hectare site in San Mateo County, CA managed by Stanford University (37.4° N, 122.2° W). JRBP has a Mediterranean climate with cool winters (mean 9.2°C), warm summers (mean 20.1°C), and annually variable precipitation that predominantly occurs in the winter and spring

(averaging 622.5 mm per year) (Ackerly *et al.* 2002). The growing season occurs during the rainy season and plants senesce in 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).

Substantial populations of *S. pulchra* occur in both serpentine and nonserpentine grasslands at JRBP (McNaughton 1968). The serpentine plant communities at JRBP are more diverse than the nearby nonserpentine plant communities and include many native grasses and forbs, in contrast to the invasive-dominated nonserpentine grasslands (McNaughton 1968; Huenneke *et al.* 1990; Field *et al.* 1996). Plant species commonly found in serpentine grasslands at JRBP include *Stipa pulchra*, *Elymus glaucus*, *Elymus multisetus, Eschscholzia californica*, and the invasive grass *Bromus hordeaceus* (McNaughton 1968; Hobbs and Mooney 1985). The nonnative annual species that dominate the nonserpentine grasslands but are absent from serpentine grasslands include *Avena barbata, Avena fatua*, *Bromus diandrus,* and *Erodium botrys* (McNaughton 1968; Hobbs and Mooney 1985; Field *et al.* 1996).

Quantification of disease

To assess plant disease burden, we quantified the percentage of living leaf area exhibiting symptoms of fungal disease in serpentine and nonserpentine populations of *S. pulchra*. We selected three serpentine grassland sites and three nonserpentine grassland sites such that each serpentine site was paired with a nearby (~170m away) nonserpentine site (Fig. 1a). Individual sites within each soil type were >300m apart and the maximum distance between any two sites was 1600m (Fig. 1a). Each year, we randomly placed four 5-meter transects at each 40m x 40m site and assessed the infection status of the *S. pulchra* individual nearest each meter mark, resulting in five individual plants per transect surveyed each year (N = 60 plants per soil type per year). A single surveyor visually estimated the percentage of living foliar tissue damaged by pathogens for six arbitrarily-selected leaves per plant. We used printed field reference guides showing known amounts of damage on leaves to standardize estimates. Damage was indicated by spots and lesions which ranged in color from dark brown to pale yellow (Fig. 1b). For damage estimates of 5% or greater, values were determined to the nearest 5%. Lower damage levels were described as either 1% or 0.1%. All survey sites were located along the ridgetop at JRBP, where

174 serpentine and nonserpentine (greenstone) soils are present in discrete adjacent bands (Fig. 1a)
175 (Oze *et al.*,2004). The relative flatness of the ridgetop ensured that all survey sites were at
176 roughly the same elevation and had similar slopes, aspects, and water availability (Oze *et al.*
177 2008).

178 We used analysis of variance (ANOVA) to test for effects of soil type, year, soil type x
179 year, and site on log-transformed mean percent disease damage by transect in serpentine and
180 nonserpentine *S. pulchra* (N = 36 serpentine and 36 nonserpentine transects). When ANOVA
181 results were statistically significant, we used t-tests for every possible pairwise combination,
182 with Bonferroni adjustments of p-values to account for multiple comparisons, to identify the
183 interactions contributing to the significant result.

184 *Isolation and molecular identification of foliar fungal pathogens*

185 To identify the fungal foliar pathogens infecting serpentine and nonserpentine *S. pulchra,*
186 we harvested one segment of symptomatic leaf tissue for culturing and identification of the
187 putatively causal fungal pathogen(s) from each surveyed individual with disease. We excised,
188 surface sterilized, and plated a 2mm^2 square of tissue from each sample at the leading edge of
189 infection, to improve the chances of isolating the causal fungal pathogen, on malt extract agar
190 with 2% chloramphenicol to prevent bacterial growth. We observed the plates for growth for
191 eight weeks and isolated morphologically distinct hyphae into axenic culture. Six segments of
192 surface sterilized, asymptomatic tissue were plated as a control and showed no growth. We
193 extracted and sequenced genomic DNA from the ITS region for each isolate. Fungal isolation
194 and sequencing methods with ITS1F and ITS4 primers followed Spear and Mordecai 2018.

195 It is possible that the growing medium used may have led to selective isolation and
196 sequencing of fungi other than those causing symptoms. Our use of small segments of leaf tissue
197 from the advancing margin of disease was intended to prevent this outcome under the
198 assumption that symptom-causing organisms should be dominant in visibly diseased tissue and
199 particularly at its expanding margin, and our fungal cultures were observed growing directly out
200 of lesions. Inoculation trials on a variety of host species described in Spear and Mordecai 2018
201 confirmed pathogenicity for 27% of tested fungi isolated from this grassland system under this
202 protocol.

203 We estimated taxonomic placement of fungal isolates by grouping sequences into

204 operational taxonomic units with 97% sequence similarity (OTUs), a proxy for species
205 delineation based on the range of intraspecific ITS divergence (O'Brien *et al.* 2005), and then
206 comparing assembled DNA sequences to the entries in the UNITE fungal database. To prepare
207 sequences for taxonomic placement, we first trimmed forward and reverse Sanger sequencing
208 reads to a maximum error rate of 5% and then automatically assembled bidirectional reads into
209 consensus sequences with a minimum of 20% overlap and 85% sequence similarity in Geneious
210 (Kearse *et al.* 2012), discarding consensus sequences less than 450 bp in length and/or less than
211 60% in read quality. We grouped the remaining internal transcribed spacer (ITS) sequences into
212 OTUs using USEARCH 10.0.240 (Edgar 2010) and estimated the taxonomic placement of each
213 OTU in mothur v.1.40.5 (http://mothur.org/; Schloss 2009) using the naïve Bayes classification
214 approach (classify.seqs with options: method='wang', cutoff=0, iters=200). We used a
215 bootstrapping confidence score of at least 80% to assign a species name and at least 60% to
216 assign taxonomy at higher ranks.

217 *Analyses of fungal community composition*

218 To characterize foliar fungal community composition in serpentine and nonserpentine *S.*
219 *pulchra* populations, we compared the diversity and assessed the similarity of communities of
220 fungi cultured from the diseased tissue of surveyed plants. To describe fungal community
221 diversity, we calculated observed taxa richness and Fisher's alpha, a widely used measure of
222 species richness that is robust to uneven sample sizes (Fisher *et al.* 1943; Magurran 2013);
223 generated taxa accumulation curves to understand sampling efficacy (Oksanen *et al.* 2016); and
224 counted the number of fungal isolates in each genus, family, order, and class from each grassland
225 type. We compared fungal community composition across grassland types and across years
226 visually, using non-metric multidimensional scaling (NMDS), and statistically, using
227 permutational multivariate analyses of variance (PERMANOVA) with pairwise Adonis
228 functions (Anderson 2001; Arbizu 2016; Oksanen *et al.* 2016). To assess spatial turnover in
229 fungal communities, we included a site distance factor in the statistical analyses, with
230 comparisons between sites <600m apart categorized as "near" and comparisons between sites
231 >600m apart categorized as "far" (Fig. 1a). To account for low culturing success size in some
232 transects, we considered the combined isolates from two nearby transects at each site in each
233 year (N = 18 serpentine and 18 nonserpentine communities, generated from 36 total transects per

234 soil type) to be distinct communities for NMDS visualization.

235 For ANOVA and PERMANOVA analyses we considered the combined isolates from
236 each site in each year to be distinct communities (N = 9 serpentine and 9 nonerspentine
237 communities). When PERMANOVA results were statistically significant, we used pairwise
238 PERMANOVAs with Bonferroni adjustments to p-values to clarify which fungal communities
239 were significantly different from one another (Arbizu 2016). For the NMDS and PERMANOVA
240 analyses, we used the function vegdist with the abundance-based Chao method, accounting for
241 unsampled species, to create a matrix of pairwise community dissimilarities (Chao *et al.* 2016;
242 Oksanen *et al.* 2016). We also used the Morisita-Horn index, which is independent of sample
243 size and diversity, to make pairwise comparisons of similarity within and between the serpentine
244 and nonserpentine fungal communities from each year (N = 265 isolates, 200 bootstrap
245 replicates) (Wolda 1981; Jost *et al.* 2011). Finally, we used ANOVA to test for effects of soil
246 type, year, site distance, year x soil type, and year x site distance interactions on fungal diversity
247 (Fisher's alpha) and richness at the site level.

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

252 *Chemical analyses of leaf tissue*

253 We analyzed C, N, Ca, Cr, Mg, Ni, and P content of the foliar tissue of *S. pulchra* grown
254 in serpentine and nonserpentine soils *in situ* and in pots (as part of a separate field experiment;
255 see Methods S1). In 2016, we collected the youngest fully expanded and entirely asymptomatic
256 leaf of each individual surveyed in the field (N = 60 serpentine, 58 nonserpentine). We dried the
257 samples at room temperature for three months, then at 60°C for 48 hours, ground them to a
258 powder, and then dried them again at 60°C for 24 hours. We measured C and N content of these
259 samples with a Carlo Erba NA-1500 elemental analyzer using atropine standards.

260 To compare Ca, Cr, Mg, Ni, and P content, which requires more tissue biomass than
261 could be collected from plants in the field, we used a set of plants grown in pots in the
262 greenhouse and then moved to JRBP for a separate experiment (Methods S1). In total, we grew

480 plants in serpentine soil sourced from our survey sites at JRBP and 480 plants in nonserpentine greenhouse soil (PROMIX HP Mycorrhizae TM High Porosity Professional Growing Medium, Premier Horticulture Inc.). Interactions with soil microbes can affect plant defenses against consumers (Pineda *et al.* 2010). Therefore, to ensure that serpentine- and nonserpentine-grown plants encountered similar soil biota, we triple autoclave-sterilized serpentine soil (40-minute cycles at 120°C) and then immediately inoculated it with greenhouse soil in a ratio of 93:7 by weight (serpentine soil treatment; Heinze *et al.* 2015) and mixed sterilized serpentine soil into greenhouse soil in the same ratio (nonserpentine soil treatment, to account for possibility of microorganisms surviving autoclaving).

Both our choice of growing medium and the effects of autoclave sterilization on physical and chemical soil properties may have affected our results. Our methods should result in a soil environment in which naturally occurring mutualists of *S. pulchra* found in serpentine soils are replaced with the biota found in the commercial house soil we used. However, artificial modification of plant-soil microbe interactions may alter plant nutrient uptake (Khan 2005). To avoid such effects, we might have used non-sterilized serpentine and nonserpentine soils from our field sites. However, the volume of soil required for the expanded experiment these plants were a part of, along with the sensitive nature of our study area, precluded this possibility.

Results from studies that show effects of autoclaving on extractable levels of the elements we measured suggest that this method is more likely to increase than decrease the similarity of nutrient availability in serpentine and nonserpentine soils. For example, autoclaving has been shown to increase extractable Ca:Mg and decrease extractable Ni (Tadros 1957; Wolf *et al.* 1989; Abou-Shanab *et al.* 2003). Therefore, any significant differences observed between the foliar chemistry of *S. pulchra* grown in autoclaved serpentine and unsterilized nonserpentine soils that indicate plant tissue chemistry matching soil chemistry should not be due to our use of autoclave sterilization.

Elemental content of these plants was measured via inductively coupled plasma optical emission spectrometry (ICP-OES). Because the minimum amount of leaf tissue for which some heavy metals can be detected via ICP-OES is 0.5g and most of our *S. pulchra* had a total aboveground biomass of less than 0.5g, each measurement represents the combined elemental content of equal weights of oven-dried foliar tissue from two plants. Plants were randomly

293 selected for analysis from among all individuals with an aboveground biomass of at least 0.29g.
294 All asymptomatic leaves were removed from each plant's base and processed as described
295 above. A 0.5g portion of each pooled sample was digested in 10ml of 70% trace metal quality
296 nitric acid with microwave assistance. Digested samples were diluted to 16.7% acid
297 concentration with DI water and the concentrations of Ca, Cr, Mg, Ni, and P were measured in
298 parts per billion by a Thermo Scientific iCAP 6300 spectrometer (Thermo Scientific, Waltham,
299 MA, USA) (Cantarelli *et al.* 2010).

300 To assess the potential for soil chemistry to mediate plant-pathogen interactions, we
301 related soil type to leaf tissue chemistry of *in situ* and potted *S. pulchra*, and chemistry of *in situ*
302 plants to disease burden. We compared the mean mol/kg dried weight of leaf tissue for Ca, Cr,
303 Mg, and Ni for potted serpentine and nonserpentine plants with Wilcoxon rank sum tests. We
304 also compared mean C : N and Ca : Mg ratios that are indicative of plant health (Ciompi *et al.*
305 1996; Brady *et al.* 2005). We used Pearson's product-moment correlation to test for significant
306 monotonic relationships between the log-transformed mean amount of diseased tissue observed
307 on individual plants in the field and their foliar C and N content.

308 All statistical analyses were done in R version 3.4.1. In addition to the R packages
309 specified in the fungal community analyses methods, we used the packages cowplot (Wilke
310 2019), data.table (Dowle and Srinivasan 2019), RColorBrewer (Neuwirth 2014), reshape2
311 (Wickham 2007), tidyverse (Wickham 2017), and xtable (Dahl *et al*. 2019) to manipulate, plot,
312 and export data and results.

313 **Results**

314 *Pathogen damage*

315 Pathogen damage was similarly ubiquitous yet low-burden in serpentine and
316 nonserpentine soils. Every plant surveyed exhibited evidence of foliar fungus-caused disease.
317 The mean percentage of diseased leaf area observed across all years was 1.63 for serpentine and
318 1.66 for nonserpentine *S. pulchra,* respectively (Fig. 1c), and the mean proportion of surveyed
319 leaves with pathogen damage was 0.83 for serpentine and 0.85 for nonserpentine plants.
320 ANOVA results indicated a significant effect of year on log-transformed mean percent damage
321 (df = 2, SS = 9.41, F-value = 5.375, p-value = 7.04×10^{-3}) (Table 1). The only significant
322 Bonferroni-adjusted p-value for t-tests for every possible pairwise combination of years was the

comparison between 2015 and 2017 (2015 mean percent damage = 1.05, 2017 = 1.94, adjusted p-value = 5.013×10^{-3}).

Fungal pathogen community

We isolated 267 unique fungal isolates from 258 plated symptomatic tissue pieces with fungal growth (144 nonserpentine and 114 serpentine tissue pieces from 36 nonserpentine and 34 serpentine transects, out of 360 total tissue pieces). Of the 267 unique fungal isolates, we successfully sequenced 256 isolates. The sequenced isolates clustered into 30 operational taxonomic units (OTUs) based on 97% sequence similarity, representing 23 genera, 15 families, 8 orders, and 5 fungal classes. Due to a lack of close sequence matches in the UNITE database and/or a lack of taxonomic information for close sequence matches, we were unable to identify 18 OTUs to the fungal species level, and four of these to the genus level (Table 2). The taxonomic results indicate that many of the OTUs represent close relatives of known plant pathogens including *Alternaria, Cladosporium, Drechslera, Phaeosphaeria, Pyrenophora, Stemphylium, Ramularia,* and *Wojnowiciella* spp. (Table 2) (Kirk *et al.* 2008; Stukenbrock and McDonald 2008; Havis *et al.* 2015; Marin-Felix *et al.* 2017, 2019). The largest OTU contained 48 isolates and the smallest OTUs consisted of one isolate each. OTUs are hereafter referred to as "species" (O'Brien et al. 2005).

The serpentine fungal community was more diverse than the nonserpentine community at every taxonomic level (Tables 2, S1-S5). Twenty-four fungal species were isolated from serpentine *S. pulchra* (Fisher's alpha = 9.324, 95% CI = 4.664, 13.983) and 22 from nonserpentine (Fisher's alpha = 7.261, 95% CI = 3.597, 10.925); 16 of these species (53%) were shared (Fig. 2; Tables 1, 2). ANOVA results showed no significant relationship between soil type, year, or year x soil type interactions and fungal richness or Fisher's alpha at the site level (Tables S6, S7). Novel genera were isolated for both community types in every year of surveying (Table S5). Species accumulation curves did not approach horizontal asymptotes for either soil type, and the serpentine curve had a steeper slope than the nonserpentine curve (Fig. S1). These curves suggest that neither fungal community was fully described, and that there were more unsampled fungal species associated with the plants growing in serpentine soil than those in nonserpentine soil.

Both fungal communities were dominated by a few abundant species (isolated >10

353 times), but the numerically dominant species differed between nonserpentine and serpentine
354 soils: these were *Alternaria* cf. *infectoria* and *Ramularia* cf. *proteae*, respectively (Table 2; Fig.
355 S2). The nonserpentine community included five abundant species, representing 62% of the
356 isolates, while the serpentine community included two abundant species, representing 51% of
357 serpentine isolates (Table 2; Fig S2). The serpentine community had a higher proportion of rare
358 species (<3 isolates) than the nonserpentine community, 63% versus 36%, respectively. One
359 *Drechslera* species was abundant in the nonserpentine community and rare in the serpentine
360 (Table 2; Fig. S2).

361 Soil type and year significantly affected fungal community composition, based on two-
362 way PERMANOVA analysis of site-level fungal communities and NMDS visualization of
363 community similarity (soil type: F-value = 5.112, R^2 = 0.163 p = 0.003; year: F-value = 5.655,
364 R^2 = 0.360 p = 0.001; Fig. 3; Table 4). Serpentine fungal communities were generally more
365 similar to one another than they were to nonserpentine fungal communities, and vice versa (Fig.
366 3; Tables 2, 3). Community composition differed significantly between 2015 and 2017, and 2016
367 and 2017, but not between 2015 and 2016 (Table S8).

368 Serpentine community composition was more consistent across years than
369 nonserpentine fungal community composition (Fig. 3; Tables 2, 3). While nonserpentine fungal
370 community similarity was unpredictable between years, with some very dissimilar communities
371 (Morisita-Horn overlap = 0.295) and some very similar ones (Morisita-Horn overlap = 0.882),
372 serpentine fungal communities were always relatively similar between years, with Morisita-Horn
373 overlap ranging from 0.707 to 0.786. Morisita-Horn overlap for nonserpentine and serpentine
374 fungal communities in each year was also variable, ranging from 0.535 to 0.725 (Table 3).

375 *Leaf tissue chemistry and disease burden*

376 As expected from soil chemistry differences, leaf chemistry in plants grown on serpentine
377 versus nonserpentine soil differed in mean C, N, Ca, Mg, Ni, and P content, and in Ca:Mg ratio.
378 Serpentine plants had significantly lower mean Ca, C, N, and P content and Ca:Mg ratio than
379 nonserpentine plants and significantly higher Ni and Mg (Fig. 4; Table S9). Cr content and C:N
380 ratio were similar for plants from both soil types. The results of Pearson's product-moment
381 correlation tests relating log-transformed mean percent symptomatic tissue to percent C, percent
382 N, and C:N ratio in asymptomatic foliar tissue were not statistically significant for serpentine or

383 nonserpentine plants (Fig. 5).

384 **Discussion**

385 Here we show that a diverse suite of foliar fungi was associated with ubiquitous, low-
386 burden damage on host individuals across growing seasons and environmental conditions. Soil
387 type and time appeared to shape the composition and diversity of fungal pathogen communities
388 in this system. These results provide some of the first evidence that local growing conditions
389 structure the pathogen communities infecting a single host species even over relatively small
390 spatial scales of a few hundred meters (Figs. 1a, 3; Table 3).

391 Compared to other plant species' interactions with both pathogenic and non-pathogenic
392 fungi, *S. pulchra* appears to host an unusually species-rich fungal community within its diseased
393 foliar tissue. Where we isolated 30 fungal species, past studies that described fungal pathogen
394 diversity for more than 100 different plant hosts consistently found fewer than 20 fungal
395 pathogens per species, and almost all hosts had fewer than ten pathogens (Mitchell and Power
396 2003; Hantsch *et al.* 2013; Rottstock *et al.* 2014). The level of fungal diversity we documented is
397 also high compared to that of fungal endophyte communities associated with asymptomatic plant
398 tissues: of 35 plant species analyzed by Arnold and colleagues, only five were found to host 30
399 or more endopyhtes (Arnold and Lutzoni 2007; Del Olmo-Ruiz and Arnold 2014). The
400 exceptional diversity of the foliar fungi that inhabit *S. pulchra* is further indicated both by
401 species accumulation curves that suggest many more species in this community remain to be
402 discovered (Fig. S1) and by the lack of close sequence matches in genetic databases for some of
403 the species isolated (Table 2). These findings suggest that the characteristics that justified the
404 identification of the California Floristic Province as a biodiversity hotspot may extend to the
405 fungal diversity associated with this flora (Myers *et al.* 2000).

406 Although disease burden was similar in both soil types, fungal communities varied by
407 soil type in both their composition and their stability over time (Figs. 1c, 2, 3, S2; Tables 1-4).
408 Higher fungal diversity and lower species turnover between years in the serpentine align with
409 patterns in serpentine versus nonserpentine grassland plant community diversity and temporal
410 stability (Fernandez-Going *et al.* 2012). The results thus imply that pathogen sharing among
411 hosts in native-dominated serpentine grasslands versus nonnative-dominated nonserpentine
412 grasslands leads to different plant-fungal interactions based on soil type. That the chemical

413 composition of *S. pulchra* reflected the chemistry of the soil in which it grew (except for Cr
414 levels) suggests that soil influence on plant phenotype may also contribute to differences
415 between fungal communities (Fig. 4, Table S9) (Oze *et al.* 2008).

416 Both the close proximity of the sampled nonserpentine and serpentine plants and the
417 finding that fungal communities were more similar between more distant sites of the same soil
418 type than they were between nearby sites of different soil types suggest that environmental
419 filtering, rather than dispersal limitation, plays a key role in fungal community assembly (Table
420 4). In addition to its effects on tissue chemistry and plant community composition, soil type
421 might impact disease dynamics if differing water availability between serpentine and
422 nonserpentine soils alters leaf physiology in ways that affect infection processes. We hypothesize
423 that some or all of these factors influence the composition of fungal communities and their
424 variation across environments.

425 Differences in nonserpentine and serpentine fungal communities may result from
426 differences in the plant communities found on these soils. Serpentine *S. pulchra* pathogen
427 communities were more diverse than nonserpentine communities at the species, genus, family,
428 order, and class levels (Fig. S2; Tables 1, S1-S5). Results from previous studies that demonstrate
429 a positive relationship between host diversity and pathogen diversity indicate that higher plant
430 species diversity in serpentine grasslands might contribute to higher fungal diversity if native
431 forbs and bunchgrasses that specialize on serpentine soils share foliar fungal pathogens with *S.*
432 *pulchra* (Hechinger and Lafferty 2005; Spear 2017). Research on five common invasive and
433 native grasses (including *S. pulchra*) at JRBP shows that multi-host foliar fungal pathogens
434 dominate this system, supporting this hypothesis (Spear and Mordecai 2018).

435 Differing heavy metal content in the leaves of nonserpentine and serpentine *S. pulchra*
436 may influence fungal community assembly through a variety of mechanisms. Soil Ca content is
437 known to influence the outcomes of plant-pathogen interactions through its roles in cellular and
438 biochemical pathways in both the plant immune system (Levine *et al.* 1996; Romeis *et al.* 2001)
439 and in fungal pathogen virulence, growth, and reproduction (e.g., Shaw and Hoch 2000; Uhm *et*
440 *al.* 2003; Brandhorst *et al.* 2005). Fungal pathogen interspecific variation in reliance on Ca for
441 successful infection and/or fungal response to Ca-dependent plant immune defenses could
442 therefore explain differences in nonserpentine and serpentine fungal community composition.

443 Previous studies showed that *Hesperolinon* sp. infection frequency and severity by the fungal
444 rust *Melampsora lini* in California grasslands depended on plant tissue Ca levels linked to
445 serpentine soil chemistry (Springer *et al.* 2006; Springer 2009). Varying tolerances of heavy
446 metals other than Ca among fungi may act as additional filters on the species present in
447 nonserpentine and serpentine communities (Iram *et al.* 2009).

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

462 Species turnover between years contributed substantially to fungal diversity in this
463 system (Fig. 3; Tables 1, S5). Interannual variation in these communities may be due to highly
464 variable yearly precipitation (2015 rainfall = 491 mm; 2016 = 604 mm, 2017 = 985 mm)
465 (Weather Underground). Timing and amount of precipitation might alter infection dynamics by
466 washing away fungal spores, transmitting spores to new hosts, and/or influencing plant
467 community composition (Hobbs and Mooney 1991; Madden 1997). Although few studies track
468 interannual variation in fungal communities on plants, the year-to-year dissimilarity of those
469 hosted by *S. pulchra* is similar to that observed across a diverse taxonomic range of plants
470 including ferns, legumes, and shrubs (Olmo-Ruiz and Arnold 2014; Cotton *et al.* 2015; Zhang *et*
471 *al.* 2016). In this context, our results add to a growing body of evidence for the general
472 importance of storage effects—in which temporally variable environmental conditions act as
473 ecological niches that facilitate species diversity—in plant-associated fungal communities

474 (Warner and Chesson 1985). This finding highlights the necessity of long-term data collection to
475 accurately characterize plant-fungal interaction networks.

476 Contrary to our hypothesis, differences in soil chemistry that extended to plant chemistry
477 did not affect disease burden (Figs. 1, 4; Table S9). Elevated heavy metal content in serpentine-
478 grown plants was far below "hyperaccumulation" levels that have been shown to inhibit
479 pathogenic infection in plants (e.g., 1.92 x 10^{-2} mol Cr/kg required for hyperaccumulation versus
480 3.30 x 10^{-4} mol Cr/kg observed in serpentine *S. pulchra*) (Jaffre *et al.* 1976; Brooks *et al.* 1977;
481 Boyd *et al.* 1994) (Table S9), but the consistently low disease burden observed in both soil types,
482 combined with evidence from recent work that symptoms in the amounts observed inflict only
483 minimal plant fitness costs, suggests that *S. pulchra* is able to effectively resist or suppress
484 damage by foliar pathogens such that heightened defenses via opportunistic hyperaccumulation
485 would provide little to no fitness benefit (Spear and Mordecai 2018; Uricchio *et al.* 2019). The
486 consistent presence of low amounts of foliar disease on *S. pulchra* implies a coevolutionary
487 relationship between this bunchgrass and its foliar fungi that has led to species interactions
488 nearer the commensal than the parasitic end of the symbiotic spectrum.

489 The variation in fungal community composition we observed on a single host species
490 with soil chemistry and over time suggests possible mechanisms preventing foliar disease from
491 regulating plant host populations in California grasslands by impacting host fitness. Transitions
492 between distinct soil types over spatial scales within the range of fungal spore dispersal may
493 prevent disease outbreaks by hampering the spread of pathogens that are more or less virulent
494 depending on leaf tissue chemistry. Variable climatic conditions favoring successful infection by
495 different fungal species each growing season (Table S5), combined with plant senescence during
496 the dry season each year, suggest temporal barriers to pathogens evolving high virulence on both
497 nonserpentine and serpentine plants.

498 The processes that limit disease burden and impact in *S. pulchra* suggest that climate and
499 growth form may explain differences results from studies in tropical rainforest systems that
500 report large fungal pathogen burdens on host populations (e.g., Augspurger and Kelly 1984;
501 Spear *et al.* 2015). For example, seasonal senescence and local environmental barriers to
502 transmission in semi-arid and Mediterranean systems may limit the severity of individual disease
503 outbreaks, in contrast to year-round suitable climates and potentially more homogenous biotic

504 and abiotic environments in tropical systems. Life histories that promote the accumulation of
505 large disease burdens, along with relatively high and constant humidity, may be important
506 determinants of the potential for fungal pathogens to regulate plant host populations. This
507 observation implies potential climate- and life history-based limits on mechanisms through
508 which disease is hypothesized to structure plant communities, including enemy escape effects
509 (Keane and Crawley 2002), density- or distance-dependent Janzen-Connell effects (Janzen 1970;
510 Connell 1971; Mordecai 2011), and epidemic-driven community shifts (Day and Monk 1974).

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

518 *Conclusions*

519 Fungi associated with foliar disease in the native bunchgrass *S. pulchra* are relatively
520 benign, likely cause minimal fitness impacts (Spear and Mordecai 2018), and contribute
521 substantially to the overall biodiversity of California grasslands. The high taxonomic diversity
522 and rarity of serpentine fungi demonstrates that this community is potentially at risk of species
523 loss via host-parasite coextinction as nonnative annual grasses continue to expand their ranges at
524 the cost of native plants, and human activities including development and increased C and N
525 deposition contribute to the loss of serpentine grasslands that already make up <2% of the
526 California's surface area (Huenneke *et al.* 1990; Vallano *et al.* 2012). This work demonstrates
527 that, in addition to >200 endemic plant species, California's serpentine grasslands support unique
528 communities of leaf-infecting fungi, providing additional motivation for active conservation of
529 native grassland communities based on the far-reaching loss of biodiversity across trophic levels
530 associated with their disappearance (Sprent 1992; Dunn *et al.* 2009).

531

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Figures

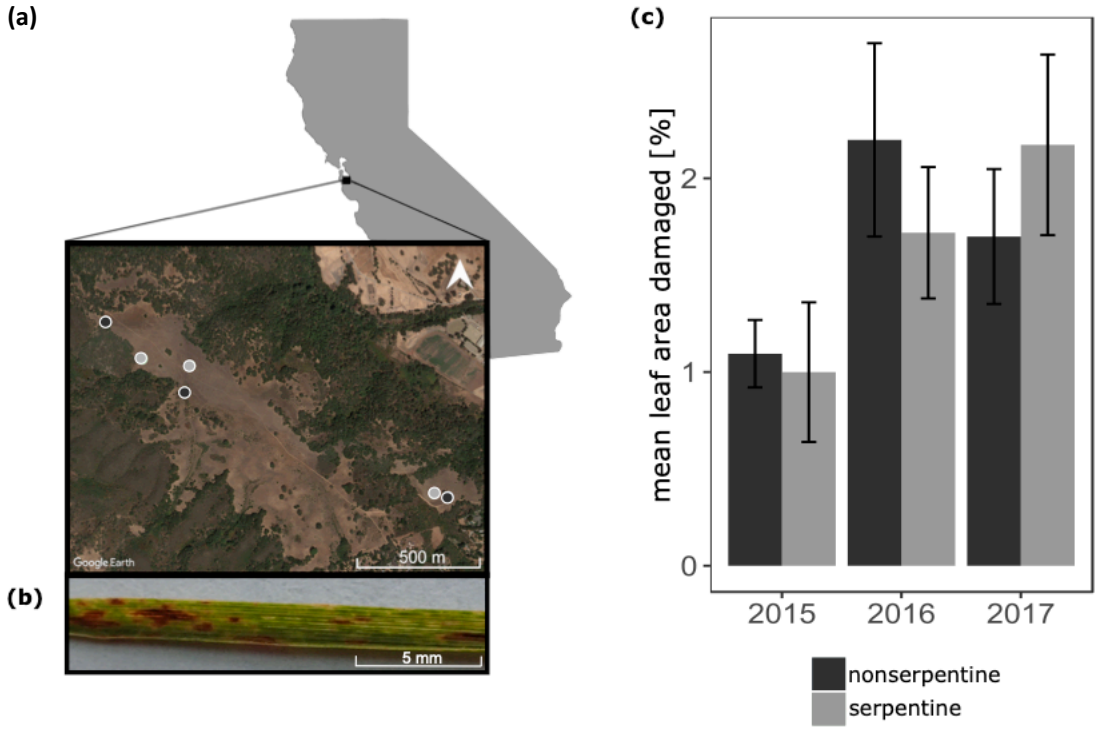

Figure 1. Pathogen damage on *Stipa pulchra* was consistently low across three years and both grassland types. Panel **(a)** maps the locations of surveyed nonserpentine sites (blue) and serpentine sites (green) along the ridgetop at JRBP. Panel **(b)** shows an example of fungal-caused pathogen foliar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

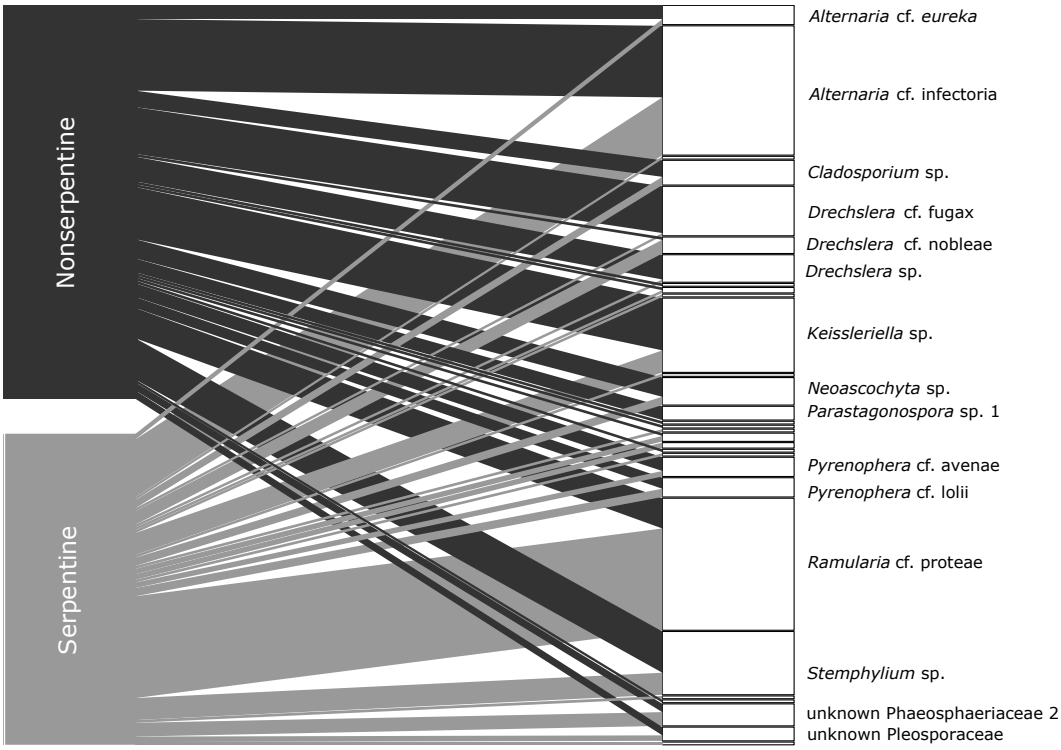

Figure 2. Pathogens isolated from *Stipa pulchra* growing in nonserpentine and serpentine grasslands. Bipartite network showing species interactions between *S. pulchra* plants (left) growing in serpentine grasslands (green) and nonserpentine grasslands (blue) and fungal species cultured from symptomatic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

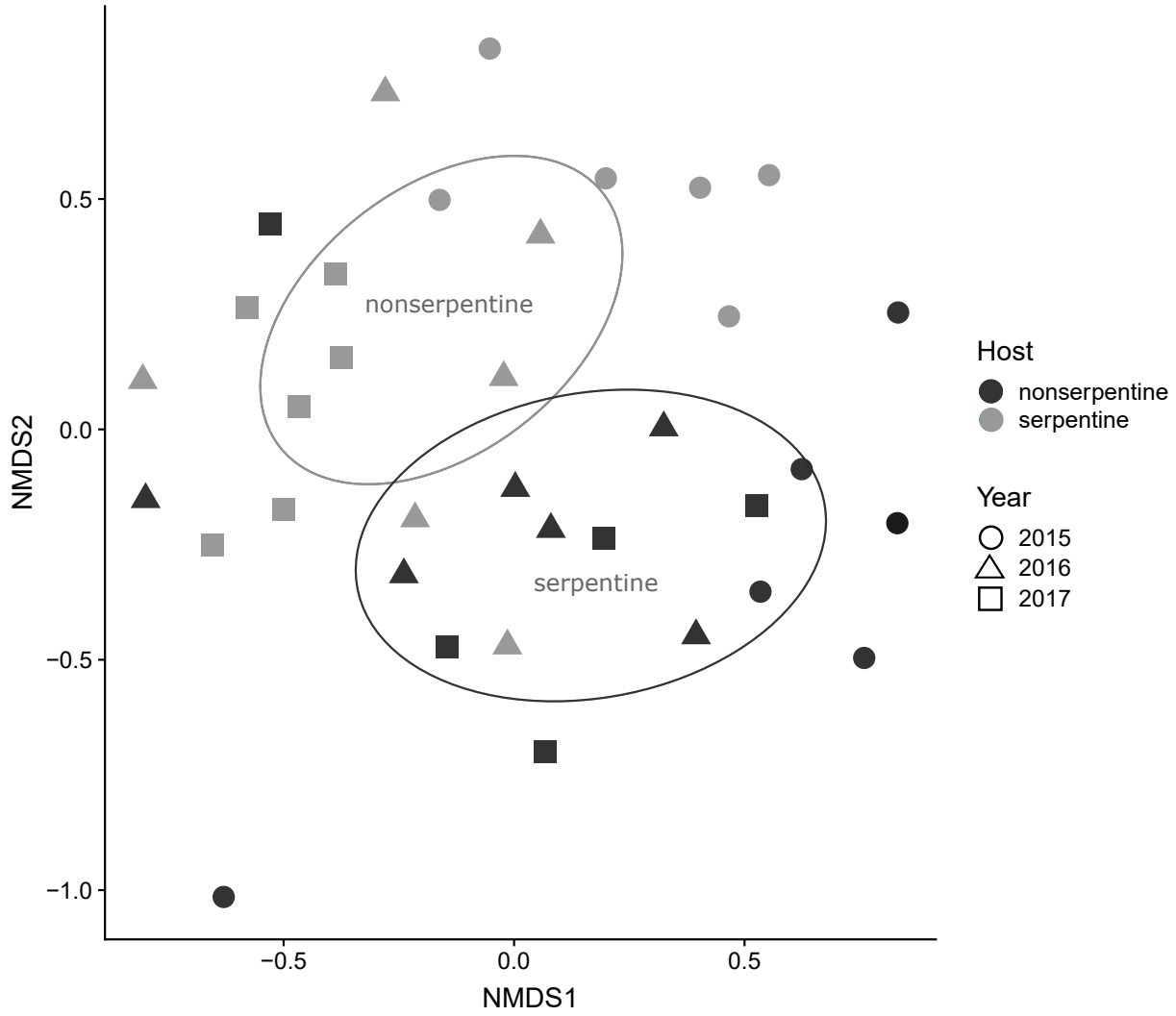

Figure 3. Distinct fungal communities isolated from *Stipa pulchra* growing in nearby serpentine and nonserpentine sites across years. Non-metric multidimensional scaling visualization of serpentine and nonserpentine fungal community similarity. Each point corresponds to the combined fungal community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

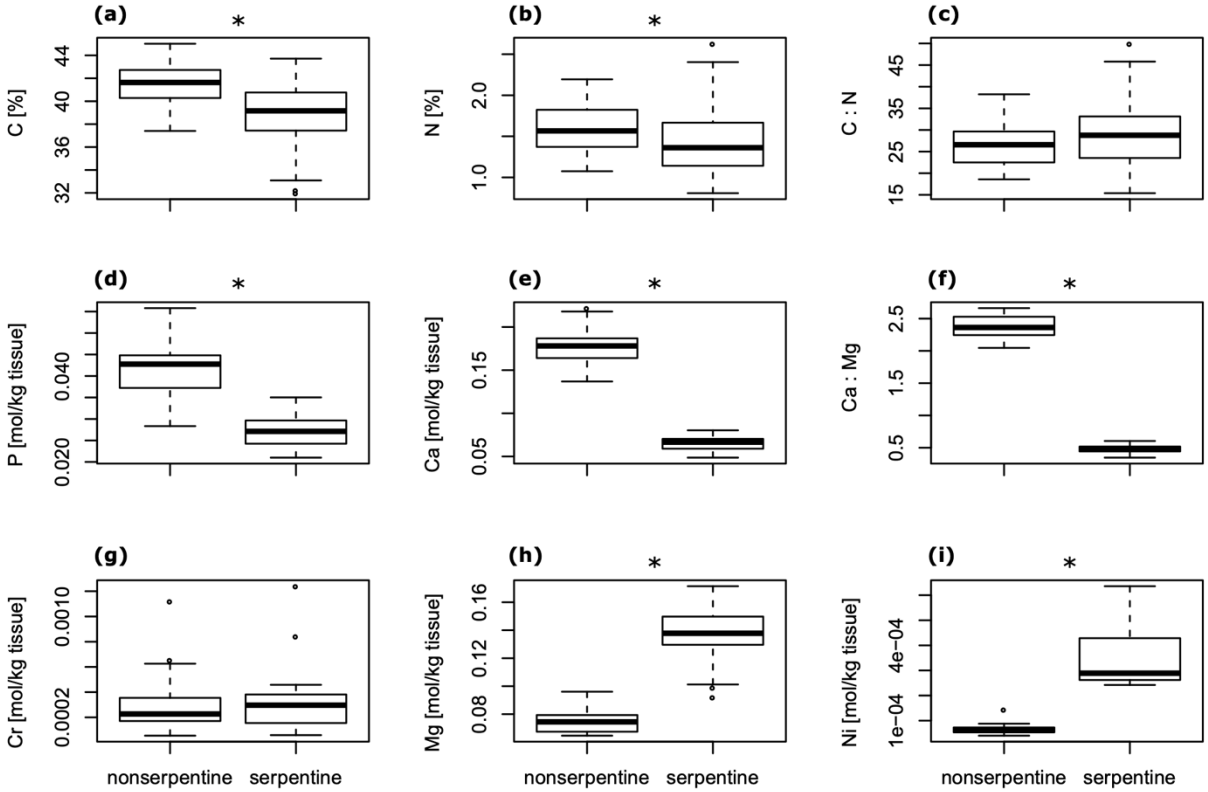

Figure 4. *Stipa pulchra* grown in nonserpentine soil had higher C, N, P, Ca, and Ca:Mg and lower Mg and Ni than those grown in serpentine soil. Box plots comparing elemental content of dried foliar tissue from nonserpentine- and serpentine-grown *S. pulchra*. In each plot, the thick black bar represents the median, the height of the box represents the interquartile range (IQR), the whiskers represent the maximum and minimum points within 1.5*IQR of the 25th and 75th percentiles, and the circles represent points outside this range. An asterisk above a panel indicates a statistically significant difference between serpentine- and nonserpentine-grown plants ($p < 0.05$) indicated by a Wilcoxon ranked-sum test. Panel **(a)** shows percent C of dried foliar tissue (W = 2713, p-value = 1.65×10^{-7}); panel **(b)**, percent N (W = 2346, p-value = 0.0011); panel **(c)**, C:N ratio (W = 1398, p-value = 0.066); panel **(d)**, moles P per kilogram dried leaf tissue (W = 390, p-value = 2.83×10^{-9}); panel **(e)**, moles Ca per kilogram dried leaf tissue (W = 400, p-value = 1.45×10^{-11}); panel **(f)**, Ca:Mg ratio (W = 400, p-value = 1.451×10^{-11}); panel **(g)**, moles Cr per kilogram dried leaf tissue (W = 192.5, p-value = 0.8498); panel **(h)**, moles Mg per kilogram dried leaf tissue (W = 1, p-value = 2.90×10^{-11}); and panel **(i)**, moles Ni per kilogram dried leaf tissue (W = 0, p-value = 6.79×10^{-8}).

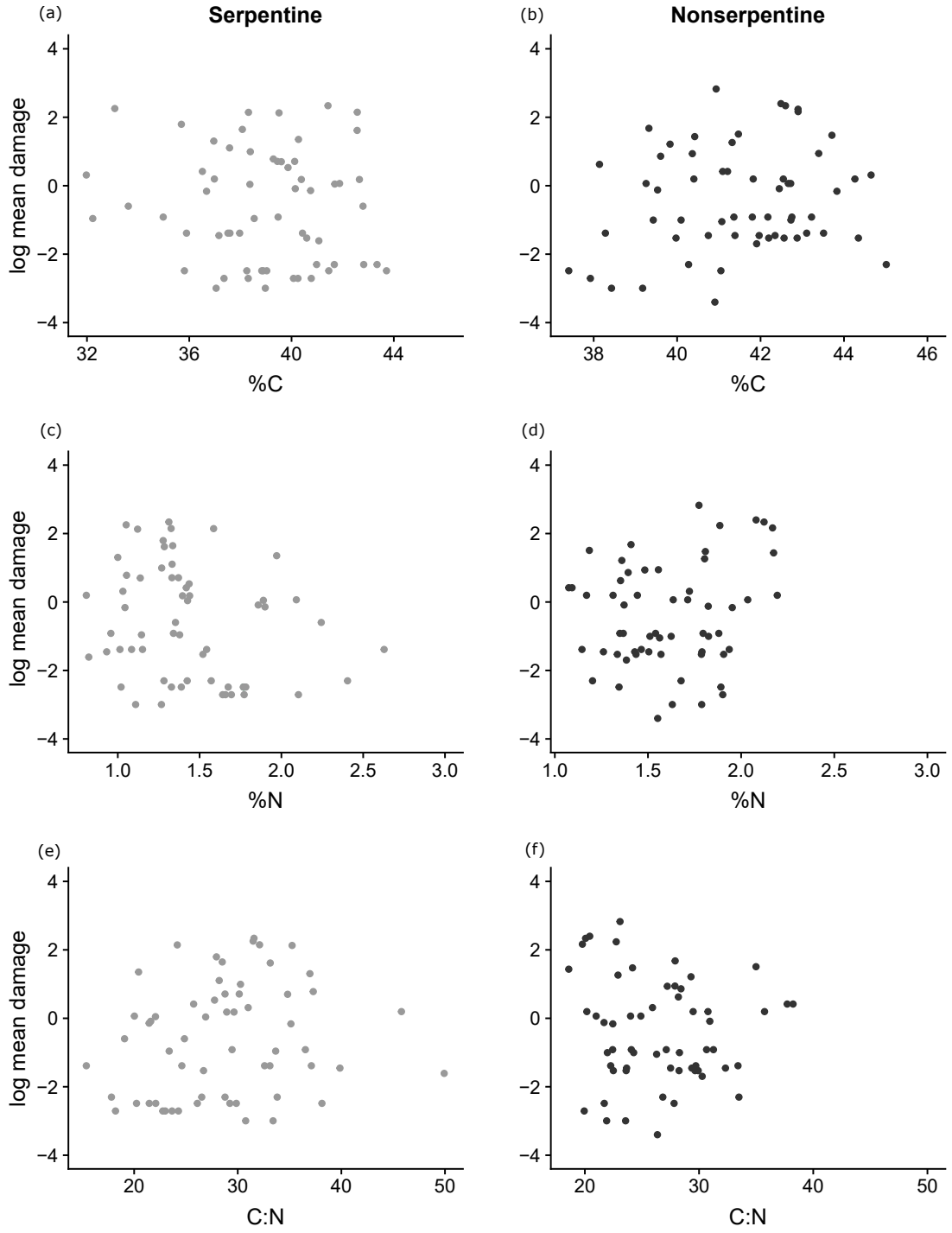

Figure 5. C, N, and C:N do not predict the mean foliar pathogen damage on *Stipa pulchra* in serpentine or nonserpentine grasslands. Foliar percent C (Panels **(a)** and **(b)**), percent N (Panels **(c)** and **(d)**) and C:N ratio (Panels **(e)** and **(f)**) versus mean percentage of diseased leaf area for serpentine and nonserpentine *S. pulchra* plants.

881 **Tables**

882 **Table 1. Pathogen damage on *Stipa pulchra* varied significantly by year, but not soil type.**

Variables	df	SS	MSE	F value	p-value
soil type	1	6.57×10^{-1}	6.57×10^{-1}	7.50×10^{-1}	0.39
year	2	9.41	4.71	5.37	7.04×10^{-3}*
site	4	6.00	1.50	1.71	1.59×10^{-1}
soil type x year	2	1.80	9.02×10^{-1}	1.03	3.63×10^{-1}

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

Table 2. Fungal species isolated from symptomatic *Stipa pulchra* leaves were unevenly distributed across soil types. Each row represents one of the species isolated. The columns 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

898 **Table 3. Fungal community diversity was higher and communities were distinct on *Stipa***
899 ***pulchra* plants growing in serpentine 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%)	

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901 Fungal community similarity by soil type and year. The leftmost column lists the fungal
902 communities considered, with the number of species in each community in parentheses. The
903 second column lists Fisher's alpha for each community. The third column shows the number of
904 shared species and, in parentheses, the percentage of total observed species that were shared for
905 each pair of communities. The rightmost column lists the estimated Morisita-Horn community
906 overlap value, based on absolute species abundances, for each pair of communities. The
907 Morisita-Horn index ranges from 0 to 1, with 1 indicating complete overlap.

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

	df	SS	MSE	F-Value	p-value
soil 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
soil type x year	2	0.3339	0.16697	1.4975	0.224
distance x year	2	0.1697	0.08485	0.6473	0.734

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911 From left to right, columns list factors tested, degrees of freedom (df), sum of squares (SS), mean
912 squared error (MSE), F-value, and p-value. Statistically significant p-values ($p < 0.05$) are
913 marked with an asterisk. The distance factor compares communities at near (<600m) vs. far
914 (>600m) distances from one another.

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927 **Supporting Information**

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

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

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

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

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

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

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

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

944 **Table S7** ANOVA table for effects of soil type, year, and soil type x year interactions on site
945 fungal species richness.

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

947 **Table S9** Results of chemical analysis of serpentine and nonserpentine *Stipa pulchra* foliar
948 tissue.

949 **Methods S1** Details regarding *Stipa pulchra* plants used for ICP-OES analysis of Ca, Cr, Mg, Ni,
950 and P.

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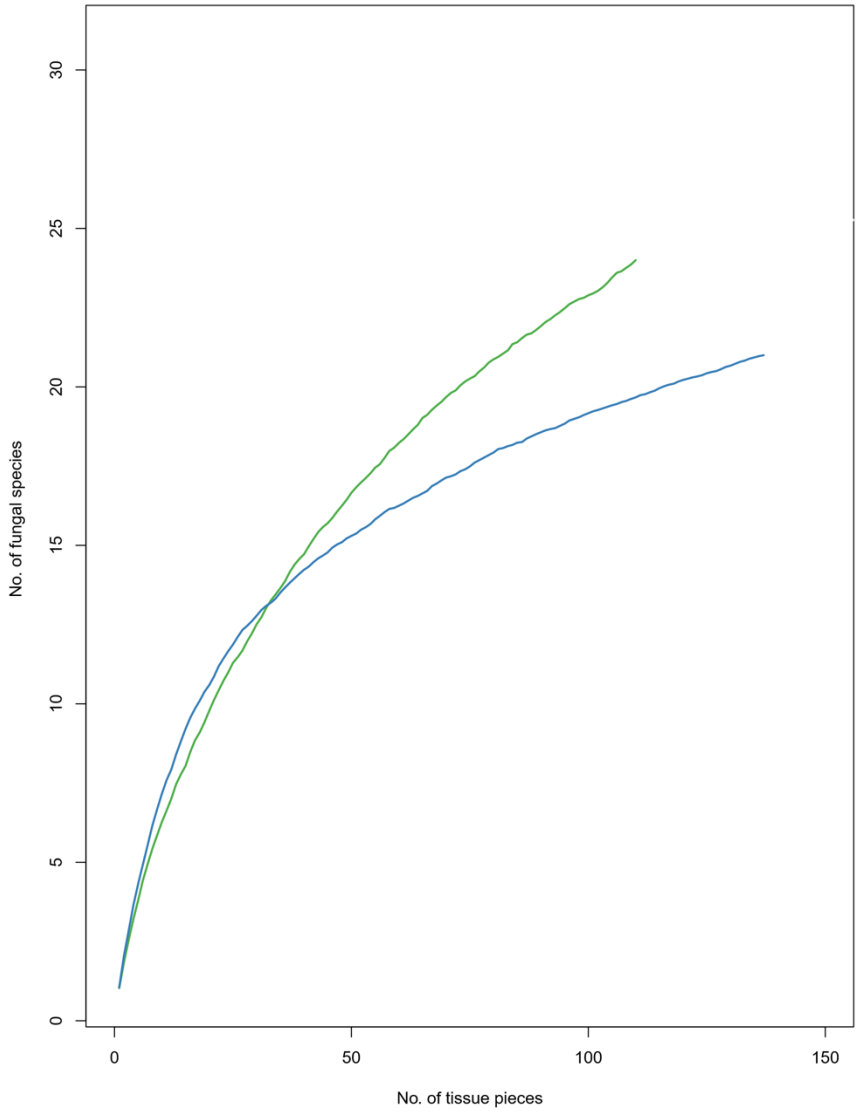
953 **Supporting Figures and Tables**



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

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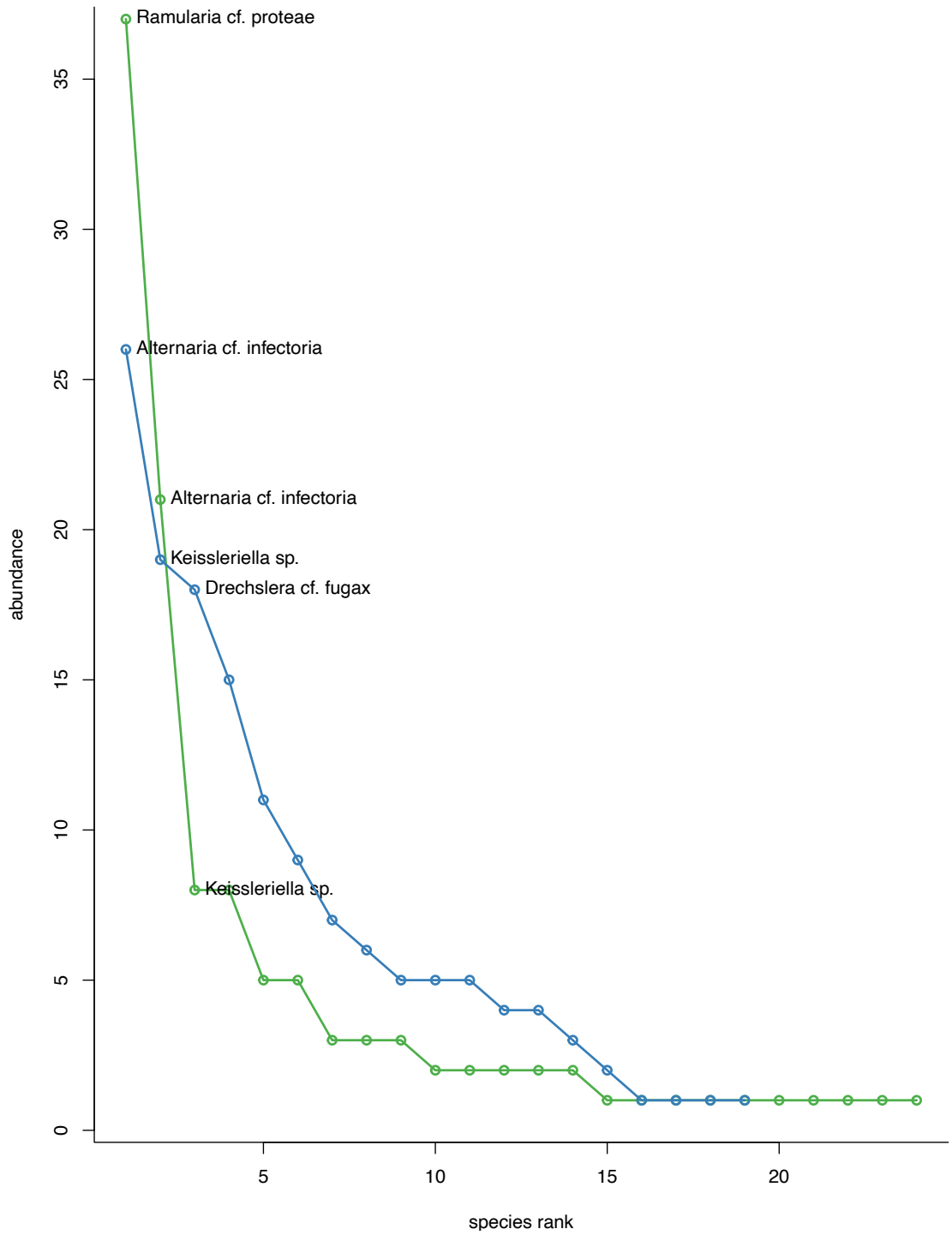

Figure S2. Fungal species abundance by rank for serpentine (green) and nonserpentine (blue) fungal communities on *Stipa pulchra* leaves. Each point represents the number of times a single species was isolated from *S. pulchra* leaves in each soil type.

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

Genus	Nonserpentine	Serpentine	Hosts	References
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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970 Numbers in each column represent the number of times a genus was isolated from *S. pulchra*
971 leaves in each soil type.

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973 **Table S2.** Family counts for nonserpentine and serpentine fungal communities isolated from
974 *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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976 Numbers in each column represent the number of times a family was isolated from *S. pulchra*
977 leaves in each soil 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 soil type.

1009 **Table S4.** Class counts for nonserpentine and serpentine fungal communities isolated from *Stipa*
1010 *pulchra* leaves.

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

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1012 Numbers in each column represent the number of times a class was isolated from *S. pulchra*
1013 leaves in each soil type.

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1030 **Table S5.** Presence (P, shaded) or absence (A, unshaded) of genera in nonserpentine and
1031 serpentine fungal communities isolated from *Stipa pulchra* leaves, by year.

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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 soil type, year, and soil type x year interactions on site-level fungal community diversity in terms of Fisher's alpha.

	df	SS	MSE	F value	p-value
Soil type	1	0.098369	0.098369	0.009695	0.923189
Year	2	3.629916	1.814958	0.178881	0.838395
Soil type x Year	2	17.34903	8.674514	0.854953	0.449656

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 soil type, year, and soil type x year interactions on site fungal species richness.

	df	SS	MS	F value	p-value
Soil type	1	6.722222	6.722222	1.070796	0.32117
Year	2	3.111111	1.555556	0.247788	0.784422
Soil type x year	2	8.444444	4.222222	0.672566	0.528628

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.

1074 **Table S8.** Pairwise PERMANOVA table for effects of year on fungal community composition.
1075 From left to right, columns list the pair of years being compared, the F-value, R^2, and the
1076 Bonferroni-adjusted p-value. P-values < 0.05 are marked with an asterisk.

Pair	F-value	R^2	Bonferroni p-value
2015 vs 2016	2.616237	0.207371	0.114
2015 vs 2017	5.983453	0.374353	0.009*
2016 vs 2017	4.396858	0.304303	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 mean mol/kg dried weight leaf tissue derived from each soil type, except for C and N, which are reported as percentage of leaf tissue by 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}	*
Ca	6.50 x 10^{-2}	*(1.89 x 10^{-3})*	1.8 x 10^{-1}	*(4.78 x 10^{-3})*	400	1.45 x 10^{-11}	*
Cr	3.30 x 10^{-4}	*(6.16 x 10^{-5})*	3.10 x 10^{-4}	*(5.73 x 10^{-5})*	192.5	8.50 x 10^{-1}	
Mg	1.40 x 10^{-1}	*(5.10 x 10^{-3})*	7.5 x 10^{-2}	*(1.84 x 10^{-3})*	1	2.90 x 10^{-11}	*
N	1.44	*(4.96 x 10^{-2})*	1.61	*(3.84 x 10^{-2})*	2346	1.10 x 10^{-3}	*
Ni	3.50 x 10^{-4}	*(2.83 x 10^{-5})*	6.57 x10^{-5}	*(4.89 x 10^{-6})*	0	6.79 x 10^{-08}	*
P	2.70 x 10^{-2}	*(8.43 x 10^{-4})*	4.2 x 10^{-2}	*(1.50 x 10^{-3})*	389	2.83 x 10^{-09}	*
Ca:Mg	4.70 x 10^{-1}	*(6.55 x 10^{-2})*	2.37	*(7.20 x 10^{-3})*	0	1.45 x 10^{-11}	*
C:N	28.72	*(8.74 x 10^{-1})*	26.54	*(6.18 x 10^{-1})*	1398	6.62 x 10^{-2}	

1113 **Methods S1**

1114 *Details regarding Stipa pulchra plants used for ICP-OES analysis of Ca, Cr, Mg, Ni, and P*

1115 We sourced foliar tissue for ICP-OES analysis of Ca, Cr, Mg, Ni, and P from *S. pulchra*
1116 plants initially grown in individual pots in greenhouse conditions and then set out at Jasper Ridge
1117 Biological Preserve (JRBP) for a separate experiment designed to assess fungal infection over
1118 geographic space in a natural setting. Potted plants were left in the field for 12 weeks (3/9/2017 –
1119 5/26/2017) and then returned to the greenhouse, surveyed for symptoms of foliar disease,
1120 separated into above- and below-ground portions, and dried. Only 22 of 480 nonserpentine-
1121 grown plants and 33 of 480 serpentine-grown plants had symptomatic leaf tissue; these plants
1122 were excluded from the pool of samples for chemical analysis.

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