Foliar pathogens are unlikely to stabilize coexistence of competing species in a California grassland

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Abstract

Pathogen infection is common in wild plants and animals, and may regulate their populations. If pathogens have narrow host ranges and increase with the density of their favored hosts, they may promote host species diversity by suppressing common species to the benefit of rare species. Yet, because many pathogens infect multiple co-occurring hosts, they may not strongly respond to the relative abundance of a single host species. Are natural communities dominated by specialized pathogens that respond to the relative abundance of a specific host or by pathogens with broad host ranges and limited responses to the relative abundance of single host? The answer determines the potential for pathogens to promote host coexistence, as often hypothesized, or to have negligible or even negative effects on host coexistence. We lack a systematic understanding of the impacts, identities, and host ranges of pathogens in natural communities. Here we characterize a community of foliar fungal pathogens and evaluate their host specificity and fitness impacts in a California grassland community of native and exotic species. We found that most of the commonly isolated fungal pathogens were multi-host, with intermediate to low specialization. The amount of pathogen damage each host experienced was independent of host species local relative abundance. Despite pathogen sharing among the host species, fungal communities slightly differed in composition across host species. Plants with high pathogen

damage tended to have lower seed production but the relationship was weak, suggesting limited fitness impacts. Moreover, seed production was not dependent on the local relative abundance of each plant species, suggesting that stabilizing coexistence mechanisms may operate at larger spatial scales in this community. Because foliar pathogens in this grassland community are multi-host and have small fitness impacts, they are unlikely to promote negative frequency-dependence or plant species coexistence in this system. Still, given that pathogen community composition differentiates across host species, some more subtle feedbacks between host relative abundance and pathogen community composition, damage, and fitness impacts are possible, which could in turn promote either coexistence or competitive exclusion.

33 **Introduction**

34 Pathogens are ubiquitous in ecological communities (Burdon 1993, Gilbert 2002,
35 Lafferty et al. 2008). Because they affect host demographic rates, pathogens are often expected
36 to regulate host species population growth (Burdon 1982, Burdon and Chilvers 1982). Most
37 pathogens infect only a subset of the available host species, so their incidence, and by extension
38 their impacts, may be host-specific (Gilbert and Webb 2007, Beckstead et al. 2014, Parker et al.
39 2015). Host-specific population regulation can promote species coexistence by suppressing
40 species when they become common and providing a relative advantage to rare species (Fig. 1).
41 This pathogen-mediated negative frequency-dependence, sometimes called the Janzen-Connell
42 hypothesis (Janzen 1970, Connell 1971), has growing support in diverse plant communities,
43 including tropical forests (Augspurger 1983, Augspurger and Kelly 1984, Gilbert 2005, Bagchi
44 et al. 2010, Bever et al. 2015), temperate forests (Packer and Clay 2000), and temperate
45 grasslands (Petermann et al. 2008).

46 At the same time, fungal pathogens of plants in natural systems often infect multiple
47 hosts (Gilbert and Webb 2007, Kluger et al. 2008, Hersh et al. 2012, Spear 2017). In diverse
48 plant communities, where the nearest neighbors may be heterospecifics, selection should favor
49 multi-host pathogens (May 1991). Thus, we hypothesize that most plant pathogens are relatively
50 host generalized (Spear et al. 2015, Spear 2017) and, by extension, that their attack rates and
51 impacts do not respond to the relative abundance of a single host species. If this is the case, then,
52 in contrast to the Janzen-Connell hypothesis, many pathogens may play little role in maintaining
53 local host diversity and may even promote competitive exclusion or spatial turnover of species
54 (Mordecai 2011, Spear et al. 2015). Few studies have assessed whether these alternatives to
55 pathogen-mediated stabilization occur in nature (but see Mordecai 2013).

For pathogens to generate negative frequency-dependence and stabilize coexistence, they must: (1) disproportionately damage relatively common hosts, such that the amount or severity of damage depends on host relative abundance; and (2) reduce host population growth (Fig. 1). Condition (1) may occur either because (A) individual pathogen species are relatively specialized or exhibit host preference, (B) communities of multi-host pathogen species are differentially structured by plant species, or (C) multi-host pathogens exert host-specific impacts. In this paper, we measure frequency-dependent damage, host specificity, and impacts on population growth for foliar pathogens that infect co-occurring native and exotic grasses in a California grassland. Specifically, across six common grass species we: (i) quantified pathogen damage and linked it to plant species relative abundance; (ii) surveyed fungal community composition and pathogen sharing across host species; and (iii) measured the response of per-capita seed output to pathogen damage and plant relative abundance.

Methods

Study site & focal grass species

We conducted the study in grasslands in Jasper Ridge Biological Preserve (JRBP) at Stanford University, a 485-ha site in San Mateo County, CA (37°24'N, 122°13'30"W; 66 - 207 m), in 2015. JRBP has a Mediterranean climate, with cool (mean 9.2°C), wet winters and warm (mean 20.1°C), dry summers (total annual precipitation ~ 622.5 mm) (Ackerly et al. 2002). Plant growth begins with the onset of winter rains and plants senesce at the onset of summer.

We assessed the identities and impacts of foliar fungal pathogens on six common grass species: three exotic annuals, *Avena barbata, Bromus hordeaceus,* and *Bromus diandrus*; one exotic perennial, *Phalaris aquatica*; and two native perennials, *Stipa pulchra* and *Elymus*

79 *glaucus.* To obtain a broader description of fungal diversity and host associations, we also
80 isolated fungi from, but did not assess damage on, the common exotic grass species *A. fatua,*
81 *Brachypodium distachyon,* and *Festuca perennis.* The exotic grasses were introduced to
82 California in the mid-19th century (Corbin and D'Antonio 2004).
83 We assessed the relationships between pathogen damage and host relative abundance
84 (Condition 1), plant seed production and pathogen damage (Condition 2), and pathogen
85 specialization and community composition (Conditions 1A-C).
86
87 *Impacts of plant species relative abundance on pathogen damage (Condition 1)*
88 To measure pathogen burden across host species relative abundance (Condition 1), we
89 visually measured the percentage of leaf area damaged by fungal pathogens (hereafter, pathogen
90 damage) for the six focal grass species across 10 transects (yellow points in Fig. S1) that were
91 established in areas where perennial species (either *S. pulchra*, *E. glaucus*, or *P. aquatica*) range
92 from rare to common in a given 1-m^2 plot; hereafter referred to as 'perennial density transects'.
93 As possible, we sampled multiple plants per transect and up to six haphazardly-selected leaves
94 per plant, calculating average pathogen damage on each plant. To measure variation in pathogen
95 damage within the growing season, we censused damage in these plots from March 11-16, 2015
96 (444 marked grass individuals) and from April 17-20, 2015 (163 of the marked grasses).
97 We tested whether pathogen damage correlated with host relative abundance in the plot
98 (Condition 1) while controlling for other potential predictors: plant species, sampling month, and
99 sampling structure (plot nested in transect as random effects), using normally-distributed errors
100 (lmer function in the lme4 package; Bates et al. 2014). We assessed the most important
101 predictors of pathogen damage by comparing the Akaike Information Criterion (AIC) values of

102 models with all or a subset of the fixed effects of sampling month * species and species *
103 frequency. We also modeled pathogen damage as a function of the dominant species at the plot
104 scale as a categorical variable, but found no significant effect.
105
106 *Isolating foliar fungal pathogens (Conditions 1A-B)*
107 To estimate fungal pathogen community composition and host ranges (Conditions 1A-B)
108 at a preserve-wide scale, we cultured fungi from grasses along 24 transects that spanned a range
109 of plant community composition, geographic location, and soil types (10 perennial density
110 transects and 14 additional transects; Fig. S1 yellow and red points, respectively). Three of the
111 pathogen survey transects ran through plots in the Jasper Ridge Global Change Experiment
112 (GCE) (Zhu et al. 2016), where we sampled in ambient and water addition plots (water addition
113 had no impact on fungal community composition). From March 19 – May 4, 2015, we collected
114 and cultured from a symptomatic leaf from 772 grass individuals. We identified fungi (as
115 described below) from 61 of the 444 marked plants from the damage survey and 219 additional
116 plants. Sampling intensity varied among the nine grass species and transects based on availability
117 of each species; the percentage of tissue pieces with growth varied among the hosts (Tables S1-
118 S2). We excised tissue (< 2 mm^2) from the advancing disease margin, surface-sterilized it in 70%
119 EtOH followed by 10% household (Clorox) bleach (60 s each), then plated it on malt extract agar
120 with 2% chloramphenicol (2% MEA). We pressed six of the segments onto 2% MEA to verify
121 effectiveness of the surface sterilization; we observed no growth. We isolated morphologically
122 distinct hyphae into pure culture on 2% MEA within 30 days. The Mordecai lab maintains
123 reference strains (California Department of Food and Agriculture permit 3160).
124

125 *Identifying fungal species by DNA sequencing (Conditions 1A-B)*

126 For each isolate, we extracted genomic DNA from fungal mycelium using REDExtract-
127 N-Amp Tissue PCR Kit (Sigma-Aldrich, Inc.), following the manufacturer's protocol. We
128 amplified and sequenced the internal transcribed spacers (ITS) 1 and 2 and the 5.8S nuclear
129 ribosomal gene using the primer pairs ITS-1F and ITS-4 (Gardes and Bruns 1993). For PCR
130 amplification, we used a T100 thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA) and
131 thermal cycling conditions following U'Ren et al. (2010). Following electrophoresis on a 1.5%
132 agarose gel, we visualized PCR products using GelRed™ (Biotium Inc., Hayward, CA) and sent
133 to them MCLAB (San Francisco, CA) for cleanup and bidirectional sequencing on an ABI 3730
134 XL sequencer.

135 We estimated the taxonomic relationships among the fungi in two ways. First, we
136 assigned operational taxonomic units (OTUs) by manually editing all reads, automatically
137 assembling bidirectional reads into consensus sequences using a minimum of 20% overlap and
138 85% sequence similarity, and clustering the 288 consensus sequences and two unidirectional
139 sequences based on a minimum of 40% overlap and 90, 95, 97 and 99% sequence similarity
140 using Sequencher (Gene Codes, Ann Arbor, MI). Second, because sequence similarity for the
141 ITS region varies across fungal species (O'Brien et al. 2005), we built phylogenetic trees for
142 groups of OTUs with similar sequences (1-52 isolates per dataset) (as described in Higginbotham
143 et al. 2014, Spear 2017). Because not all sequences mapped onto named fungal species, we
144 assigned each operational species a unique species code (Table S3). We treated isolates
145 belonging to a species complex as a single species. All sequence data will be submitted to
146 GenBank (accession numbers XXXX-XXXX).

147

148 *Analyses of fungal community composition and host associations (Conditions 1A-B)*

149 To describe the fungal community (*N* = 290 isolates), we calculated (i) sampling efficacy
150 using taxon accumulation curves, (ii) observed taxon richness, (iii) estimated lower bound of true
151 richness, accounting for unseen taxa and correcting for under-sampling in highly diverse
152 assemblages, with the iChao1 estimator (Chiu et al. 2014), (iv) rank-abundance distribution, and
153 (v) diversity of taxa with Fisher's alpha, which is robust to unequal sample sizes (Fisher et al.
154 1943, Magurran 2013) and the effective number of species (Jost 2006). We defined fungal
155 species isolated ten or more times as abundant. We then contrasted fungal community
156 composition across five grass species (Conditions 1A-B) using permutational multivariate
157 analyses of variance (PERMANOVAs; Anderson 2001), with the adonis and
158 pairwise.perm.manova functions (Oksanen et al. 2016, Hervé 2017). We visualized the
159 differences using non-metric multidimensional scaling (NMDS). For these analyses (*N* = 99
160 isolates), we: (i) considered each grass species-by-perennial density transect combination to be a
161 distinct community, excluding those communities with fewer than three isolates and *B.*
162 *hordeaceus*, which only had one community with the three or more isolates; and (ii) created a
163 matrix of pairwise community dissimilarities using the function vegdist with the Chao method,
164 which is abundance-based and adjusted to consider unseen species (Chao et al. 2005, Oksanen et
165 al. 2016). Finally, we made pairwise comparisons of the pathogen communities of the nine
166 sampled grass species based on the observed number of shared species and using the Morisita-
167 Horn index (*N* = 290 isolates, 200 bootstrap replicates).

168 To assess the specialization of the non-singleton fungal species (*N* = 228 isolates)
169 (Condition 1A), we calculated the weighted specialization index d', the degree to which a species
170 deviates from random host associations, adjusted for the preserve-wide relative abundance of the

171 hosts *A. barbata*, *B. diandrus*, *B. hordeaceus*, *S. pulchra*, and *E. glaucus* (see R code for data)
172 (Blüthgen et al. 2006, Dormann et al. 2016). We classified the d' values as low (0-0.33),
173 moderate (0.34-0.67), and high (0.68-1) specialization (Blüthgen et al. 2006, Dormann et al.
174 2016).

175 All pathogen community analyses were conducted in *R* version 3.4.2, using the packages
176 RVAideMemoire (Hervé 2017), vegan (Oksanen et al. 2016), SpadeR (Chao et al. 2016),
177 bipartite (Dormann et al. 2016), fossil (Vavrek 2011), BiodiversityR (Kindt and Coe 2005), rich
178 (Rossi 2011) and with custom commands (Gardener 2014).

179

180 *Pathogenicity tests (Conditions 1A-B)*

181 We verified the pathogenicity of the fungal isolates by experimentally inoculating 99
182 isolates, representing 35 fungal species, onto the host species from which they were originally
183 isolated (Table S4). In a greenhouse, we secured colonized or uncolonized (for paired controls)
184 2% ME agar plugs to healthy leaves using Parafilm (Sinclair and Dhingra 1995). We censused
185 leaves for symptoms within one week, and compared the proportion of diseased leaves for each
186 isolate to its paired control using bias-reduced generalized linear models (brglm function;
187 Kosmidis 2013), with binomial errors and probit link functions (Table S4).

188

189 *Impacts of pathogen damage on per-capita seed production (Condition 1C, 2)*

190 To assess the relationship between fecundity, community composition, and pathogen
191 burden, we harvested seeds from 350 of the 444 grass individuals from which we had surveyed
192 pathogen damage in the perennial density transects. We measured per-capita seed production,
193 density of all host species, and pathogen damage for all focal species except *P. aquatica*, which

occurs in monotypic stands with little variation in relative abundance. We standardized per-capita seed output and pathogen damage in either March or April by species using z-scores, and regressed pathogen damage on seed output (models with unstandardized variables produced similar results). We built separate models for pathogen damage in March versus April because they used the same individuals with a single seed output. Because variance in seed production differed across damage levels, we used quantile regression on the 25th, 50th, and 75th percentiles of seed production (rq function, Koenker 2017). Finally, we linked fungal genera to pathogen damage in March and/or April (N = 65 isolates) and seed output (N = 56 isolates) (Condition 1C; we did not have enough samples to assess damage and seed output by fungal species). More comprehensively assessing fitness impacts would require measuring demographic rates on experimentally infected plants.

All pathogen damage and seed production statistical analyses were performed in *R* version 3.2.3 (R Development Core Team 2014) using the packages plyr (Wickham 2011), reshape (Wickham 2007), plotrix (Lemon 2006), ggplot2 (Wickham 2009), quantreg (Koenker 2017), lmerTest (Kuznetsova et al. 2016), lqmm (Geraci 2016), lme4 (Bates et al. 2014), piecewiseSEM (Lefcheck 2016), and lsmeans (Lenth and Love 2017).

Results

Pathogen damage across host species and relative abundance (Condition 1)

Focal species relative abundance was not significantly related to pathogen damage, counter to Condition 1 (Figs. 1 and 2). Instead, the strongest predictors of pathogen damage were host species and sampling month (Tables S5-S6). *A. barbata* and *B. hordeaceus* had the highest

216 pathogen damage (Table S5). Pathogen damage was higher in April than in March (Fig. S2;
217 paired two-tailed *t*-tests: mean difference = 0.0265, $t = -4.1019$, $df = 162$, $p = 6.47 \times 10^{-5}$).
218
219 *Fungal pathogen identities and diversity*
220 We observed a diverse fungal community (Fig. S3). We cultivated 302 isolates (290
221 successfully sequenced) from 772 symptomatic leaves from nine plant species (Table S1).
222 Considering 90 - 99% sequence similarity, the isolates represented 27 – 48 operational
223 taxonomic units (OTUs), respectively (Fisher's alpha index for diversity: 7.28 - 16.39).
224 Hereafter, we designate all fungal species taxonomic affiliations based on the phylogenetic
225 analyses. The fungal isolates represented 41 fungal species (iChao1 estimated species richness:
226 285.78, 95% CI = 85.91, 1375.24; Fisher's alpha for diversity: 13.03, 95% CI = 8.91, 18.58;
227 effective number of species = 18.29, 95% CI = 15.45, 21.14). Most fungal species were rare
228 (56% were singletons or doubletons) and few were abundant (22% isolated >10 times) (Fig. S4).
229 *Pyrenophora*, *Ramularia*, *Alternaria*, and *Parastagonospora* were the most common genera
230 (Fig. 3 and Table S3). We experimentally confirmed the pathogenicity of 27% of the 99 isolates
231 tested, representing eight of the nine common fungal species (Fig 3; Tables S3-S4).
232
233 *Fungal pathogen host associations and community similarity (Conditions 1A-B)*
234 Most fungi were isolated from multiple hosts, counter to Condition 1A that fungal species
235 are host-specific: 74% of the 19 non-singleton fungal species infected multiple hosts, averaging
236 four host species (Fig. 3). Two fungi, *Pyrenophora lolii* (A2) and *Alternaria infectoria* species–
237 group (C1), were isolated from seven of the nine grass species (Fig. 3). Concordantly, most non-
238 singleton fungal species had low to moderate specificity (d' median = 0.346; Table S7). An

239 exception with high specificity (d' = 0.924) was *Pyrenophora tritici-repentis* (E), which was
240 isolated 20/21 times from *E. glaucus* and once from an *S. pulchra* individual in an *E. glaucus*-
241 dominated plot; it comprised 61% of the 33 fungal isolates from *E. glaucus* leaves (Fig. 3; Table
242 S7; Condition 1A). Two additional likely specialists were only isolated from the under-sampled
243 grass species *P. aquatica* (N = 18 isolates): *Pyrenophora* cf. *dactylidis* (L) and
244 *Parastagonospora caricis* (AM), comprising 39% of that host's isolates (Fig. 3; Condition 1A).
245 The eight best-sampled grasses species shared one to eight fungal species with other
246 grass species (median = 3; Table S8; Fig. 3). The average estimated pairwise similarity between
247 host species was moderate (42%; min = 5% for *E. glaucus – P. aquatica*; max = 100% for *F.*
248 *perennis – S. pulchra* and *A. barbata – A. fatua;* Table S8). By contrast, two grass species had
249 low estimated fungal community similarity with other species: *E. glaucus* (5% – 11%), and *P.*
250 *aquatica* (5% – 70%; Table S8; Fig. 3). Despite extensive pathogen sharing, the fungal pathogen
251 communities of five of the focal grass species were significantly different, supporting Condition
252 1B that communities of shared pathogens are structured by host species (N = 99 isolates,
253 PERMANOVA: $F_{4,15} = 3.682$, $R^2 = 0.495$, $p = 0.001$; Fig. 4).
254
255 *Relationship between pathogen damage and per-capita seed output (Condition 2)*
256 The relationships between pathogen damage and seed production were generally
257 negative but highly variable, providing only weak support for Condition 2 that pathogens impact
258 host fitness (Fig. 5a-b). Quantile regression showed that increasing pathogen damage in April
259 was associated with reduced seed output at the 50th percentile (i.e., for average-output
260 individuals), but had no significant relationship for the 25th or 75th percentiles (i.e., for high- and
261 low-output individuals) (Table S9; Fig. 5b). Contrary to expectations for negative frequency-

262 dependence at local scales, focal species frequency had no association with either seed output
263 (Fig. 5d) or pathogen damage (Fig. 2).
264 Particular fungal pathogen genera were not significantly associated with higher damage
265 in April or lower seed production (Condition 1C), based on z-scores across host species ($N = 65$
266 individuals: 13 *A. barbata*, 8 *B. diandrus*, 17 *E. glaucus*, 22 *S. pulchra*, and 5 *P. aquatica*; Fig.
267 S5). Small sample sizes precluded testing for host-specific impacts of all observed fungal
268 species. Anecdotally, a single *S. pulchra* infected with *Sordaria* sp. (a singleton) had high
269 pathogen damage and low seed production, consistent with a large pathogen impact.
270
271 **Discussion**
272 *Limited potential for pathogen-mediated coexistence*
273 Although pathogens are often hypothesized to promote plant species coexistence by
274 generating negative frequency dependence (Gillett 1962, Augspurger 1983, Packer and Clay
275 2000, Petermann et al. 2008, Allan et al. 2010, Mangan et al. 2010, Bagchi et al. 2014, Bever et
276 al. 2015, Whitaker et al. 2017), diverse natural communities should favor multi-host pathogens,
277 decoupling pathogen abundance from the density of individual host species (May 1991, Spear et
278 al. 2015). The degree of pathogen specialization and their role in promoting frequency
279 dependence remains unresolved (Mordecai 2011). In a California grassland, we found ubiquitous
280 pathogen damage: most plants and 57% of all surveyed leaves had pathogen damage. Yet the
281 observed pathogen damage had no relationship with host relative abundance (contrary to
282 Condition 1 for pathogens to cause negative frequency dependence; Fig. 1). Moreover, foliar
283 pathogen damage was only weakly associated with lower seed production (Fig. 5a-b), providing
284 limited support for pathogen impacts on plant fitness (Condition 2 in Fig. 1). While pathogens

285 could also affect the outcome of competition by altering fitness differences between species,
286 observational data (Fig. 5a-b) and preliminary experiments (Mordecai et al., unpublished) do not
287 support strong, differential fitness impacts of foliar pathogen infection in this system.

288 The lack of a relationship between pathogen damage and host relative abundance (Fig. 2)
289 is unsurprising given the extensive pathogen sharing among grass species (Table S8; Fig. 3; in
290 contrast to Condition 1A in Fig. 1). Many pathogens are specific to a genus or family (Gilbert
291 and Webb 2007, Barrett et al. 2009); thus, for the study grasses (all Poaceae), pathogen damage
292 should be decoupled from the relative abundance of a single host species. It is also possible that
293 pathogen damage responds to host relative abundance at the regional, rather than local, scale
294 (Mitchell et al. 2002).

295 Despite pathogen sharing (Table S8; Fig. 3), fungal community composition subtly
296 varied across grass species, partially supporting Condition 1B that pathogen communities differ
297 across host species (Figs. 1, 3-4). In particular, the recently invading perennial grass *P. aquatica*
298 ("Oakmead Herbarium: Arrivals, Weeds | Jasper Ridge Biological Preserve" 2018) had low
299 pathogen damage and a distinct pathogen community, including two fungi with strong host
300 affinities: *Pyrenophora cf. dactylidis* (L) and *Parastagonospora caricis* (AM). Moreover, several
301 common fungi were relatively specialized (partially supporting Condition 1A [Fig. 1] that
302 pathogens are host-specific), including *Pyrenophora tritici-repentis* (E) which was almost
303 exclusively isolated from the native perennial *E. glaucus*. With limited sample sizes for each
304 pathogen – host pair, we were unable to measure host-specific impacts of shared pathogens
305 (Condition 1C) conclusively. In sum, although the multi-host fungal pathogens that dominate this
306 California grassland community varied in their host affinities (Condition 1A), and pathogen

307 communities were partially structured by host species (Condition 1B), neither mechanism was
308 strong enough to generate frequency-dependent pathogen damage (Condition 1).
309 Our study focused on culturable fungi, which may be disproportionately host generalist,
310 and on foliar pathogens, which may exert smaller fitness impacts than seedling damping off
311 pathogens, root pathogens, and pathogens that castrate plants (Gilbert 2002). Those pathogens
312 that impact different life stages and/or tissue types may shape the outcome of competition
313 between plant species. A fuller assessment would require measuring fitness effects across life
314 stages in experimental infections and incorporating them into population growth models.
315 The broad host ranges and limited fitness impacts of pathogens in this system contrast
316 sharply with pathogen impacts on closely-related cultivated cereals such as barley, wheat, and
317 oats. The fungal pathogen species we encountered are congeners of important cereal pathogens,
318 including *Pyrenophora, Parastagonospora,* and *Ramularia* spp., which cause major yield losses
319 and often specialize on host genotypes (Havis et al. 2015, McDonald and Stukenbrock 2016).
320 Our results imply that naturally occurring host genetic and species diversity may mitigate the
321 spread of highly virulent pathogens in wild grassland systems (McDonald and Stukenbrock
322 2016). Foliar pathogen load has declined with increased plant community diversity in natural
323 grassland and old-field systems (Mitchell et al. 2002, Rottstock et al. 2014). Further, broad host
324 ranges and minimal host impacts are well suited to pathogen persistence and spread in seasonal,
325 mixed species grasslands like our California grassland site. Particularly in annual-dominated
326 stands, pathogens must recolonize and spread during the limited winter and spring growing
327 season each year, giving a selective advantage to multi-host pathogens.
328
329 *Conclusions*

330 In one of the first studies to directly measure pathogen-mediated frequency-dependence
331 and connect it to pathogen community composition, we showed that, contrary to prevailing
332 hypotheses in other plant systems, foliar fungal pathogens are unlikely to promote plant
333 coexistence in an invaded California grassland. Much of the evidence that pathogens maintain
334 plant community diversity is based on spatial and temporal patterns of conspecific negative
335 density-dependent mortality (e.g., Packer and Clay 2000, Klironomos 2002, Bell et al. 2006,
336 Petermann et al. 2008, Bagchi et al. 2010, Mangan et al. 2010, Comita et al. 2010), with limited
337 examination of fungal identity and host affinity (but see Parker and Gilbert 2007, Gilbert and
338 Webb 2007, Hersh et al. 2012, Schweizer et al. 2013, Spear 2017). By contrast, our work
339 suggests that pathogens have limited impacts on the outcome of competition when the burden is
340 frequency-independent and fitness costs are minimal, which may commonly occur in natural
341 systems.

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347

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510 **Figure Captions**

511 **Figure 1**. Pathogen-mediated frequency-dependence requires that: (1) pathogen damage increase
512 with host species relative abundance, via mechanisms A-C, and (2) pathogen damage reduces
513 fitness. The resulting decline in per-capita population growth rates with relative abundance can
514 stabilize plant species coexistence.

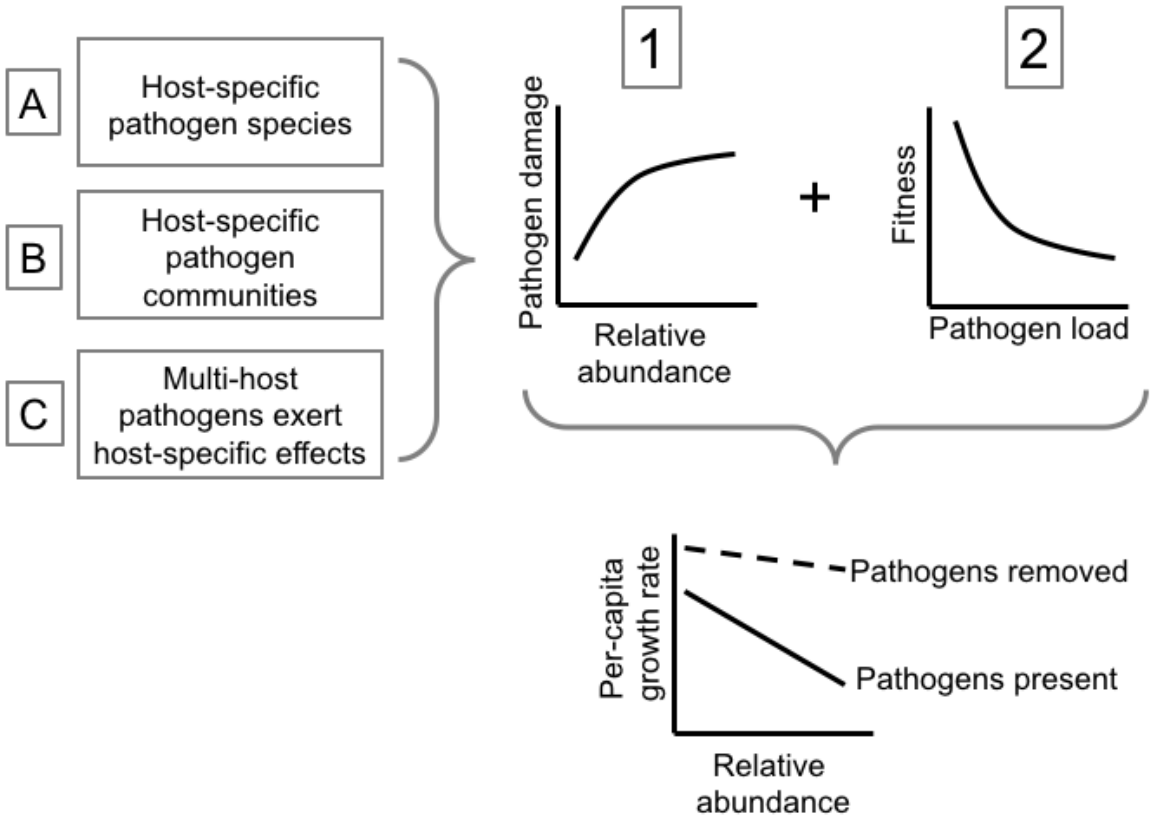
515 **Figure 2.** Relationship between focal species frequency in the plot and pathogen damage in
516 March, for six focal species. Marginal $R^2 < 0.02$ for all species.

517 **Figure 3.** Bipartite network of 41 fungal pathogen species (right; $N = 290$ isolates) isolated from
518 nine grass species (left), with widths proportional to isolation frequency. Fungal specificity (d') is
519 indicated for 17 common species (L = low, M = moderate, H = high). Experimentally confirmed
520 pathogenicity is indicated with an asterisk.

521 **Figure 4.** Dissimilarity of fungal pathogen community composition ($N = 99$ isolates, 21 fungal
522 species) among five grass species (stress = 0.101). Data points represent fungal community
523 composition across grass species-by-transect combinations, with 95% confidence ellipses around
524 the centroid of each grass species. The fungal community of: (i) *S. pulchra* (SP) was
525 significantly different from those of *A. barbata* (AB) ($p_{adj} = 0.037$), *B. diandrus* (BD) ($p_{adj} =$
526 0.037), *E. glaucus* (EG) ($p_{adj} = 0.04$), and *P. aquatica* (PA) ($p_{adj} = 0.037$); (ii) PA was
527 significantly different from that of BD ($p_{adj} = 0.05$); and (iii) BD was significantly different from
528 that of AB ($p_{adj} = 0.05$).

529 **Figure 5.** Pathogen damage in March versus seed production (a), pathogen damage in April
530 versus seed production (b), pathogen damage in March versus April (c), and species frequency
531 versus seed production (d). All values are expressed as z-scores calculated by species. Marginal
532 $R^2 < 0.03$ for all plotted relationships.

533 **Figure 1**



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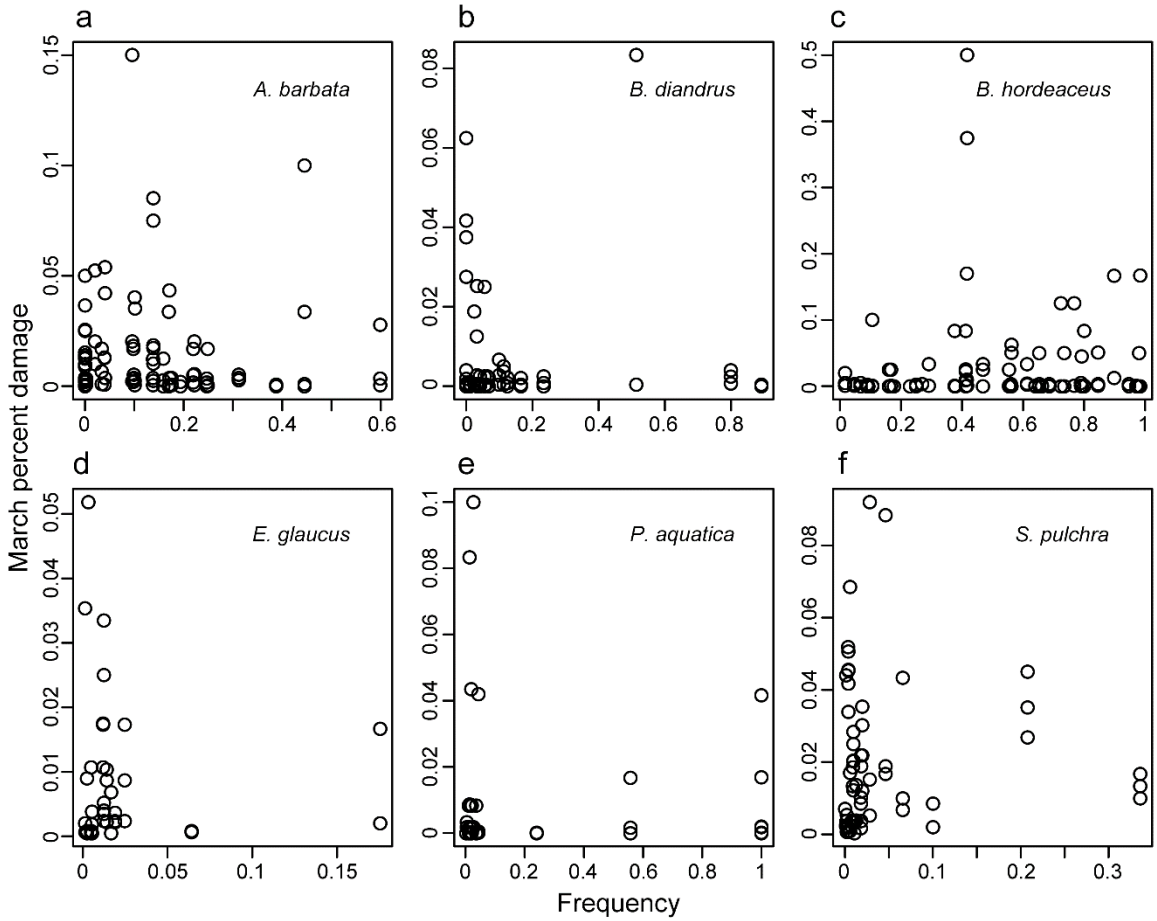
536 **Figure 2**



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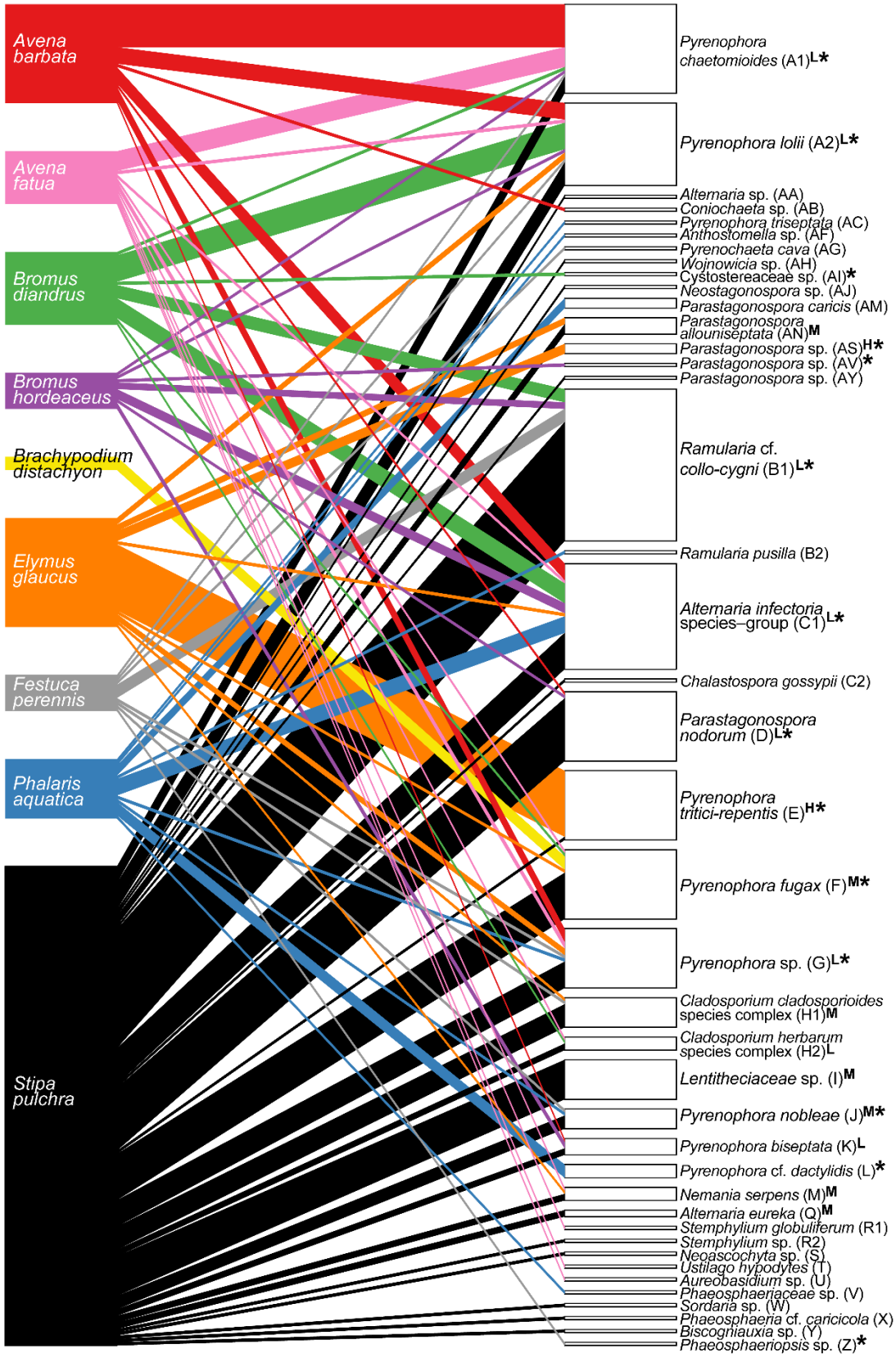
539 **Figure 3**



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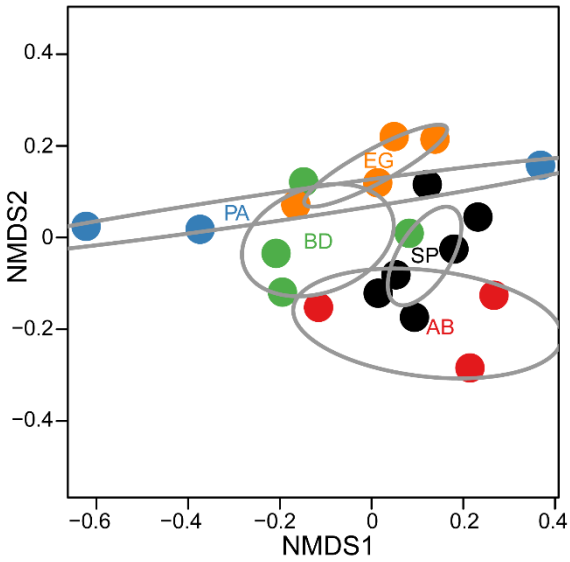

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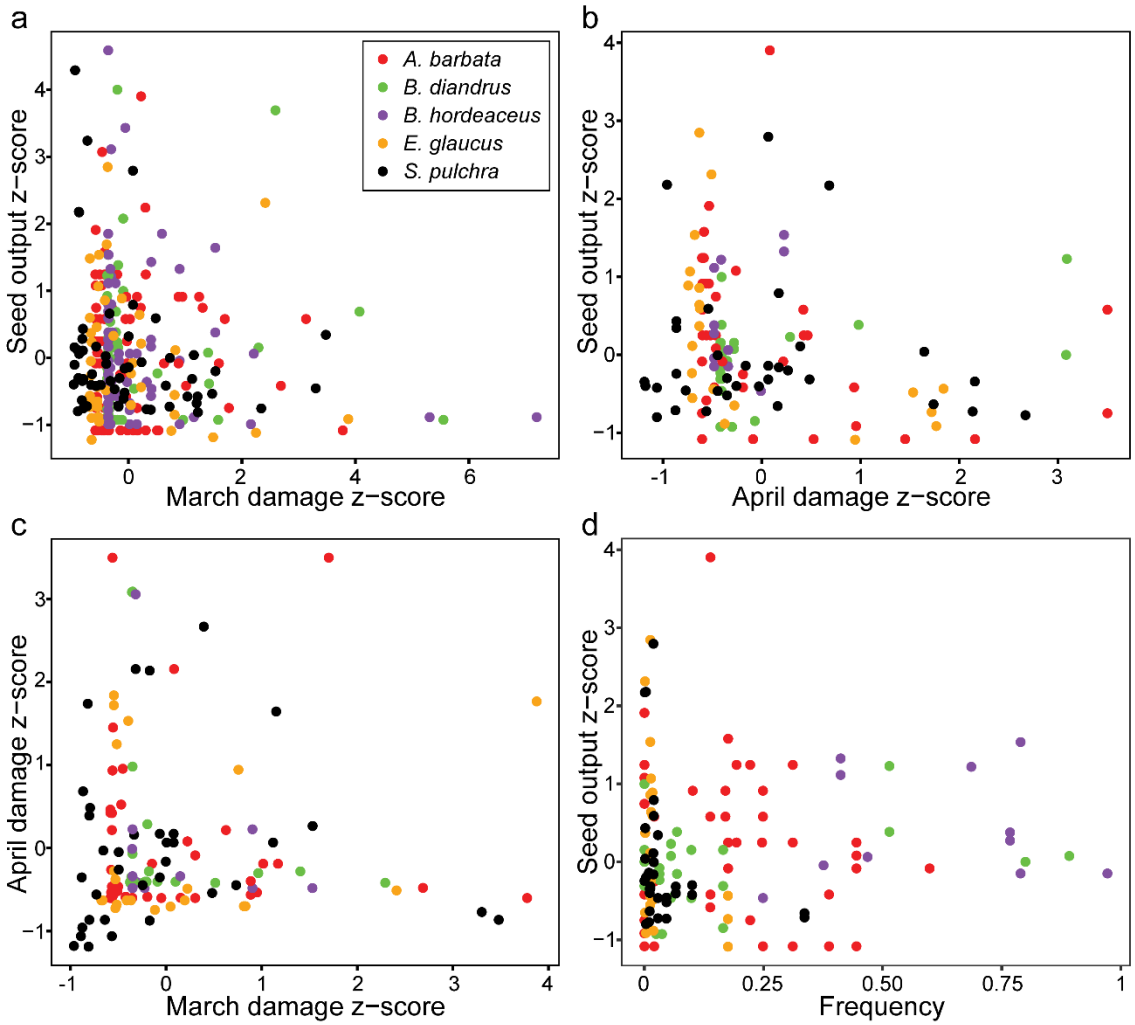
544 **Figure 5**



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