Foliar pathogens of California grasses infect multiple hosts: implications for grassland diversity

Erin R. Spear[*] and Erin A. Mordecai[*†]

[*]Biology Department, Stanford University, Stanford, CA 94305

[†]Corresponding author: emordeca@stanford.edu

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 differentially limiting common species while giving rare species advantages. 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 with species-specific responses to host density or by pathogens with broad host ranges and limited responses to host density? The answer determines the potential for pathogens to promote host diversity, as hypothesized in many plant communities, or to have negligible or even negative effects on host diversity. We lack a systematic understanding of the impacts, identities, and host ranges of pathogens in natural communities. Here we characterize the community of fungal pathogens associated with symptomatic grass leaves and evaluate their host specificity and fitness impacts in a California grassland community of native and exotic grass species. We found that most of the commonly isolated fungal pathogens were multi-host and exhibited intermediate to low specialization. The amount of pathogen damage each host experienced was independent of the local relative abundance of each host species. 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 of pathogen damage. Moreover, seed production was not dependent on the local relative abundance of each plant species, suggesting that coexistence mechanisms may operate at larger spatial scales in this community. In sum, pathogens in this grassland community are multi-host and have small fitness impacts. As a result, foliar pathogens are unlikely to promote negative frequency-dependence, nor to promote plant species coexistence, in this system. Still, given that pathogen community composition differentiates across host species (effective specialization), 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.

38 **Introduction**

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

52 At the same time, there is mounting empirical evidence that fungal pathogens of plants in
53 natural systems infect multiple hosts (Gilbert and Webb 2007, Kluger et al. 2008, Hersh et al.
54 2012, Spear 2017). Most plant pathogens (particularly fungi and bacteria) are passively
55 dispersed. In diverse plant communities where the nearest neighbors may be heterospecifics,
56 selection should favor pathogens that can infect multiple host species (i.e., multi-host pathogens)
57 (May 1991). Broad host ranges can preclude any relationship between the relative abundance of
58 a single host species and pathogen attack rates. Thus we hypothesize that most plant pathogens
59 are relatively host generalized (Spear et al. 2015, Spear 2017) and that their impacts do not
60 strongly respond to the relative abundance of a single host species. If this is the case, then, in

61 contrast to predictions of the Janzen-Connell hypothesis, many pathogens may play little role in
62 maintaining local host diversity and may even promote competitive exclusion or spatial turnover
63 of species (Mordecai 2011, Spear et al. 2015).

64 Even if pathogens have relatively broad ranges, several mechanisms could lead to
65 frequency-dependent population regulation, and simulation models suggest that strict host
66 specialization is not necessary for pathogens to promote coexistence (Sedio and Ostling 2013).
67 Individual pathogens may exhibit host affinity, infecting certain host species more often than
68 others, or have differential fitness consequences among hosts, severely impacting some hosts
69 while having minimal impact on others (Fig. 1; Mordecai 2011, Spear et al. 2015). In addition,
70 communities of pathogens (as opposed to individual pathogen species) may differ in composition
71 and impacts in response to the relative abundance of a host species (Fig. 1; Benítez et al. 2013).
72 For differentiation across host species to promote coexistence, it must result in
73 disproportionately high pathogen burden on locally common host species and local escape for
74 rare host species, and the damage incurred must have fitness costs (Fig. 1). Few studies have
75 tested whether these patterns occur in nature.

76 In sum, pathogens stabilize host species coexistence when they disproportionately reduce
77 the population growth of common species, thereby benefiting rare species. This requires that: (1)
78 pathogens disproportionately damage relatively common hosts, such that the amount or severity
79 of damage responds to host relative abundance; and (2) pathogen damage reduces host
80 population growth (Fig. 1). Condition (1) may occur either because (A) individual pathogen
81 species are relatively specialized or exhibit host preference, (B) communities of multi-host
82 pathogen species are differentially structured by plant species, or (C) multi-host pathogens exert
83 host-specific impacts. In this paper, we empirically evaluate the evidence for these conditions—

84 frequency-dependent pathogen damage, pathogen host specificity, and pathogen impacts on
85 population growth—for foliar pathogens that infect co-occurring native and exotic grasses in a
86 Northern California grassland. Specifically, we evaluated these conditions across six common
87 plant species by (i) surveying pathogen damage and linking it to plant species relative
88 abundance, (ii) surveying fungal community composition and pathogen sharing across host
89 species, and (iii) measuring the response of per-capita seed output to pathogen damage and plant
90 relative abundance.
91
92 **Methods**
93 *Study site & focal grass species*
94 We conducted the study in the grasslands of Stanford University's Jasper Ridge
95 Biological Preserve (JRBP), a 485-ha site in San Mateo County, CA (37°24'N, 122°13'30"W; 66
96 - 207 m). JRBP has a Mediterranean climate, with cool (mean temperature 9.2°C), wet winters
97 and warm (mean temperature 20.1°C), dry summers (total annual precipitation ~ 622.5 mm)
98 (Ackerly et al. 2002). Plant growth begins with the onset of winter rains and plants senesce at the
99 onset of summer. We conducted our study during the 2015 growing season, from March 19 –
100 May 4.
101 Our study focused on the identities and impacts of fungal pathogens of six locally
102 common grass species: three exotic annuals, *Avena barbata, Bromus hordeaceus,* and *Bromus*
103 *diandrus*; one exotic perennial, *Phalaris aquatica*; and two native perennials, *Stipa pulchra* and
104 *Elymus glaucus.* In addition to the six focal species, we isolated fungi from but did not assess
105 damage for three locally common exotic grass species: *Avena fatua, Brachypodium distachyon,*
106 and *Festuca perennis. A. barbata, A. fatua, B. distachyon, B. hordeaceus, B. diandrus, F.*

107 *perennis* and *P. aquatica* are exotic annual and perennial grasses introduced to California in the
108 mid-19th century (Corbin and D'Antonio 2004).
109 We assessed pathogen damage in response to host relative abundance (Condition 1), plant
110 seed production in response to pathogen damage (Condition 2), and pathogen specialization and
111 community composition (Conditions 1A-B). To evaluate the impact of pathogens on grasses
112 under varying host species relative abundance (Condition 1), we assessed pathogen-caused
113 damage across 10 transects (yellow points in Fig. S1) that were established in areas where
114 perennial species (either *S. pulchra*, *E. glaucus*, or *P. aquatica*) vary in their abundance, ranging
115 from rare to common in a given 1-m^2 area; hereafter referred to as 'perennial density transects'.
116 We also harvested seeds from a subset of marked individuals on which we also surveyed damage
117 (Condition 2). The marked grasses from which seeds were harvested had various levels of
118 damage and local abundances. We surveyed fungal pathogen community composition
119 (Conditions 1A-B) by culturing fungi from symptomatic leaves collected along the 10 perennial
120 density transects and an additional 14 transects that spanned a range of plant community
121 composition, geographic location, and soil types, capturing a broader range of growing
122 conditions (24 transects in total; yellow and red points in Fig. S1). Three of the pathogen survey
123 transects ran through the Jasper Ridge Global Change Experiment (GCE), a multifactorial
124 experiment manipulating water, nitrogen, temperature, and CO_2, where we sampled in ambient
125 and water addition plots. Because water addition had no impact on fungal community
126 composition (results not reported), we include isolates from the water addition plots in the full
127 fungal community dataset.
128
129 *Impacts of plant species relative abundance on pathogen damage (Condition 1)*

130 We visually censused fungal pathogen-caused foliar damage for the six focal grass
131 species (*A. barbata, B. diandrus, B. hordeaceus, E. glaucus, P. aquatica,* and *S. pulchra*) along
132 the 10 perennial density transects (yellow points in Fig. S1), documenting percent leaf area
133 damaged. We surveyed damage using a hierarchical sampling scheme: as possible, multiple
134 plants per transect and multiple (up to six) haphazardly-selected leaves per plant. To quantify
135 variation in pathogen damage within the growing season, the 10 perennial density transects were
136 censused twice: first, between March 11 and 16 (444 marked grass individuals) and second,
137 between April 17 and 20 (163 of the marked grasses). During each census, we sampled plants of
138 all focal species present in each plot, averaging a total of 8.9 samples from 3.2 species per plot in
139 March and 5.4 samples from 2.5 species per plot in April. For each plant, we calculated an
140 average percent damage estimate across all sampled leaves (our main response variable) and a
141 proportion of sampled leaves that had damage.
142 We tested whether pathogen damage responded to host relative abundance (Condition 1)
143 while controlling for other potential predictors: plant species, time within the growing season,
144 and the sampling structure. The response variables for the models were mean percent leaf area
145 damaged, which we refer to as 'mean damage' hereafter, and proportion of leaves damaged (i.e.,
146 any amount of pathogen damage). We used mixed-effects linear models (lmer function in the
147 lme4 package; Bates et al. 2014) to test for interactive effects of host species, plant species
148 relative abundance at the plot scale (i.e., frequency), and sampling date, with plot number nested
149 within transect as random effects (i.e., random intercepts). For models of mean damage, we used
150 normally-distributed errors because this measurement represents an average of up to six values
151 between zero and one. For models of proportion of leaves damaged we used binomial errors. We
152 assessed the most important predictors of pathogen damage by comparing the Akaike

153 Information Criterion (AIC) values of nested models that included the nested random effects
154 accounting for sampling structure and the following combinations of fixed effects: sampling
155 month * species + frequency; sampling month * species; species + frequency; sampling month +
156 species; species.

157 Because plant species composition may influence pathogen damage at several scales, we
158 also built models of pathogen damage as a function of plant composition at a larger scale by
159 including a categorical variable indicating plant species dominance at the sub-transect scale.
160 Since plant composition at this scale had no influence on pathogen damage, we do not discuss
161 the results further (the model is included in the R code file that accompanies this paper).

162

163 *Isolating foliar fungal pathogens (Conditions 1A-B)*

164 We assessed the host specialization and community structuring of putative fungal
165 pathogens causing leaf damage by isolating fungi in pure culture. Between March 19 and May 4,
166 2015, we collected 772 segments of symptomatic foliar tissue (e.g., with chlorotic spots, necrotic
167 lesions, etc.) from nine grass species along 24 transects in JRBP (yellow and red points in Fig.
168 S1). We collected one piece of symptomatic leaf tissue per grass individual. Sampling intensity
169 varied among grass species and transects (Tables 1 and S1). To isolate the putative fungal
170 pathogens in culture, we excised a small portion of tissue (< 2 mm^2) from the advancing margin
171 of disease for each leaf piece collected, surface-sterilized the excised tissue (sequential washes of
172 70% EtOH and 10% bleach; 60 s each), and plated it on malt extract agar with 2%
173 chloramphenicol (2% MEA). We monitored fungal growth under ambient lab conditions for up
174 to four weeks. We isolated emergent hyphae into axenic culture on 2% MEA based on
175 morphology and pigmentation. To verify the efficacy of the surface sterilization, we pressed six

176 treated leaf pieces onto 2% MEA. After two weeks, we observed no growth from the imprints.
177 Reference strains are maintained in a living culture collection in the Mordecai lab, Department of
178 Biology, Stanford University (Stanford, CA, USA). The California Department of Food and
179 Agriculture approved the collection, transport, and storage of isolates (permit no. 3160).
180
181 *Identifying fungal species by DNA sequencing (Conditions 1A-B)*
182 For each isolate, we scraped fungal mycelium from living culture, transferred it to a
183 microcentrifuge tube, and extracted genomic DNA using REDExtract-N-Amp Tissue PCR Kit
184 (Sigma-Aldrich, Inc.), following the manufacturer's protocol. We amplified and sequenced the
185 internal transcribed spacers (ITS) 1 and 2 and the 5.8S nuclear ribosomal gene using the primer
186 pairs ITS-1F and ITS-4 (Gardes and Bruns 1993). For PCR amplification, we used a T100
187 thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA) and thermal cycling conditions
188 following U'Ren et al. (2010). Following electrophoresis on a 1.5% agarose gel, we visualized
189 PCR products using GelRed™ (Biotium Inc., Hayward, CA) and sent to them MCLAB (San
190 Francisco, CA) for cleanup and bidirectional sequencing on an ABI 3730 XL sequencer.
191 We manually inspected and edited all reads. Bidirectional reads were automatically
192 assembled into a consensus sequence, using a minimum of 20% overlap and 85% sequence
193 similarity. We then clustered the 288 consensus sequences and two unidirectional sequences into
194 operational taxonomic units (OTUs) based on a minimum of 40% overlap and 90, 95, 97 and
195 99% sequence similarity. We used Sequencher (Gene Codes, Ann Arbor, MI) for sequence
196 editing and assembly and OTU designations. All sequence data will be submitted to GenBank
197 (accession numbers XXXX-XXXX).

198 Because percent sequence similarity for the ITS region varies within versus between
199 fungal species (as described in O'Brien et al. 2005) and given the limitations of GenBank (Kang
200 et al. 2010, U'Ren et al. 2010), we conducted phylogenetic analyses to improve our inference of
201 taxonomic placement. We partitioned OTUs with similar sequences into datasets (1-52 JRBP
202 isolates per dataset), and generated a phylogenetic tree for each dataset following the procedures
203 described in Higginbotham et al. (2014) and Spear (2017). Because not all sequences mapped
204 onto named fungal species, we assigned each operational species a unique species code, which
205 we list in parentheses after the lowest estimated taxonomic placement (Table S2). We treated
206 isolates belonging to a species complex as a single species.
207
208 *Analyses of fungal community composition and host associations (Conditions 1A-B)*
209 We conducted several analyses of the fungal community. First, we describe sampling
210 efficacy and fungal species richness and diversity. Second, we compare fungal communities
211 across host species. Third, we explicitly consider the frequency of shared pathogens from the
212 grass species' perspective. Finally, we evaluate host specialization from the fungal species'
213 perspective.
214 For the full fungal community dataset ($N = 290$), we report (i) sampling efficacy, (ii) the
215 observed richness of taxa (and percent singletons), (iii) the estimated lower bound of true species
216 richness, (iv) the relative-abundance distributions, and (v) the diversity of taxa. To assess
217 sampling efficacy (i), we generated accumulation curves, for the OTUs based on sequence
218 similarity and for the fungal species based on phylogenetic analyses, with a method that finds the
219 mean richness when sites are added in random order. We estimated the lower bound of true
220 species richness (iii) with the iChao1 estimator, which relies on the number of single-, double-,

221 triple- and quadrupletons to estimate the number of unseen species in the community and correct
222 for the negative bias associated with under-sampling in highly diverse assemblages (Chiu et al.
223 2014). We visualized the richness, evenness, and dominant species of the fungal community by
224 plotting species abundance versus species rank abundance (iv). Fungal species isolated ten or
225 more times were defined as abundant. We estimated diversity (v) based on Fisher's alpha, which
226 is robust to unequal sample sizes (Fisher et al. 1943, Magurran 2013), the Shannon index, which
227 is sensitive to sample size (Magurran 2013), and the effective number of species (Jost 2006) to
228 allow for comparison with other studies.

229 To compare fungal pathogen communities across host species (Conditions 1A-B), we
230 report richness and diversity metrics (ii-v) for each grass species ($N = 290$ isolates). Additionally,
231 we assessed differences in community composition among grass species (Conditions 1A-B) via a
232 permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) and visualized
233 the differences using non-metric multidimensional scaling (NMDS). The PERMANOVA (adonis
234 function) tested for significant differences in fungal community composition among the grass
235 species (Oksanen et al. 2016). As adonis is sensitive to heterogeneity of variances, we used the
236 betadisper function and ANOVAs to test whether the fungal communities of grass species had
237 similar dispersions (Oksanen et al. 2016). We identified significant differences in fungal
238 community composition between pairs of grass species using pairwise PERMANOVAs with the
239 False Discovery Rate (FDR) adjustment for multiple comparisons (Hervé 2017). For the NMDS,
240 PERMANOVAs, and tests of homogeneity of variance, we considered each focal grass species-
241 by-perennial density transect combination to be a distinct community. We excluded grass
242 species-by-transect communities for which fewer than three isolates were collected and *B.*
243 *hordeaceus* communities because only one species-by-transect community had the minimum

244 number of isolates (in total, 20 communities, 21 fungal species, 5 grass species, and 99 isolates
245 were included in the dataset). From the resulting raw data matrix, we created a matrix of pairwise
246 dissimilarities in fungal community composition for use with the NMDS, PERMANOVAs, and
247 homogeneity of variances test. The dissimilarity matrix was created via function vegdist with the
248 Chao method, which implements an abundance-based Jaccard index that was adjusted to
249 consider unseen species (Chao et al. 2005, Oksanen et al. 2016).

250 To evaluate pathogen sharing among grass species, we first characterized the observed
251 number of shared fungal species between pairs of grass species. Second, we estimated the
252 similarity of pairs of grass species (i.e., fungal community overlap) based on the relative
253 abundances of the fungal species for the eight grass species for which 10 or more isolates were
254 collected, using the Morisita-Horn index (200 bootstrap replicates).

255 To assess the specialization of the fungal species (Condition 1A) and the uniqueness of
256 each grass species' fungal community (Condition 1B), we visualized all interactions (links) with
257 a bipartite plot and then calculated species-level interaction specialization via the weighted
258 specialization index d'. The index illustrates the degree to which a species deviates from
259 expected, random interactions by comparing interaction frequencies to the overall availability of
260 potential partners (the marginal frequencies) (Blüthgen et al. 2006). As we did not sample the
261 grass species proportional to their relative abundances in JRBP, the total number of fungal
262 isolates cultivated from a grass species (marginal frequency) was not a suitable proxy for its
263 availability. Thus, to improve the accuracy of our calculations, we only included the five focal
264 grass species (*A. barbata*, *B. diandrus*, *B. hordeaceus*, *S. pulchra*, and *E. glaucus*) with
265 independent, preserve-wide estimates of their relative abundances (based on 30 10-m diameter
266 circular plots; see the accompanying R code for the abundance values) in the dataset. We then

267 incorporated the abundance estimates into the analysis in lieu of the number of isolates collected
268 per grass species (Blüthgen et al. 2006, Dormann et al. 2016). Additionally, we excluded
269 singleton fungal species as their host range and degree of specialization were likely to be
270 misinterpreted (Dormann 2011) (dataset for network analysis: $N = 228$ isolates, representing 17
271 fungal species). The d' index ranges from zero (perfect generalist) to one (perfect specialist)
272 (Dormann 2011). Consistent with Blüthgen et al. (2006), we considered 0-0.33, 0.34-0.67, and
273 0.68-1 to be low, moderate, and high specialization, respectively.
274 All pathogen community analyses were conducted in *R* version 3.4.2, using the packages
275 RVAideMemoire (Hervé 2017), vegan (Oksanen et al. 2016), SpadeR (Chao et al. 2016),
276 bipartite (Dormann et al. 2016), fossil (Vavrek 2011), BiodiversityR (Kindt and Coe 2005), rich
277 (Rossi 2011) and with custom commands (Gardener 2014).
278
279 *Pathogenicity tests (Conditions 1A-B)*
280 One advantage of our culture-based approach to fungal identification was that it allowed
281 us to verify the pathogenicity of the fungal isolates through experimental inoculations, since
282 taxonomic affiliations do not necessarily indicate pathogenicity (Delaye et al. 2013). We
283 experimentally inoculated 99 isolates from symptomatic leaves onto plants of the host species
284 from which they were originally isolated (Table S3). The isolates tested represented 35 of the 41
285 observed fungal species, including all of the common (isolated ≥10 times) fungal species, and
286 originated from eight of the nine grass species surveyed. Disease development is contingent on
287 the presence of a susceptible host and competent pathogen, and environmental conditions that
288 compromise the host and favor the pathogen (Barrett et al. 2009). Given natural intraspecific
289 variation in host susceptibility and pathogen aggressiveness and that our experiments may not

290 have replicated the abiotic conditions that originally led to disease development in JRBP, we
291 tested multiple isolates of some of the non-singleton fungal species to avoid misclassifying a
292 fungal species as nonpathogenic. In Stanford greenhouses, we inoculated apparently healthy
293 grass leaves by securing colonized 2% ME agar plugs against leaves with small strips of
294 Parafilm (Sinclair and Dhingra 1995). Uncolonized agar plugs were used as negative paired
295 controls. We completed two rounds of inoculations. In round one, we inoculated each plant with
296 4-5 isolates and a negative control, with each treatment applied to a unique leaf (5-6 treated
297 leaves/plant; 9-12 leaves/treatment). In round two, we applied a single isolate or control
298 treatment to each individually-potted plant (4 unique leaves per plant and 5 plants per treatment,
299 totaling 20 leaves per treatment). Thirteen of the 99 isolates tested were tested in both rounds
300 (Table S3). After monitoring plants for up to one week, we assessed pathogenicity by censusing
301 leaves for obvious symptoms of disease (e.g., lesions or necrosis). To compare the proportion of
302 leaves with disease for a given fungal isolate versus its paired control (same plant species and
303 inoculation date) we used bias-reduced generalized linear models (brglm function; Kosmidis
304 2013), assuming binomial error distributions and probit link functions (Table S3). The analyses
305 excluded all leaves for which it was unclear if tissue damage was the result of disease, resulting
306 in 5 to 20 leaves per treatment (Table S3). We deemed an isolate pathogenic if a significantly
307 greater proportion of inoculated leaves had pathogen damage than paired control leaves (using α
308 $= 0.05$; Table S3).
309
310 *Impacts of pathogen damage on per-capita seed production (Condition 2)*
311 Pathogen damage may have fitness consequences for the host, including reduced
312 survival, growth, and seed output. To explore the relationship between plant demographics,

313 competition, and pathogen impact, we harvested seeds from 350 of the marked grasses from the
314 perennial density transects. We measured per-capita seed production as a function of conspecific
315 and heterospecific density and percent leaf area damaged for *Avena barbata, Bromus*
316 *hordeaceus, Bromus diandrus, Stipa pulchra,* and *Elymus glaucus*. We did not measure seed
317 production of *Phalaris aquatica* because it occurs in monotypic stands with little variation in
318 relative abundance.

319 Some pathogen species may be more damaging, and in turn have higher fitness costs, to
320 their hosts than others. We addressed this issue by linking fungal species identity to the mean
321 leaf damage ($N = 65$ fungal isolates, representing 16 fungal species) and seed output ($N = 56$
322 fungal isolates, representing 12 fungal species) on marked plant individuals in the perennial
323 density transects. All had percent damage estimates in March and all but one had percent damage
324 estimates in April.

325 We used regression models to assess the extent to which pathogen damage correlated
326 with per-capita seed output. To compare variation across species, we standardized both seed
327 output and March and April percent damage by calculating z-scores (i.e., by subtracting the
328 within-species mean and dividing by the within-species standard deviation). By doing so, we
329 asked whether deviations from average pathogen damage correlated with deviations from
330 average seed production, which would suggest pathogen fitness impacts. We modeled the
331 relationships between standardized March or April damage estimates and standardized seed
332 production (we did not include both March and April damage together in the same model
333 because these were assessed on the same individuals with only a single seed output value).
334 Models in which seed production was standardized by species but damage was not produced
335 similar results (shown in the R code accompanying the paper). The variance in seed production

was not constant across the range of damage, so we used quantile regression to calculate regression coefficients (slopes and intercepts) for the 25th, 50th, and 75th percentiles of seed production, using the function rq in the quantreg package in R (Koenker 2017). A fuller understanding of 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), and lme4 (Bates et al. 2014).

Results

Pathogen damage across host species and host species frequency (Condition 1)

Host species differed in the amount of pathogen-mediated damage that they experienced, and *A. barbata*, *B. diandrus*, and *E. glaucus* experienced higher mean damage in April than in March (Fig. S2; Table S4). Grass species significantly differed in their proportion of leaves damaged: the two native perennials, *S. pulchra* and *E. glaucus*, had the highest proportion of leaves damaged (81% and 75%, respectively; Table S4; Fig. S2). For individual plants sampled twice, mean damage and proportion of leaves damaged were both higher in April than in March (paired two-tailed *t*-tests for mean damage: mean difference = 0.0265, $t = -4.1019$, $df = 162$, $p = 6.47 \times 10^{-5}$; for proportion damaged: mean difference = 0.146, $t = -5.256$, $df = 162$, $p = 4.587 \times 10^{-7}$).

The strongest predictors of pathogen damage (based on model AIC) were species, sampling date, and their interaction, although we also tested for interactive effects of host species

359 and plant community at the plot and sub-transect scale (Tables S4-S5). *A. barbata* and *E. glaucus*
360 had higher percent damage, particularly at the later sampling point (April). All species, except *A.*
361 *barbata,* had a higher proportion of leaves damaged in April than in March, suggesting that
362 pathogen damage accumulates through the growing season. Counter to Condition 1 for
363 pathogens to promote coexistence, focal species frequency within the plot did not significantly
364 affect pathogen damage (Fig. 2). The model that included that term had an AIC value only
365 slightly above the minimum AIC, indicating similar performance (the model that includes
366 frequency is shown in Table S4 for illustration).
367
368 *Fungal pathogen identities and diversity (Conditions 1A-B)*
369 We isolated fungi from 38% of the 772 foliar tissue pieces that we collected along 24
370 transects across Jasper Ridge Biological Preserve (JRBP). We successfully sequenced 290 of the
371 resulting 302 isolates (Table 1). Fungi were isolated from all nine grass species. However, the
372 grass species were unequally sampled and the isolation frequency (i.e., percent of tissue pieces
373 with growth) differed among the hosts (Table 1).
374 The accumulation curves for the fungal species and OTUs (based on phylogenetic
375 analyses and percent sequence similarity, respectively) were non-asymptotic, indicating
376 incomplete sampling and a diverse community (N = 290; Fig. S3). Considering 90 - 99%
377 sequence similarity, the isolates represented 28 - 49 operational taxonomic units (OTUs),
378 respectively (Fisher's alpha index for diversity: 7.28 - 16.39). Hereafter, we designate all fungal
379 species taxonomic affiliations based on the phylogenetic analyses. The fungal isolates (N = 290)
380 represented 41 fungal species (iChao1 estimated species richness: 285.78, 95% CI = 85.91,
381 1375.24; Fisher's alpha for diversity: 13.03, 95% CI = 8.91, 18.58; Shannon diversity = 2.91,

382 95% CI = 2.75, 3.06; effective number of species = 18.29, 95% CI = 15.45, 21.14). Most of the
383 41 observed fungal species were rare (56% were singletons or doubletons) and relatively few
384 were abundant (22% were observed more than 10 times) (Fig. S4). The most commonly
385 observed genera included *Pyrenophora*, *Ramularia*, *Alternaria*, and *Parastagonospora* (Fig. 3
386 and Table S2). Using greenhouse-based inoculation experiments, we experimentally confirmed
387 the pathogenicity of 27% of the 99 isolates tested, representing 14 of the 35 fungal species tested,
388 eight of the nine common fungal species, and the most commonly observed genera in our survey
389 (Fig 3; Tables S2-S3).
390
391 *Fungal pathogen host associations (Conditions 1A-B)*
392 The observed and estimated lower bound of fungal species richness per grass species
393 (iChao1) ranged from one to 26 and from one to 43.27, respectively (Table 1; Figs. 3 and S5).
394 Unsurprisingly, the greatest richness (26 fungal species) was observed for the best-sampled grass
395 species with the highest isolation frequency, *S. pulchra* (Table 1). Controlling for sample size via
396 Fisher's alpha index, fungal pathogen community diversity was greatest for *F. perennis, A. fatua,*
397 and *S. pulchra* (Table 1). For the eight grass species for which multiple fungal species were
398 observed (i.e., excluding *B. distachyon*; Table 1), most of the fungal species were observed at
399 intermediate to low frequencies (Table 1; Fig. S5).
400 The majority (74%) of the 19 non-singleton fungal species infected multiple hosts (Fig.
401 3). On average, the multi-host fungi were isolated from four grass species. Two of the fungal
402 species, *Pyrenophora lolii* (A2) and *Alternaria infectoria* species–group (C1), were isolated from
403 seven of the nine grass species that we sampled (Fig. 3). Concordantly, the weighted
404 specialization index d' values suggest that the majority of the fungi had moderate to low

405 specificity (d' from 0.021 to 0.465, median d' = 0.346; for the nine common fungal species,
406 median d' = 0.3; Table 2).
407 While the majority of the fungal species for which d' could be calculated had low to
408 moderate host specificity, two had high host specificity: *Pyrenophora tritici-repentis* (E) and
409 *Parastagonospora* sp. (AS) (d' = 0.924 and 1, respectively; Table 2; Fig. 3). *Pyrenophora tritici-*
410 *repentis* (E) was isolated almost entirely from *Elymus glaucus* (the only exception was a single
411 isolate from a *Stipa pulchra* individual growing in an *E. glaucus*-dominated transect plot) and it
412 comprised 61% of the 33 fungal isolates from *E. glaucus* leaves, exhibiting strong host
413 preference (Condition 1A). Two additional likely specialists were only isolated from the under-
414 sampled grass species *Phalaris aquatica* (*N* = 18 isolates): *Pyrenophora* cf. *dactylidis* (L) and
415 *Parastagonospora caricis* (AM) (Fig. 3). While we could not confidently calculate specialization
416 index d' values, *Pyrenophora* cf. *dactylidis* (L) and *Parastagonospora caricis* (AM) made up
417 39% of the few isolates originating from *P. aquatica* leaves, suggesting strong host preference
418 (Condition 1A).
419
420 *Pathogen sharing and community similarity among grass species (Conditions 1A-B)*
421 The eight grasses species from which 10 or more isolates were collected shared at least
422 one and up to eight fungal pathogen species with every other grass species (median = 3; Table
423 S6; Fig. 3). As estimated by the Morisita-Horn index, the average estimated similarity between
424 pairs of host species was moderate (42%) (min = 5% for *E. glaucus* and *P. aquatica*; max =
425 100% for *F. perennis* and *S. pulchra* and for *A. barbata* and *A. fatua;* Table S6). Six of the grass
426 species shared their numerically dominant fungal species with one of the other grass species
427 (Figs. 3 and S5).

428 Two of the grass species had a relatively low fungal community similarity with the other
429 grass species in our study: *E. glaucus,* the second best-sampled grass species (N = 33; Morisita-
430 Horn similarity index 5% to 11%), and *P. aquatica* (N = 18; Morisita-Horn similarity index 5%
431 to 70%; Table S6; Fig. 3). Congruously, the weighted specificity index d' for *E. glaucus* suggests
432 that the grass species hosted a relatively unique assemblage of fungal pathogens (d' = 0.665;
433 Table 1).
434 The fungal pathogen communities associated with the leaves of the five focal grass
435 species included in the PERMANOVA (N = 99 isolates) were significantly dissimilar ($F_{4,15}$ =
436 3.682, R^2 = 0.495, p = 0.001; Fig. 4). Specifically, (i) the fungal community associated with *S.*
437 *pulchra* (32 isolates) was significantly different from those of *A. barbata* (11 isolates) (P_{adj} =
438 0.037), *B. diandrus* (16 isolates) (P_{adj} = 0.037), *E. glaucus* (31 isolates) (P_{adj} = 0.04), and *P.*
439 *aquatica* (9 isolates) (P_{adj} = 0.037); (ii) the fungal community associated with *P. aquatica* (9
440 isolates) was significantly different from that of *B. diandrus* (16 isolates) (P_{adj} = 0.05); and (iii)
441 the fungal community associated with *B. diandrus* (16 isolates) was significantly different from
442 that of *A. barbata* (11 isolates) (P_{adj} = 0.05) (Fig. 4). The fungal communities of the grass species
443 had similar dispersions ($F_{4,15}$ = 0.035, p = 0.997; Fig. 4).
444
445 *Impacts of pathogen damage on per-capita seed output (Condition 2)*
446 The relationships between pathogen damage and seed production were generally
447 negative but highly variable, particularly at the low levels of pathogen damage that most of the
448 plants in the survey experienced (Fig. 5). Because the variance was higher at low pathogen
449 damage, we used quantile regression to estimate how the relationship between damage and seed
450 production varied across individuals in the lower, middle, and upper quantile of the seed

451 production distribution. We found that increasing pathogen damage in April was associated with
452 reduced seed output at the 50th percentile, but that the negative effect was not statistically
453 significant for the 25th or 75th percentiles (Table S7; Fig. 5). In other words, plants of average
454 seed production had significantly reduced seed output with increasing pathogen damage, but
455 both high- and low- seed output individuals did not have a significant response to pathogen
456 damage. The effect of March damage was weakly negative but not statistically significant for
457 any quantile (Fig. 5). Focal species frequency had no association with either seed output (Fig. 5)
458 or pathogen damage (Fig. 2). Much of the variation in seed production was not explained by
459 pathogen damage in either month.

460 Fungal pathogen species may differ in their ability to cause damage, and ultimately
461 fitness consequences, to their hosts. To compare pathogen damage caused across host species we
462 compared the z-score of percent damage in April (calculated by host species) based on the
463 known fungal species identity infecting that host individual (N = 65 host individuals: 13 *A.*
464 *barbata*, 8 *B. diandrus*, 17 *E. glaucus*, 22 *S. pulchra*, and 5 *P. aquatica*). We performed a similar
465 analysis for the z-score of seed production (calculated by host species) against fungal species
466 identity. None of the common fungal species or genera were notably associated with higher than
467 average damage or lower than average seed production (Fig. S6). Anecdotally, a single *Stipa*
468 *pulchra* individual infected with *Sordaria* sp. (a singleton) had high pathogen damage and low
469 seed production, consistent with a large pathogen impact.

470

471 **Discussion**

472 *Limited potential for pathogen-mediated coexistence*

473 Pathogens are widely hypothesized to promote plant community diversity by generating
474 negative frequency dependence (Augspurger 1983, Packer and Clay 2000, Petermann et al. 2008,
475 Allan et al. 2010, Mangan et al. 2010, Bagchi et al. 2014, Bever et al. 2015, Whitaker et al.
476 2017). Yet diverse natural communities should favor multi-host pathogens with broad host
477 ranges, decoupling pathogen abundance from the density of a single host species (May 1991,
478 Spear et al. 2015). As a result, the degree of pathogen specialization and their role in promoting
479 frequency dependence remains unresolved (Mordecai 2011). In this grassland system, we found
480 ubiquitous pathogen damage: the majority of plants and 57% of all surveyed leaves had pathogen
481 damage (Fig. S2) as has been observed in many other ecosystems (Gilbert 2002, Gilbert and
482 Webb 2007, Parker et al. 2015). Yet we found limited empirical evidence for foliar fungal
483 pathogens generating negative frequency dependence, thereby maintaining plant species
484 diversity (Fig. 1).
485 Consistent with grassland seed pathogens (Beckman et al. 2014), foliar pathogen damage
486 did not strongly respond to host relative abundance (Fig. 2) in a way that would promote
487 negative frequency dependence (Condition 1). This is perhaps unsurprising given that the grass
488 species extensively shared foliar pathogens, and that most of the common pathogens infected
489 several species (Table 2; Fig. 3; in contrast to Condition 1A). Many diseases are specific to a
490 genus or family (Gilbert and Webb 2007, Barrett et al. 2009); thus, for the study grasses (all
491 Poaceae), pathogen damage may be decoupled from the relative abundance of a single host
492 species. It is also possible that pathogen damage responds to host relative abundance at the
493 regional, rather than local, scale (Mitchell et al. 2002).
494 Despite pathogen sharing among the grass species (Table S6; Fig. 3), fungal community
495 composition subtly varied across grass species (partially supporting Condition 1B; Table 1; Figs.

496 4 and S5). Moreover, several common fungi were relatively specialized (partially supporting
497 Condition 1A). *Pyrenophora tritici-repentis* (E) occurred mainly on the native perennial *E.*
498 *glaucus* and was the most common fungus infecting that species. The only other occurrence of
499 this pathogen in our surveys was on a single native *S. pulchra* individual located within an *E.*
500 *glaucus* stand, suggesting a potential pathogen spillover event. Moreover, the recently invading
501 perennial grass *P. aquatica* (http://jrbp.stanford.edu/content/oakmead-herbarium-arrivals-
502 weeds#Arrival) had low pathogen damage and a relatively distinct pathogen community,
503 including two fungi exhibiting host preference, *Pyrenophora cf. dactylidis* (L) and
504 *Parastagonospora caricis* (AM). Taken together, these results suggest that although this
505 Northern California grassland fungal pathogen community is dominated by multi-host
506 pathogens, they vary in their affinity for each host species (Condition 1A) and pathogen
507 communities may be structured, at least in part, by host species identity (Condition 1B).
508 The low pathogen damage and relatively distinct pathogen community of *P. aquatica*
509 suggest that only a few of the resident pathogens have been able to make the ecological or
510 evolutionary jump to infect *P. aquatica* since its introduction. Exotics that are slow to
511 accumulate pathogens in their naturalized range tend to be more noxious (Mitchell and Power
512 2003). In JRBP, *P. aquatica* is an aggressive species that forms monotypic stands that suppress
513 native plant species. By contrast, the other exotic species are all annuals that co-occur with
514 native perennial species and have been naturalized in the area for over one hundred years,
515 potentially allowing more time for pathogen host switches to occur.
516 Although higher pathogen damage was weakly associated with lower seed production
517 (Fig. 5), foliar pathogens, which reduce photosynthetic function, did not appear to dramatically
518 affect fitness (Condition 2). Nonetheless, the ability to detect any relationship between percent

519 damage and seed production is notable given the range of other sources of variation, including
520 individual size, competition with the surrounding community, and microhabitat suitability.
521 However, a fuller assessment would require measuring fitness effects across life stages in
522 experimental infections. Although some of the fungal species exhibited strong host affinity
523 (Condition 1A; Fig. 3; Table 2) and pathogen community composition subtly varied across plant
524 species (Condition 1B; Table 1; Figs. 4 and S5), supporting effective specialization (Hersh et al.
525 2012, Benítez et al. 2013), the foliar pathogens in this system are not likely to respond strongly
526 enough to host relative abundance (Condition 1) or to have large enough fitness impacts to
527 promote plant species coexistence by generating negative frequency-dependence (Condition 2).
528 More fully understanding the role of pathogens in shaping the outcome of competition
529 between plant species requires measuring plant – pathogen interactions and their consequences
530 across demographic rates, and incorporating those measurements into population growth models
531 to understand emergent outcomes. For example, seedling damping off pathogens, root pathogens,
532 and pathogens that castrate plants by infecting flowers and/or seeds may prove to be more
533 important players (Gilbert 2005). Studies manipulating fungal communities with fungicides and
534 soil sterilization have suggested that pathogens promote plant community diversity (Allan et al.
535 2010, Mangan et al. 2010, Bagchi et al. 2014).
536 In addition to considering all plant demographic rates, it is necessary to investigate the
537 entire pathogen community. We focused on local lesion diseases of leaves and on culturable
538 foliar fungi, which may be disproportionately host generalist due to their necrotrophic lifestyle
539 and ability to grow on malt extract agar. Our study did not identify potentially important
540 biotrophic fungi, viruses, bacteria, or parasitic nematodes that may occur in roots or stems.
541 Biotrophic, obligate plant pathogens are generally expected to exhibit higher host specificity than

542 facultative pathogens (Gilbert 2005), making them more likely to respond to host density and
543 thereby maintain plant community diversity. Further, our study did not determine whether a
544 given multi-host pathogen exerts host-specific impacts (Condition 1C), which has been observed
545 in other systems (Sarmiento et al. 2017) and could contribute to the maintenance of local
546 diversity.

547 The broad host ranges and minimal demographic impacts of pathogens in this wild
548 grassland system contrast sharply with pathogen impacts on phylogenetically-related cultivated
549 agricultural grasses such as barley, wheat, and oats. The fungal pathogen species we encountered
550 are closely related to important cereal pathogens, such as *Pyrenophora, Parastagonospora,* and
551 *Ramularia* spp., that have caused major yield losses (Havis et al. 2015, McDonald and
552 Stukenbrock 2016). Yet, in agricultural systems, pathogens are often specialized on host
553 genotypes and cause major damage that translates into reduced seed output (McDonald and
554 Stukenbrock 2016). More broadly, foliar pathogen load declines with plant community diversity
555 in natural grassland and old-field systems (Mitchell et al. 2002, Rottstock et al. 2014). This
556 highlights the importance of naturally occurring host genetic and species diversity for mitigating
557 the spread of highly virulent pathogen genotypes in wild grassland systems (McDonald and
558 Stukenbrock 2016). In other words, pathogens may be diverse, relatively unspecialized, and have
559 low fitness impacts because host diversity prevents the invasion and dominance of virulent
560 strains or, conversely, because pathogens prevent any single host species or genotype from
561 becoming dominant.

562 The generalist strategy of broad host ranges and minimal host impacts is well suited to
563 persistence and spread in seasonal, high-density mixed species grasslands like our study system.
564 Particularly in annual-dominated stands, pathogens must quickly recolonize and spread during

565 the limited winter and spring growing season, giving a selective advantage to flexible, multi-host
566 infection strategies. Despite extensive theoretical and empirical work on host-specialist
567 pathogens and their impacts on host plant communities, there is little theoretical or empirical
568 framework for understanding the impact of diverse communities of generalist pathogens on host
569 communities (but see Mordecai 2011, Hersh et al. 2012, Benítez et al. 2013, Sarmiento et al.
570 2017). Developing empirically testable theory for how diverse, generalist pathogens with subtle
571 impacts on host fitness may impact the outcome of competition in host communities is a key
572 direction for future research.
573
574 *Conclusions*
575 Much of the research suggesting that host-specific pathogens maintain the local diversity
576 of plants is based on spatial and temporal patterns of conspecific negative density-dependent
577 mortality (e.g., Packer and Clay 2000, Klironomos 2002, Bell et al. 2006, Petermann et al. 2008,
578 Bagchi et al. 2010, Mangan et al. 2010, Comita et al. 2010) and there is a dearth of information
579 about the identities and host affinities of the fungal pathogens in natural systems (but see Parker
580 and Gilbert 2007, Gilbert and Webb 2007, Hersh et al. 2012, Schweizer et al. 2013, Spear 2017).
581 By examining 290 fungal isolates from 280 individual symptomatic plants, we present one of the
582 largest surveys of California grassland fungal pathogen composition. Additionally, few studies
583 have concurrently identified the pathogen communities, described the amount of pathogen-
584 mediated damage, and linked that damage to host species relative abundance and fitness costs
585 (seed production) across host species in common habitat (Parker and Gilbert 2007, Roy et al.
586 2014).

587 Our holistic approach illustrated that foliar pathogens in this system are multi-host, do not
588 strongly respond to host species relative abundance, and have minor impacts on host fitness.
589 Together, these conditions make it unlikely that foliar pathogens promote coexistence via
590 negative frequency-dependence in this system. More broadly, this and previous studies suggest
591 that foliar pathogens may not maintain grassland diversity (Peters and Shaw 1996, Mitchell
592 2003; but see Allan et al. 2010). However, given conflicting results, more work is required to
593 clarify the role of foliar pathogens in plant species coexistence, particularly relative to other
594 potentially important factors.

595 Identifying the factors that shape the relative abundances and coexistence of species,
596 potentially including pathogens, is particularly important for grasslands. Grasslands are one of
597 the most diverse and widespread habitats in the West Coast of the U.S., yet they are also heavily
598 invaded, degraded, and exposed to variable and changing climate (Harpole et al. 2007). As these
599 disturbances increasingly shift environmental conditions, predicting how plant – pathogen
600 interactions respond is a critical future research avenue for anticipating ecosystem change.

601

602 **Acknowledgements**

603 We appreciate field assistance from Reuben Brandt, Joe Sertich, Johannah Farner, Fletcher
604 Halliday, and JRBP volunteers including Bill Gomez and Meredith McClintock; laboratory
605 assistance from Kabir Peay's lab, especially Nora Dunkirk; data collection assistance from Ryan
606 Tabibi, Divya Ramani, and Caroline Daws; and support from JRBP staff including Nona
607 Chiariello, Philippe Cohen, Cary Tronson, and Steve Gomez. We received funding from the
608 Jasper Ridge Kennedy Endowment and the National Science Foundation (DEB-1518681).

609

610 **References**

611 Ackerly, D., C. Knight, S. Weiss, K. Barton, and K. Starmer. 2002. Leaf size, specific leaf area
612 and microhabitat distribution of chaparral woody plants: contrasting patterns in species
613 level and community level analyses. Oecologia 130:449–457.

614 Allan, E., J. van Ruijven, and M. J. Crawley. 2010. Foliar fungal pathogens and grassland
615 biodiversity. Ecology 91:2572–2582.

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

618 Arbizu, P. M. 2016, January 4. How can I do PerMANOVA pairwise contrasts in R? [Msg 2].

619 Augspurger, C. K. 1983. Seed dispersal of the tropical tree, Platypodium elegans, and the escape
620 of its seedlings from fungal pathogens. The Journal of Ecology 71:759–771.

621 Augspurger, C. K., and C. K. Kelly. 1984. Pathogen mortality of tropical tree seedlings:
622 experimental studies of the effects of dispersal distance, seedling density, and light
623 conditions. Oecologia 61:211–217.

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

627 Bagchi, R., T. Swinfield, R. E. Gallery, O. T. Lewis, S. Gripenberg, L. Narayan, and R. P.
628 Freckleton. 2010. Testing the Janzen-Connell mechanism: pathogens cause
629 overcompensating density dependence in a tropical tree. Ecology Letters 13:1262–1269.

630 Barrett, L. G., J. M. Kniskern, N. Bodenhausen, W. Zhang, and J. Bergelson. 2009. Continua of
631 specificity and virulence in plant host–pathogen interactions: causes and consequences.
632 New Phytologist 183:513–529.

633 Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models
634 using Eigen and S4.
635 Beckman, N. G., R. Dybzinski, and G. D. Tilman. 2014. Neighborhoods have little effect on
636 fungal attack or insect predation of developing seeds in a grassland biodiversity
637 experiment. Oecologia 174:521–532.
638 Beckstead, J., S. E. Meyer, K. O. Reinhart, K. M. Bergen, S. R. Holden, and H. F. Boekweg.
639 2014. Factors affecting host range in a generalist seed pathogen of semi-arid shrublands.
640 Plant Ecology 215:427–440.
641 Bell, T., R. P. Freckleton, and O. T. Lewis. 2006. Plant pathogens drive density-dependent
642 seedling mortality in a tropical tree. Ecology Letters 9:569–574.
643 Benítez, M.-S., M. H. Hersh, R. Vilgalys, and J. S. Clark. 2013. Pathogen regulation of plant
644 diversity via effective specialization. Trends in Ecology & Evolution 28:705–711.
645 Bever, J. D., S. A. Mangan, and H. M. Alexander. 2015. Maintenance of plant species diversity
646 by pathogens. Annual Review of Ecology, Evolution, and Systematics 46:305–325.
647 Blüthgen, N., F. Menzel, and N. Blüthgen. 2006. Measuring specialization in species interaction
648 networks. BMC Ecology 6:9.
649 Burdon, J. 1993. The structure of pathogen populations in natural plant communities. Annual
650 Review of Phytopathology 31:305–323.
651 Burdon, J., and G. Chilvers. 1982. Host density as a factor in plant disease ecology. Annual
652 Review of Phytopathology 20:143–166.
653 Burdon, J. J. 1982. The effect of fungal pathogens on plant communities. The plant community
654 as a working mechanism:99–112.

Chao, A., R. L. Chazdon, R. K. Colwell, and T. J. Shen. 2005. A new statistical approach for assessing similarity of species composition with incidence and abundance data. Ecology Letters 8:148–159.

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

Chiu, C.-H., Y.-T. Wang, B. A. Walther, and A. Chao. 2014. An improved nonparametric lower bound of species richness via a modified good–turing frequency formula. Biometrics 70:671–682.

Comita, L. S., H. C. Muller-Landau, S. Aguilar, and S. P. Hubbell. 2010. Asymmetric Density Dependence Shapes Species Abundances in a Tropical Tree Community. Science 329:330–332.

Connell, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. Dynamics of populations:298–312.

Corbin, J. D., and C. M. D'Antonio. 2004. Competition between native perennial and exotic annual grasses: Implications for an historical invasion. Ecology 85:1273–1283.

Delaye, L., G. García-Guzmán, and M. Heil. 2013. Endophytes versus biotrophic and necrotrophic pathogens—are fungal lifestyles evolutionarily stable traits? Fungal Diversity 60:125–135.

Dormann, C. F. 2011. How to be a specialist? Quantifying specialisation in pollination networks - ProQuest. Network Biology 1.1:1.

Dormann, C. F., J. Fruend, and B. Gruber. 2016. bipartite: Visualising Bipartite Networks and Calculating Some (Ecological) Indices.

Fisher, R. A., A. S. Corbet, and C. B. Williams. 1943. The Relation Between the Number of Species and the Number of Individuals in a Random Sample of an Animal Population. Journal of Animal Ecology 12:42–58.

Gardener, M. 2014. Community Ecology: Analytical Methods Using R and Excel. Pelagic Publishing Ltd.

Gardes, M., and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113–118.

Geraci, M. 2016. lqmm: Linear Quantile Mixed Models.

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

Gilbert, G. S. 2005. Biotic interactions in the tropics: their role in the maintenance of species diversity. Pages 141–164 Dimensions of plant disease in tropical forests.

Gilbert, G. S., and C. O. Webb. 2007. Phylogenetic signal in plant pathogen–host range. Proceedings of the National Academy of Sciences 104:4979–4983.

Harpole, W. S., D. L. Potts, and K. N. Suding. 2007. Ecosystem responses to water and nitrogen amendment in a California grassland. Global Change Biology 13:2341–2348.

Havis, N. D., J. K. M. Brown, G. Clemente, P. Frei, M. Jedryczka, J. Kaczmarek, M. Kaczmarek, P. Matusinsky, G. R. D. McGrann, S. Pereyra, M. Piotrowska, H. Sghyer, A. Tellier, and M. Hess. 2015. Ramularia collo-cygni—An Emerging Pathogen of Barley Crops. Phytopathology 105:895–904.

Hersh, M. H., R. Vilgalys, and J. S. Clark. 2012. Evaluating the impacts of multiple generalist fungal pathogens on temperate tree seedling survival. Ecology 93:511–520.

Hervé, M. 2017. RVAideMemoire: Testing and Plotting Procedures for Biostatistics.

700 Higginbotham, S., W. R. Wong, R. G. Linington, C. Spadafora, L. Iturrado, and A. E. Arnold.
701 2014. Sloth Hair as a Novel Source of Fungi with Potent Anti-Parasitic, Anti-Cancer and
702 Anti-Bacterial Bioactivity. PLOS ONE 9:e84549.
703 Janzen, D. H. 1970. Herbivores and the number of tree species in tropical forests. The American
704 Naturalist 104:501–528.
705 Jost, L. 2006. Entropy and diversity. Oikos 113:363–375.
706 Kang, S., M. A. Mansfield, B. Park, D. M. Geiser, K. L. Ivors, M. D. Coffey, N. J. Grünwald, F.
707 N. Martin, C. A. Lévesque, and J. E. Blair. 2010. The Promise and Pitfalls of Sequence-
708 Based Identification of Plant-Pathogenic Fungi and Oomycetes. Phytopathology
709 100:732–737.
710 Kindt, R., and R. Coe. 2005. BiodiversityR. Tree diversity analysis. A manual and software for
711 common statistical methods for ecological and biodiversity studies. World Agroforestry
712 Centre (ICRAF), Nairobi.
713 Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in
714 communities. Nature 417:67–70.
715 Kluger, C. G., J. W. Dalling, R. E. Gallery, E. Sanchez, C. Weeks-Galindo, and A. E. Arnold.
716 2008. Host generalists dominate fungal communities associated with seeds of four
717 neotropical pioneer species. Journal of Tropical Ecology 24:351–354.
718 Koenker, R. 2017. quantreg: Quantile Regression. R, Vienna.
719 Kosmidis, I. 2013. brglm: Bias reduction in binomial-response Generalized Linear Models.
720 Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2016. lmerTest: Tests in Linear
721 Mixed Effects Models.

722 Lafferty, K. D., S. Allesina, M. Arim, C. J. Briggs, G. De Leo, A. P. Dobson, J. A. Dunne, P. T.
723 J. Johnson, A. M. Kuris, D. J. Marcogliese, N. D. Martinez, J. Memmott, P. A. Marquet,
724 J. P. McLaughlin, E. A. Mordecai, M. Pascual, R. Poulin, and D. W. Thieltges. 2008.
725 Parasites in food webs: the ultimate missing links. Ecology Letters 11:533–546.
726 Lemon, J. 2006. Plotrix: a package in the red light district of R. R-News 6:8–12.
727 Magurran, A. E. 2013. Measuring Biological Diversity. John Wiley & Sons.
728 Mangan, S. A., S. A. Schnitzer, E. A. Herre, K. M. L. Mack, M. C. Valencia, E. I. Sanchez, and
729 J. D. Bever. 2010. Negative plant-soil feedback predicts tree-species relative abundance
730 in a tropical forest. Nature 466:752–755.
731 May, R. M. 1991. A fondness for fungi. Nature 352:475–476.
732 McDonald, B. A., and E. H. Stukenbrock. 2016. Rapid emergence of pathogens in agro-
733 ecosystems: global threats to agricultural sustainability and food security. Phil. Trans. R.
734 Soc. B 371:20160026.
735 Mitchell, C. E., D. Tilman, and J. V. Groth. 2002. Effects of grassland plant species diversity,
736 abundance, and composition on foliar fungal disease. Ecology 83:1713–1726.
737 Mordecai, E. A. 2011. Pathogen impacts on plant communities: unifying theory, concepts, and
738 empirical work. Ecological Monographs 81:429–441.
739 O'Brien, H. E., J. L. Parrent, J. A. Jackson, J.-M. Moncalvo, and R. Vilgalys. 2005. Fungal
740 Community Analysis by Large-Scale Sequencing of Environmental Samples. Applied
741 and Environmental Microbiology 71:5544–5550.
742 Oksanen, J., R. Kindt, P. Legendre, and B. O'Hara. 2016. vegan: Community Ecology Package.
743 Packer, A., and K. Clay. 2000. Soil pathogens and spatial patterns of seedling mortality in a
744 temperate tree. Nature 404:278–281.

745 Parker, I. M., and G. S. Gilbert. 2007. When there is no escape: the effects of natural enemies on
746 native, invasive, and noninvasive plants. Ecology 88:1210–1224.
747 Parker, I. M., M. Saunders, M. Bontrager, A. P. Weitz, R. Hendricks, R. Magarey, K. Suiter, and
748 G. S. Gilbert. 2015. Phylogenetic structure and host abundance drive disease pressure in
749 communities. Nature 520:542–544.
750 Petermann, J. S., A. J. F. Fergus, L. A. Turnbull, and B. Schmid. 2008. Janzen-Connell effects
751 are widespread and strong enough to maintain diversity in grasslands. Ecology 89:2399–
752 2406.
753 R Development Core Team. 2014. R: A Language and Environment for Statistical Computing. R
754 Foundation for Statistical Computing, Vienna, Austria.
755 Rossi, J.-P. 2011. rich: an R package to analyse species richness. Diversity 3:112–120.
756 Rottstock, T., J. Joshi, V. Kummer, and M. Fischer. 2014. Higher plant diversity promotes higher
757 diversity of fungal pathogens, while it decreases pathogen infection per plant. Ecology
758 95:1907–1917.
759 Roy, B. A., K. Hudson, M. Visser, and B. R. Johnson. 2014. Grassland fires may favor native
760 over introduced plants by reducing pathogen loads. Ecology 95:1897–1906.
761 Sarmiento, C., P.-C. Zalamea, J. W. Dalling, A. S. Davis, S. M. Stump, J. M. U'Ren, and A. E.
762 Arnold. 2017. Soilborne fungi have host affinity and host-specific effects on seed
763 germination and survival in a lowland tropical forest. Proceedings of the National
764 Academy of Sciences:201706324.
765 Schweizer, D., G. S. Gilbert, and K. D. Holl. 2013. Phylogenetic ecology applied to enrichment
766 planting of tropical native tree species. Forest Ecology and Management 297:57–66.

767 Sedio, B. E., and A. M. Ostling. 2013. How specialised must natural enemies be to facilitate
768 coexistence among plants? Ecology Letters 16:995–1003.
769 Sinclair, J. B., and O. D. Dhingra. 1995. Basic Plant Pathology Methods. CRC Press, Boca
770 Raton, FL.
771 Spear, E. R. 2017. Phylogenetic relationships and spatial distributions of putative fungal
772 pathogens of seedlings across a rainfall gradient in Panama. Fungal Ecology 26:65–73.
773 Spear, E. R., P. D. Coley, and T. A. Kursar. 2015. Do pathogens limit the distributions of tropical
774 trees across a rainfall gradient? Journal of Ecology 103:165–174.
775 U'Ren, J. M., F. Lutzoni, J. Miadlikowska, and A. E. Arnold. 2010. Community Analysis
776 Reveals Close Affinities Between Endophytic and Endolichenic Fungi in Mosses and
777 Lichens. Microbial Ecology 60:340–353.
778 Vavrek, M. J. 2011. fossil: palaeoecological and palaeogeographical analysis tools.
779 Palaeontologia Electronica 14:1T.
780 Whitaker, B. K., J. T. Bauer, J. D. Bever, and K. Clay. 2017. Negative plant-phyllosphere
781 feedbacks in native Asteraceae hosts – a novel extension of the plant-soil feedback
782 framework. Ecology Letters in press:n/a-n/a.
783 Wickham, H. 2007. Reshaping data with the reshape package. Journal of Statistical Software 21.
784 Wickham, H. 2009. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.
785 Wickham, H. 2011. The split-apply-combine strategy for data analysis. Journal of Statistical
786 Software 40:1–29.
787

788 **Table 1.** Collection details, richness and diversity of fungal species, and specialization for the grass species sampled. The grass
789 species were not evenly sampled (10 to 223 tissue pieces per host, median = 61). The isolation frequency (percent of tissue pieces with
790 fungal growth) also differed among hosts (11.9% to 63.2%, median = 28.8%). As a result, 50% of the 290 successfully sequenced
791 isolates originated from *Stipa pulchra.*

Grass spp.	**No. of unique tissue pieces collected**	**Isolation frequency**	**No. of sequenced isolates**	**Obs. richness of fungal spp. (% singletons)**	**iChao1 est. lower bound of richness (95% CI)**	**Fisher's alpha index of diversity (SE)**	**Specialization index (d')[a]**	**No. of transects from which tissue pieces were collected (and sequenced isolates originated)**
Avena barbata†	116	28.4%	30‡	7 (43%)	9.9 (7.35, 31.02)	2.87 (1.37)	0.462	16 (12)
Avena fatua	59	28.8%	16‡	10 (80%)	40 (14.53, 208.51)	11.41 (6.24)	NA	7 (5)
Brachypodium distachyon	10	40%	4	1 (0%)	1 (1, 1.28)	0.43 (0.55)	NA	1 (1)
Bromus diandrus†	107	19.6%	22	7 (57%)	12.73 (7.92, 42.78)	3.54 (1.78)	0.302	17 (8)

Bromus hordeaceus†	101	11.9%	11‡	7 (57%)	11.39 (7.76, 32.25)	8.29 (5.5)	0.2	17 (7)
Elymus glaucus†	53	60.4%	33‡	9 (44%)	12.21 (9.59, 26.52)	4.08 (1.76)	0.665	7 (6)
Festuca perennis	42	26.2%	11	8 (88%)	27.09 (12.68, 85.87)	13.19 (9.33)	NA	13 (9)
Phalaris aquatica†	61	31.1%	18‡	9 (67%)	24.67 (13.21, 67.25)	7.16 (3.64)	NA	8 (6)
Stipa pulchra†	223	63.2%	145‡	26 (42%)	43.27 (32.35, 72.95)	9.23 (2.22)	0.327	21 (19)
All	772	37.6%	290‡	41 (54%)	285.78 (85.91, 1375.24)	13.03 (2.44)	NA	24 (24)

We were unable to successfully sequence 12 of the original 302 fungal isolates. For two of the 290 successfully sequenced isolates, we could only generate unidirectional reads.

†The six focal grass species for which pathogen-caused damage was censused.

‡The number of successfully sequenced isolates was lower than the number of unique isolates cultivated.

[a] The specialization index d' ranges from 0 (perfect generalist) to 1 (perfect specialist) (Dormann 2011).

798 **Table 2.** Host specialization of the 17 non-singleton fungal species from symptomatic leaves of
799 five grass species with independent estimates of relative abundance in JRBP. Fungal species are
800 sorted by abundance from common to rare. Thirteen (76%) of the non-singleton fungal species
801 were isolated from multiple hosts. The specialization index d' represents the extent to which the
802 grass species attacked deviated from random given the availability of the grass species, varying
803 from 0 (perfect generalist) to 1 (perfect specialist) (Dormann 2011).

Estimated taxonomic placement	**Fungal sp. code**	**No. of isolates**	**No. of obs. host spp.**	**Specialization of fungi on grasses (d')**
Ramularia cf. *collo-cygni*	B1	42	3	0.3
Alternaria infectoria species–group	C1	26	5	0.107
Pyrenophora lolii	A2	23	5	0.176
Parastagonospora nodorum	D	21	3	0.301
Pyrenophora tritici-repentis	E	21	2	0.924
Pyrenophora chaetomioides	A1	20	4	0.149
Pyrenophora fugax	F	16	3	0.346
Pyrenophora sp.	G	14	3	0.241
Lentitheciaceae sp.	I	12	1	0.409
Cladosporium cladosporioides species complex	H1	8	2	0.384
Pyrenophora biseptata	K	5	3	0.021
Parastagonospora allouniseptata	AN	5	2	0.465
Pyrenophora nobleae	J	4	1	0.398
Cladosporium herbarum species complex	H2	3	2	0.307
Nemania serpens	M	3	2	0.423
Parastagonospora sp.	AS	3	1	1
Alternaria eureka	Q	2	1	0.347

804 **Figure Captions**

805 **Figure 1**. Mechanism by which host-specific pathogen species or communities can promote
806 diversity via the Janzen-Connell hypothesis. The necessary conditions for pathogens to promote
807 host community diversity are: (1) pathogen damage must increase with host species relative
808 abundance, and (2) pathogen damage must reduce fitness, and in turn population growth.
809 Frequency-dependent pathogen damage (Condition 1) may occur because: (A) pathogen species
810 are host-specialized, (B) pathogen communities differentiate among host species, or (C) multi-
811 host pathogens cause host-specific impacts. When these conditions are met, pathogens cause per-
812 capita population growth rates to decline with relative abundance, stabilizing species
813 coexistence. If pathogens were removed, per-capita growth rates would decline less steeply with
814 relative abundance, making stable coexistence less likely.

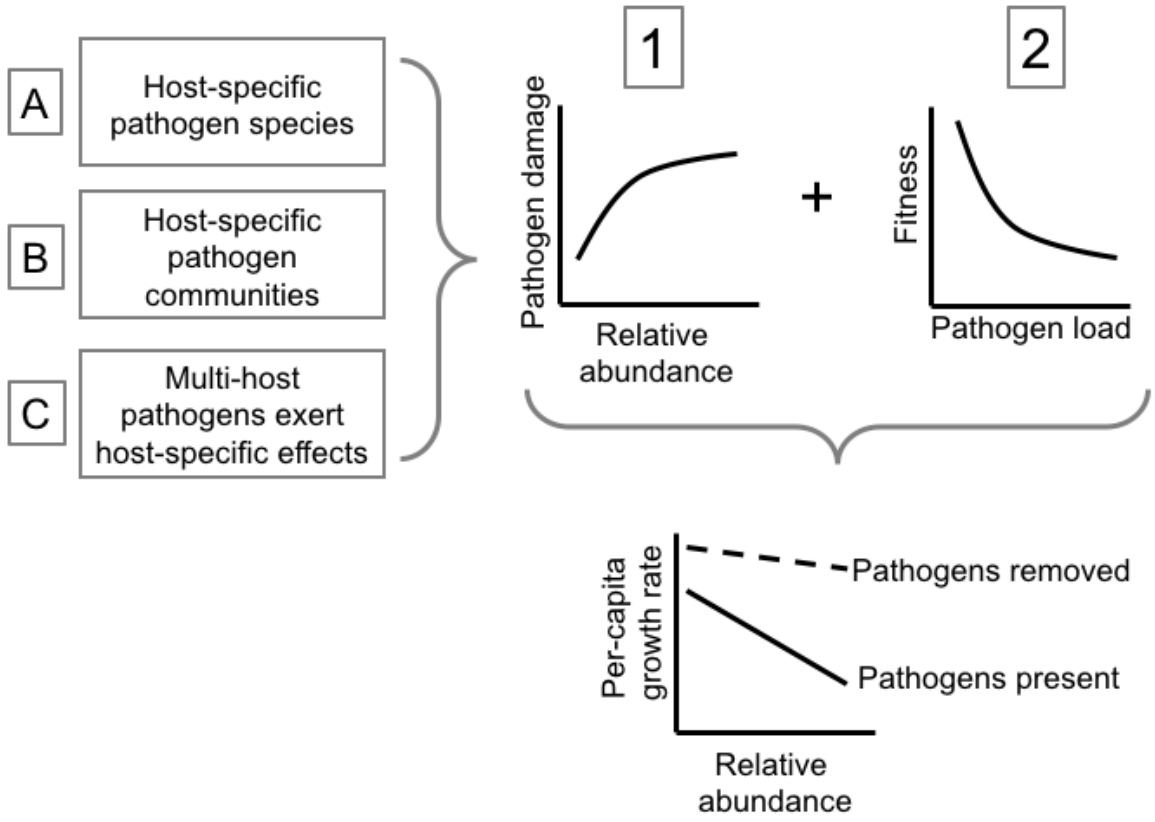
815 **Figure 2.** Relationship between focal species frequency in the plot and mean percent of leaf area
816 damaged in March on individual plants growing in the perennial transects, for six focal species.

817 **Figure 3.** Bipartite network between the nine sampled grass species (left) and the 41 observed
818 fungal pathogen species (right) in JRBP (N = 290 isolates). The widths of the connections are
819 proportional to the number of times a given fungal species was isolated from a given grass
820 species. The 17 fungal species for which the weighted specialization index d' could be calculated
821 are marked with a letter code indicative of their specificity (L = low, M = moderate, H = high).
822 The 14 fungal species for which pathogenicity was experimentally confirmed are marked with an
823 asterisk.

824 **Figure 4.** Non-metric multidimensional scaling plot depicting differences in fungal pathogen
825 community composition (N = 99 isolates, 21 fungal species) among five grass species (stress =
826 0.101). Data points represent grass species-by-transect combinations, with 95% confidence

827 ellipses around the centroid of each grass species. The point sizes represent the goodness of fit
828 for each point (i.e., the correlation between the plotted distances and the observed community
829 dissimilarities [distances]), with smaller points indicating a better fit.
830 **Figure 5.** Relationships between pathogen damage in March and April, species frequency, and
831 seed production, represented as z-scores. Z-scores were calculated by subtracting the species-
832 level mean and dividing the difference by the species-level standard deviation, for ease of
833 comparison across species. Mean percent damage in March versus seed production (top left),
834 mean percent damage in April versus seed production (bottom left), mean percent damage in
835 March versus April (top right), and species frequency in the community versus seed production
836 (bottom right). Focal species are abbreviated as *Avena barbata* (AB; light blue), *Bromus*
837 *diandrus* (BD; dark blue), *Bromus hordeaceus* (BH; light green), *Elymus glaucus* (EG; dark
838 green), and *Stipa pulchra* (SP; red) (seed production was not measured on *Phalaris aquatica*).
839
840

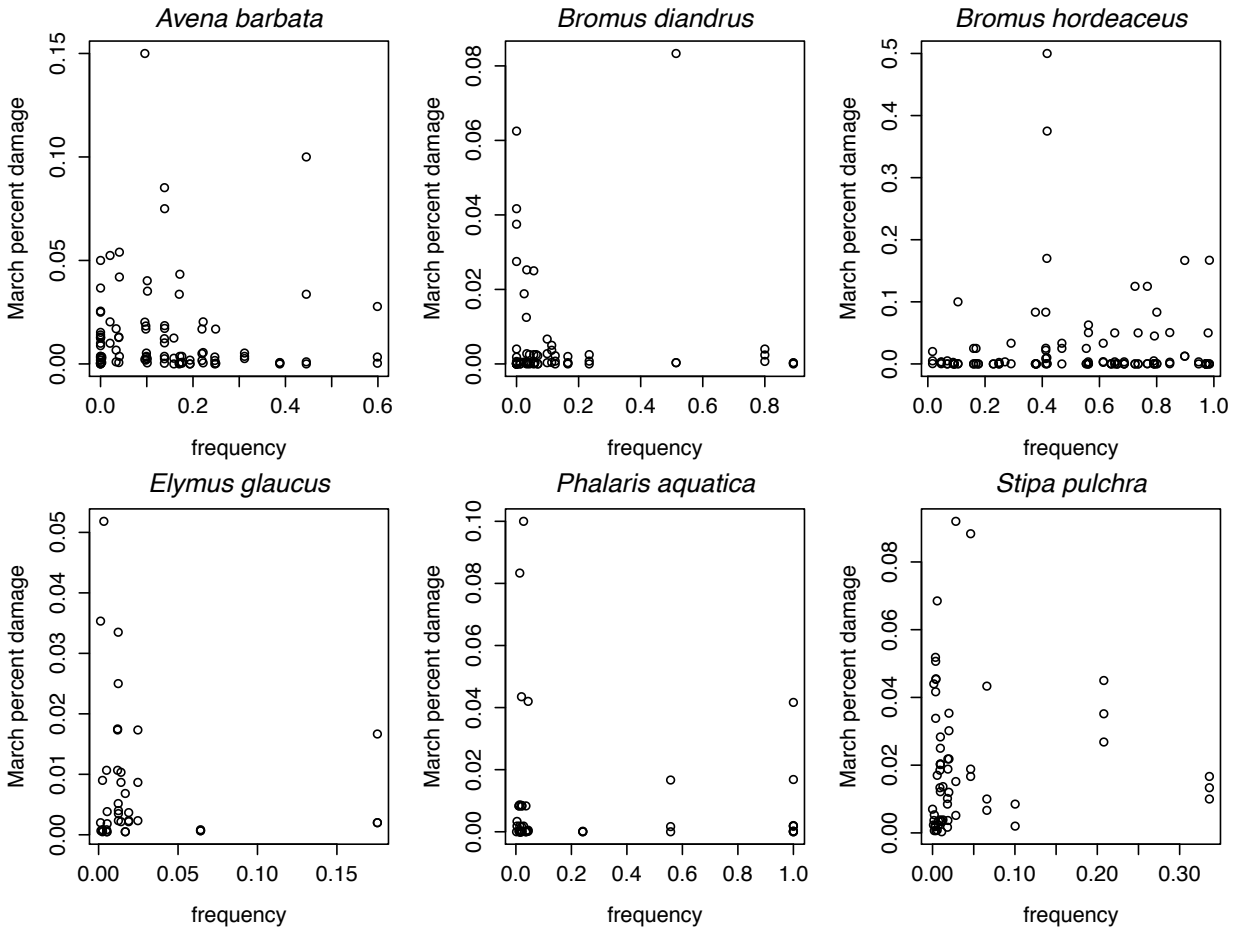
841 **Figure 1**



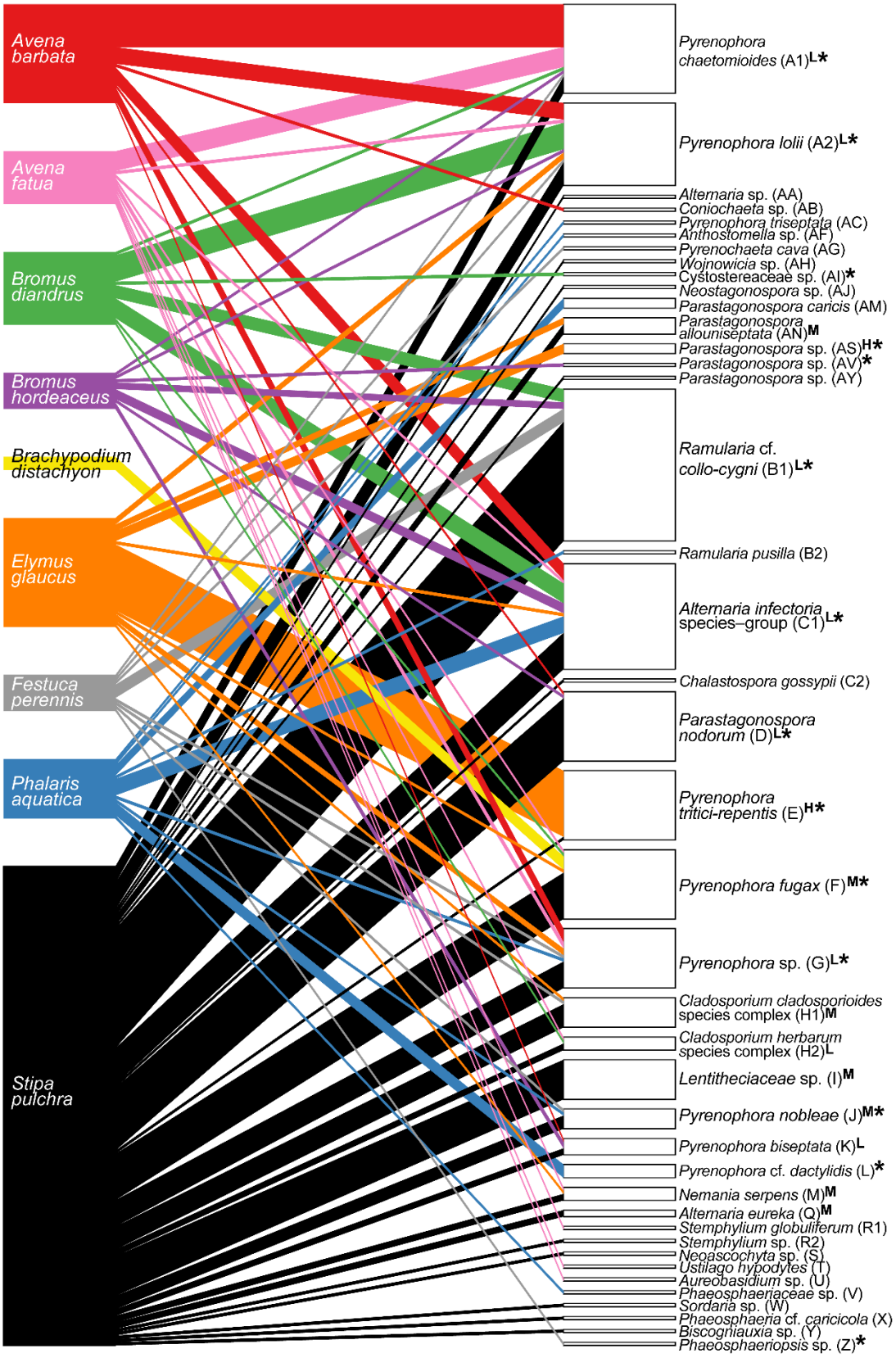
842

843

Figure 2

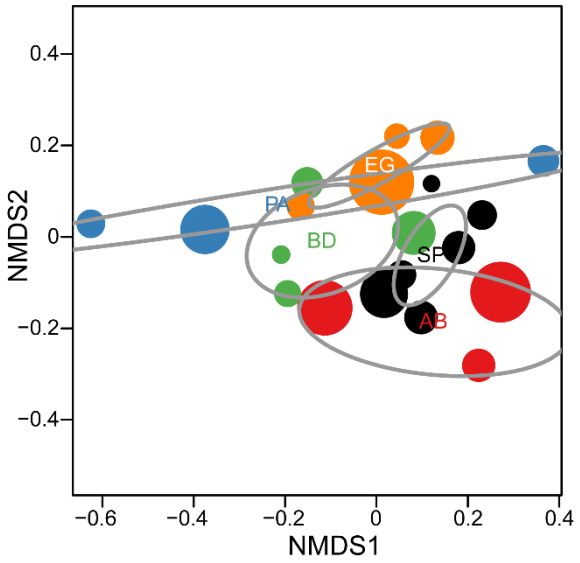

Avena barbata
Bromus diandrus
Bromus hordeaceus
Elymus glaucus
Phalaris aquatica
Stipa pulchra
March percent damage
frequency

855 **Figure 3**



856

857 **Figure 4**



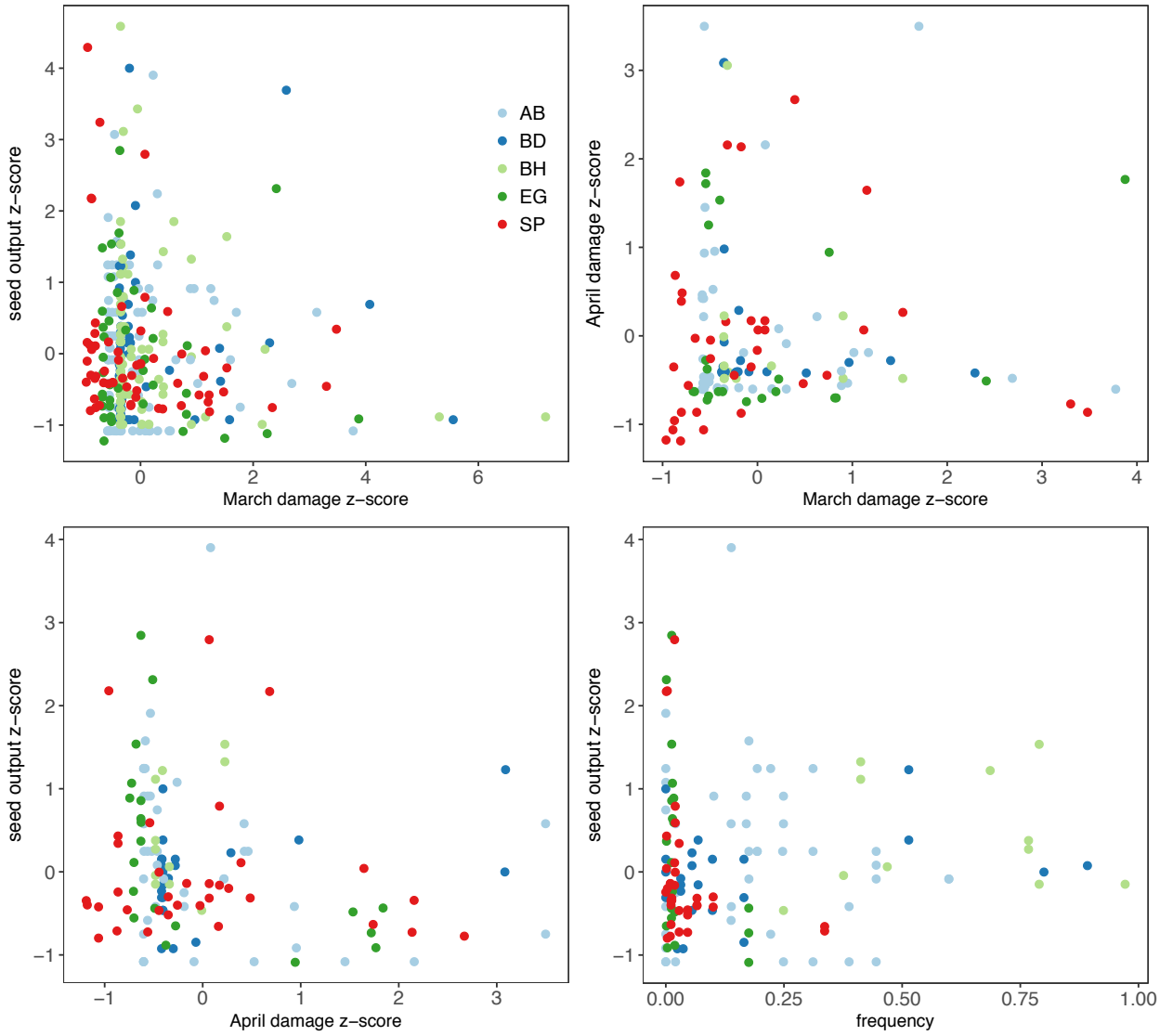
858

859

860

861

Figure 5


AB
BD
BH
EG
SP
seed output z−score
March damage z−score
April damage z−score
frequency