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

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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 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.
Introduction

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

At the same time, there is mounting empirical evidence that fungal pathogens of plants in natural systems infect multiple hosts (Gilbert and Webb 2007, Kluger et al. 2008, Hersh et al. 2012, Spear 2017). Most plant pathogens (particularly fungi and bacteria) are passively dispersed. In diverse plant communities where the nearest neighbors may be heterospecifics, selection should favor pathogens that can infect multiple host species (i.e., multi-host pathogens) (May 1991). Broad host ranges can preclude any relationship between the relative abundance of a single host species and pathogen attack rates. Thus we hypothesize that most plant pathogens are relatively host generalized (Spear et al. 2015, Spear 2017) and that their impacts do not strongly respond to the relative abundance of a single host species. If this is the case, then, in
contrast to predictions of the Janzen-Connell hypothesis, many pathogens may play little role in maintaining local host diversity and may even promote competitive exclusion or spatial turnover of species (Mordecai 2011, Spear et al. 2015).

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

In sum, pathogens stabilize host species coexistence when they disproportionately reduce the population growth of common species, thereby benefiting rare species. This requires that: (1) pathogens disproportionately damage relatively common hosts, such that the amount or severity of damage responds to host relative abundance; and (2) pathogen damage reduces 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 empirically evaluate the evidence for these conditions—
frequency-dependent pathogen damage, pathogen host specificity, and pathogen impacts on population growth—for foliar pathogens that infect co-occurring native and exotic grasses in a Northern California grassland. Specifically, we evaluated these conditions across six common plant species by (i) surveying pathogen damage and linking it to plant species relative abundance, (ii) surveying fungal community composition and pathogen sharing across host species, and (iii) measuring 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 the grasslands of Stanford University’s Jasper Ridge Biological Preserve (JRBP), a 485-ha site in San Mateo County, CA (37°24’N, 122°13’30”W; 66-207 m). JRBP has a Mediterranean climate, with cool (mean temperature 9.2°C), wet winters and warm (mean temperature 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 conducted our study during the 2015 growing season, from March 19 – May 4.

Our study focused on the identities and impacts of fungal pathogens of six locally 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 glaucus*. In addition to the six focal species, we isolated fungi from but did not assess damage for three locally common exotic grass species: *Avena fatua*, *Brachypodium distachyon*, and *Festuca perennis*. *A. barbata, A. fatua, B. distachyon, B. hordeaceus, B. diandrus, F.*
**Impacts of plant species relative abundance on pathogen damage (Condition 1)**

Perennis and *P. aquatica* are exotic annual and perennial grasses introduced to California in the mid-19th century (Corbin and D’Antonio 2004).

We assessed pathogen damage in response to host relative abundance (Condition 1), plant seed production in response to pathogen damage (Condition 2), and pathogen specialization and community composition (Conditions 1A-B). To evaluate the impact of pathogens on grasses under varying host species relative abundance (Condition 1), we assessed pathogen-caused damage across 10 transects (yellow points in Fig. S1) that were established in areas where perennial species (either *S. pulchra*, *E. glaucus*, or *P. aquatica*) vary in their abundance, ranging from rare to common in a given 1-m² area; hereafter referred to as ‘perennial density transects’.

We also harvested seeds from a subset of marked individuals on which we also surveyed damage (Condition 2). The marked grasses from which seeds were harvested had various levels of damage and local abundances. We surveyed fungal pathogen community composition (Conditions 1A-B) by culturing fungi from symptomatic leaves collected along the 10 perennial density transects and an additional 14 transects that spanned a range of plant community composition, geographic location, and soil types, capturing a broader range of growing conditions (24 transects in total; yellow and red points in Fig. S1). Three of the pathogen survey transects ran through the Jasper Ridge Global Change Experiment (GCE), a multifactorial experiment manipulating water, nitrogen, temperature, and CO₂, where we sampled in ambient and water addition plots. Because water addition had no impact on fungal community composition (results not reported), we include isolates from the water addition plots in the full fungal community dataset.
We visually censused fungal pathogen-caused foliar damage for the six focal grass species (A. barbata, B. diandrus, B. hordeaceus, E. glaucus, P. aquatica, and S. pulchra) along the 10 perennial density transects (yellow points in Fig. S1), documenting percent leaf area damaged. We surveyed damage using a hierarchical sampling scheme: as possible, multiple plants per transect and multiple (up to six) haphazardly-selected leaves per plant. To quantify variation in pathogen damage within the growing season, the 10 perennial density transects were censused twice: first, between March 11 and 16 (444 marked grass individuals) and second, between April 17 and 20 (163 of the marked grasses). During each census, we sampled plants of all focal species present in each plot, averaging a total of 8.9 samples from 3.2 species per plot in March and 5.4 samples from 2.5 species per plot in April. For each plant, we calculated an average percent damage estimate across all sampled leaves (our main response variable) and a proportion of sampled leaves that had damage.

We tested whether pathogen damage responded to host relative abundance (Condition 1) while controlling for other potential predictors: plant species, time within the growing season, and the sampling structure. The response variables for the models were mean percent leaf area damaged, which we refer to as ‘mean damage’ hereafter, and proportion of leaves damaged (i.e., any amount of pathogen damage). We used mixed-effects linear models (lmer function in the lme4 package; Bates et al. 2014) to test for interactive effects of host species, plant species relative abundance at the plot scale (i.e., frequency), and sampling date, with plot number nested within transect as random effects (i.e., random intercepts). For models of mean damage, we used normally-distributed errors because this measurement represents an average of up to six values between zero and one. For models of proportion of leaves damaged we used binomial errors. We assessed the most important predictors of pathogen damage by comparing the Akaike
Information Criterion (AIC) values of nested models that included the nested random effects accounting for sampling structure and the following combinations of fixed effects: sampling month * species + frequency; sampling month * species; species + frequency; sampling month + species; species. Because plant species composition may influence pathogen damage at several scales, we also built models of pathogen damage as a function of plant composition at a larger scale by including a categorical variable indicating plant species dominance at the sub-transect scale. Since plant composition at this scale had no influence on pathogen damage, we do not discuss the results further (the model is included in the R code file that accompanies this paper).

Isolating foliar fungal pathogens (Conditions 1A-B)

We assessed the host specialization and community structuring of putative fungal pathogens causing leaf damage by isolating fungi in pure culture. Between March 19 and May 4, 2015, we collected 772 segments of symptomatic foliar tissue (e.g., with chlorotic spots, necrotic lesions, etc.) from nine grass species along 24 transects in JRBP (yellow and red points in Fig. S1). We collected one piece of symptomatic leaf tissue per grass individual. Sampling intensity varied among grass species and transects (Tables 1 and S1). To isolate the putative fungal pathogens in culture, we excised a small portion of tissue (< 2 mm²) from the advancing margin of disease for each leaf piece collected, surface-sterilized the excised tissue (sequential washes of 70% EtOH and 10% bleach; 60 s each), and plated it on malt extract agar with 2% chloramphenicol (2% MEA). We monitored fungal growth under ambient lab conditions for up to four weeks. We isolated emergent hyphae into axenic culture on 2% MEA based on morphology and pigmentation. To verify the efficacy of the surface sterilization, we pressed six
treated leaf pieces onto 2% MEA. After two weeks, we observed no growth from the imprints.

Reference strains are maintained in a living culture collection in the Mordecai lab, Department of Biology, Stanford University (Stanford, CA, USA). The California Department of Food and Agriculture approved the collection, transport, and storage of isolates (permit no. 3160).

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

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

We manually inspected and edited all reads. Bidirectional reads were automatically assembled into a consensus sequence, using a minimum of 20% overlap and 85% sequence similarity. We then clustered the 288 consensus sequences and two unidirectional sequences into operational taxonomic units (OTUs) based on a minimum of 40% overlap and 90, 95, 97 and 99% sequence similarity. We used Sequencher (Gene Codes, Ann Arbor, MI) for sequence editing and assembly and OTU designations. All sequence data will be submitted to GenBank (accession numbers XXXX-XXXX).
Because percent sequence similarity for the ITS region varies within versus between fungal species (as described in O’Brien et al. 2005) and given the limitations of GenBank (Kang et al. 2010, U’Ren et al. 2010), we conducted phylogenetic analyses to improve our inference of taxonomic placement. We partitioned OTUs with similar sequences into datasets (1-52 JRBP isolates per dataset), and generated a phylogenetic tree for each dataset following the procedures described in Higginbotham et al. (2014) and Spear (2017). Because not all sequences mapped onto named fungal species, we assigned each operational species a unique species code, which we list in parentheses after the lowest estimated taxonomic placement (Table S2). We treated isolates belonging to a species complex as a single species.

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

We conducted several analyses of the fungal community. First, we describe sampling efficacy and fungal species richness and diversity. Second, we compare fungal communities across host species. Third, we explicitly consider the frequency of shared pathogens from the grass species’ perspective. Finally, we evaluate host specialization from the fungal species’ perspective.

For the full fungal community dataset \( (N = 290) \), we report (i) sampling efficacy, (ii) the observed richness of taxa (and percent singletons), (iii) the estimated lower bound of true species richness, (iv) the relative-abundance distributions, and (v) the diversity of taxa. To assess sampling efficacy (i), we generated accumulation curves, for the OTUs based on sequence similarity and for the fungal species based on phylogenetic analyses, with a method that finds the mean richness when sites are added in random order. We estimated the lower bound of true species richness (iii) with the iChao1 estimator, which relies on the number of single-,
triple- and quadrupletons to estimate the number of unseen species in the community and correct for the negative bias associated with under-sampling in highly diverse assemblages (Chiu et al. 2014). We visualized the richness, evenness, and dominant species of the fungal community by plotting species abundance versus species rank abundance (iv). Fungal species isolated ten or more times were defined as abundant. We estimated diversity (v) based on Fisher’s alpha, which is robust to unequal sample sizes (Fisher et al. 1943, Magurran 2013), the Shannon index, which is sensitive to sample size (Magurran 2013), and the effective number of species (Jost 2006) to allow for comparison with other studies.

To compare fungal pathogen communities across host species (Conditions 1A-B), we report richness and diversity metrics (ii-v) for each grass species ($N = 290$ isolates). Additionally, we assessed differences in community composition among grass species (Conditions 1A-B) via a permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) and visualized the differences using non-metric multidimensional scaling (NMDS). The PERMANOVA (adonis function) tested for significant differences in fungal community composition among the grass species (Oksanen et al. 2016). As adonis is sensitive to heterogeneity of variances, we used the betadisper function and ANOVAs to test whether the fungal communities of grass species had similar dispersions (Oksanen et al. 2016). We identified significant differences in fungal community composition between pairs of grass species using pairwise PERMANOVAs with the False Discovery Rate (FDR) adjustment for multiple comparisons (Hervé 2017). For the NMDS, PERMANOVAs, and tests of homogeneity of variance, we considered each focal grass species-by-perennial density transect combination to be a distinct community. We excluded grass species-by-transect communities for which fewer than three isolates were collected and $B. hordeaceus$ communities because only one species-by-transect community had the minimum
number of isolates (in total, 20 communities, 21 fungal species, 5 grass species, and 99 isolates were included in the dataset). From the resulting raw data matrix, we created a matrix of pairwise dissimilarities in fungal community composition for use with the NMDS, PERMANOVAs, and homogeneity of variances test. The dissimilarity matrix was created via function vegdist with the Chao method, which implements an abundance-based Jaccard index that was adjusted to consider unseen species (Chao et al. 2005, Oksanen et al. 2016).

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

To assess the specialization of the fungal species (Condition 1A) and the uniqueness of each grass species’ fungal community (Condition 1B), we visualized all interactions (links) with a bipartite plot and then calculated species-level interaction specialization via the weighted specialization index d'. The index illustrates the degree to which a species deviates from expected, random interactions by comparing interaction frequencies to the overall availability of potential partners (the marginal frequencies) (Blüthgen et al. 2006). As we did not sample the grass species proportional to their relative abundances in JRBP, the total number of fungal isolates cultivated from a grass species (marginal frequency) was not a suitable proxy for its availability. Thus, to improve the accuracy of our calculations, we only included the five focal grass species (A. barbata, B. diandrus, B. hordeaceus, S. pulchra, and E. glaucus) with independent, preserve-wide estimates of their relative abundances (based on 30 10-m diameter circular plots; see the accompanying R code for the abundance values) in the dataset. We then
incorporated the abundance estimates into the analysis in lieu of the number of isolates collected per grass species (Blüthgen et al. 2006, Dormann et al. 2016). Additionally, we excluded singleton fungal species as their host range and degree of specialization were likely to be misinterpreted (Dormann 2011) (dataset for network analysis: \( N = 228 \) isolates, representing 17 fungal species). The d' index ranges from zero (perfect generalist) to one (perfect specialist) (Dormann 2011). Consistent with Blüthgen et al. (2006), we considered 0-0.33, 0.34-0.67, and 0.68-1 to be low, moderate, and high specialization, respectively.

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

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

One advantage of our culture-based approach to fungal identification was that it allowed us to verify the pathogenicity of the fungal isolates through experimental inoculations, since taxonomic affiliations do not necessarily indicate pathogenicity (Delaye et al. 2013). We experimentally inoculated 99 isolates from symptomatic leaves onto plants of the host species from which they were originally isolated (Table S3). The isolates tested represented 35 of the 41 observed fungal species, including all of the common (isolated \( \geq 10 \) times) fungal species, and originated from eight of the nine grass species surveyed. Disease development is contingent on the presence of a susceptible host and competent pathogen, and environmental conditions that compromise the host and favor the pathogen (Barrett et al. 2009). Given natural intraspecific variation in host susceptibility and pathogen aggressiveness and that our experiments may not
have replicated the abiotic conditions that originally led to disease development in JRBP, we
tested multiple isolates of some of the non-singleton fungal species to avoid misclassifying a
fungal species as nonpathogenic. In Stanford greenhouses, we inoculated apparently healthy
grass leaves by securing colonized 2% ME agar plugs against leaves with small strips of
Parafilm (Sinclair and Dhingra 1995). Uncolonized agar plugs were used as negative paired
controls. We completed two rounds of inoculations. In round one, we inoculated each plant with
4-5 isolates and a negative control, with each treatment applied to a unique leaf (5-6 treated
leaves/plant; 9-12 leaves/treatment). In round two, we applied a single isolate or control
treatment to each individually-potted plant (4 unique leaves per plant and 5 plants per treatment,
totaling 20 leaves per treatment). Thirteen of the 99 isolates tested were tested in both rounds
(Table S3). After monitoring plants for up to one week, we assessed pathogenicity by censusing
leaves for obvious symptoms of disease (e.g., lesions or necrosis). To compare the proportion of
leaves with disease for a given fungal isolate versus its paired control (same plant species and
inoculation date) we used bias-reduced generalized linear models (brglm function; Kosmidis
2013), assuming binomial error distributions and probit link functions (Table S3). The analyses
excluded all leaves for which it was unclear if tissue damage was the result of disease, resulting
in 5 to 20 leaves per treatment (Table S3). We deemed an isolate pathogenic if a significantly
greater proportion of inoculated leaves had pathogen damage than paired control leaves (using $\alpha$
$= 0.05$; Table S3).

**Impacts of pathogen damage on per-capita seed production (Condition 2)**

Pathogen damage may have fitness consequences for the host, including reduced
survival, growth, and seed output. To explore the relationship between plant demographics,
competition, and pathogen impact, we harvested seeds from 350 of the marked grasses from the perennial density transects. We measured per-capita seed production as a function of conspecific and heterospecific density and percent leaf area damaged for *Avena barbata*, *Bromus hordeaceus*, *Bromus diandrus*, *Stipa pulchra*, and *Elymus glaucus*. We did not measure seed production of *Phalaris aquatica* because it occurs in monotypic stands with little variation in relative abundance.

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

We used regression models to assess the extent to which pathogen damage correlated with per-capita seed output. To compare variation across species, we standardized both seed output and March and April percent damage by calculating z-scores (i.e., by subtracting the within-species mean and dividing by the within-species standard deviation). By doing so, we asked whether deviations from average pathogen damage correlated with deviations from average seed production, which would suggest pathogen fitness impacts. We modeled the relationships between standardized March or April damage estimates and standardized seed production (we did not include both March and April damage together in the same model because these were assessed on the same individuals with only a single seed output value). Models in which seed production was standardized by species but damage was not produced 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
and plant community at the plot and sub-transect scale (Tables S4-S5). *A. barbata* and *E. glaucus* had higher percent damage, particularly at the later sampling point (April). All species, except *A. barbata*, had a higher proportion of leaves damaged in April than in March, suggesting that pathogen damage accumulates through the growing season. Counter to Condition 1 for pathogens to promote coexistence, focal species frequency within the plot did not significantly affect pathogen damage (Fig. 2). The model that included that term had an AIC value only slightly above the minimum AIC, indicating similar performance (the model that includes frequency is shown in Table S4 for illustration).

Fungal pathogen identities and diversity (Conditions 1A-B)

We isolated fungi from 38% of the 772 foliar tissue pieces that we collected along 24 transects across Jasper Ridge Biological Preserve (JRBP). We successfully sequenced 290 of the resulting 302 isolates (Table 1). Fungi were isolated from all nine grass species. However, the grass species were unequally sampled and the isolation frequency (i.e., percent of tissue pieces with growth) differed among the hosts (Table 1).

The accumulation curves for the fungal species and OTUs (based on phylogenetic analyses and percent sequence similarity, respectively) were non-asymptotic, indicating incomplete sampling and a diverse community (*N* = 290; Fig. S3). Considering 90 - 99% sequence similarity, the isolates represented 28 - 49 operational taxonomic units (OTUs), respectively (Fisher’s alpha index for diversity: 7.28 - 16.39). Hereafter, we designate all fungal species taxonomic affiliations based on the phylogenetic analyses. The fungal isolates (*N* = 290) represented 41 fungal species (iChao1 estimated species richness: 285.78, 95% CI = 85.91, 1375.24; Fisher’s alpha for diversity: 13.03, 95% CI = 8.91, 18.58; Shannon diversity = 2.91,
95% CI = 2.75, 3.06; effective number of species = 18.29, 95% CI = 15.45, 21.14). Most of the
41 observed fungal species were rare (56% were singletons or doubletons) and relatively few
were abundant (22% were observed more than 10 times) (Fig. S4). The most commonly
observed genera included *Pyrenophora, Ramularia, Alternaria*, and *Parastagonospora* (Fig. 3
and Table S2). Using greenhouse-based inoculation experiments, we experimentally confirmed
the pathogenicity of 27% of the 99 isolates tested, representing 14 of the 35 fungal species tested,
eight of the nine common fungal species, and the most commonly observed genera in our survey
(Fig 3; Tables S2-S3).

**Fungal pathogen host associations (Conditions 1A-B)**

The observed and estimated lower bound of fungal species richness per grass species
(iChao1) ranged from one to 26 and from one to 43.27, respectively (Table 1; Figs. 3 and S5).
Unsurprisingly, the greatest richness (26 fungal species) was observed for the best-sampled grass
species with the highest isolation frequency, *S. pulchra* (Table 1). Controlling for sample size via
Fisher’s alpha index, fungal pathogen community diversity was greatest for *F. perennis, A. fatua,*
and *S. pulchra* (Table 1). For the eight grass species for which multiple fungal species were
observed (i.e., excluding *B. distachyon*; Table 1), most of the fungal species were observed at
intermediate to low frequencies (Table 1; Fig. S5).

The majority (74%) of the 19 non-singleton fungal species infected multiple hosts (Fig.
3). On average, the multi-host fungi were isolated from four grass species. Two of the fungal
species, *Pyrenophora lolii* (A2) and *Alternaria infectoria* species–group (C1), were isolated from
seven of the nine grass species that we sampled (Fig. 3). Concordantly, the weighted
specialization index d’ values suggest that the majority of the fungi had moderate to low
specificity (d' from 0.021 to 0.465, median d' = 0.346; for the nine common fungal species, median d' = 0.3; Table 2).

While the majority of the fungal species for which d' could be calculated had low to moderate host specificity, two had high host specificity: *Pyrenophora tritici-repentis* (E) and *Parastagonospora* sp. (AS) (d' = 0.924 and 1, respectively; Table 2; Fig. 3). *Pyrenophora tritici-repentis* (E) was isolated almost entirely from *Elymus glaucus* (the only exception was a single isolate from a *Stipa pulchra* individual growing in an *E. glaucus*-dominated transect plot) and it comprised 61% of the 33 fungal isolates from *E. glaucus* leaves, exhibiting strong host preference (Condition 1A). Two additional likely specialists were only isolated from the undersampled grass species *Phalaris aquatica* (N = 18 isolates): *Pyrenophora cf. dactylidis* (L) and *Parastagonospora carici* (AM) (Fig. 3). While we could not confidently calculate specialization index d' values, *Pyrenophora cf. dactylidis* (L) and *Parastagonospora carici* (AM) made up 39% of the few isolates originating from *P. aquatica* leaves, suggesting strong host preference (Condition 1A).

**Pathogen sharing and community similarity among grass species (Conditions 1A-B)**

The eight grasses species from which 10 or more isolates were collected shared at least one and up to eight fungal pathogen species with every other grass species (median = 3; Table S6; Fig. 3). As estimated by the Morisita-Horn index, the average estimated similarity between pairs of host species was moderate (42%) (min = 5% for *E. glaucus* and *P. aquatica*; max = 100% for *F. perennis* and *S. pulchra* and for *A. barbata* and *A. fatua*; Table S6). Six of the grass species shared their numerically dominant fungal species with one of the other grass species (Figs. 3 and S5).
Two of the grass species had a relatively low fungal community similarity with the other grass species in our study: *E. glaucus*, the second best-sampled grass species (*N* = 33; Morisita-Horn similarity index 5% to 11%), and *P. aquatica* (*N* = 18; Morisita-Horn similarity index 5% to 70%; Table S6; Fig. 3). Congruously, the weighted specificity index *d*' for *E. glaucus* suggests that the grass species hosted a relatively unique assemblage of fungal pathogens (*d*' = 0.665; Table 1).

The fungal pathogen communities associated with the leaves of the five focal grass species included in the PERMANOVA (*N* = 99 isolates) were significantly dissimilar (*F*<sub>4,15</sub> = 3.682, *R*<sup>2</sup> = 0.495, *p* = 0.001; Fig. 4). Specifically, (i) the fungal community associated with *S. pulchra* (32 isolates) was significantly different from those of *A. barbata* (11 isolates) (*P*<sub>adj</sub> = 0.037), *B. diandrus* (16 isolates) (*P*<sub>adj</sub> = 0.037), *E. glaucus* (31 isolates) (*P*<sub>adj</sub> = 0.04), and *P. aquatica* (9 isolates) (*P*<sub>adj</sub> = 0.037); (ii) the fungal community associated with *P. aquatica* (9 isolates) was significantly different from that of *B. diandrus* (16 isolates) (*P*<sub>adj</sub> = 0.05); and (iii) the fungal community associated with *B. diandrus* (16 isolates) was significantly different from that of *A. barbata* (11 isolates) (*P*<sub>adj</sub> = 0.05) (Fig. 4). The fungal communities of the grass species had similar dispersions (*F*<sub>4,15</sub> = 0.035, *p* = 0.997; Fig. 4).

**Impacts of pathogen damage on per-capita seed output (Condition 2)**

The relationships between pathogen damage and seed production were generally negative but highly variable, particularly at the low levels of pathogen damage that most of the plants in the survey experienced (Fig. 5). Because the variance was higher at low pathogen damage, we used quantile regression to estimate how the relationship between damage and seed production varied across individuals in the lower, middle, and upper quantile of the seed
production distribution. We found that increasing pathogen damage in April was associated with reduced seed output at the 50th percentile, but that the negative effect was not statistically significant for the 25th or 75th percentiles (Table S7; Fig. 5). In other words, plants of average seed production had significantly reduced seed output with increasing pathogen damage, but both high- and low- seed output individuals did not have a significant response to pathogen damage. The effect of March damage was weakly negative but not statistically significant for any quantile (Fig. 5). Focal species frequency had no association with either seed output (Fig. 5) or pathogen damage (Fig. 2). Much of the variation in seed production was not explained by pathogen damage in either month.

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

Discussion

Limited potential for pathogen-mediated coexistence
Pathogens are widely hypothesized to promote plant community diversity by generating negative frequency dependence (Augspurger 1983, Packer and Clay 2000, Petermann et al. 2008, Allan et al. 2010, Mangan et al. 2010, Bagchi et al. 2014, Bever et al. 2015, Whitaker et al. 2017). Yet diverse natural communities should favor multi-host pathogens with broad host ranges, decoupling pathogen abundance from the density of a single host species (May 1991, Spear et al. 2015). As a result, the degree of pathogen specialization and their role in promoting frequency dependence remains unresolved (Mordecai 2011). In this grassland system, we found ubiquitous pathogen damage: the majority of plants and 57% of all surveyed leaves had pathogen damage (Fig. S2) as has been observed in many other ecosystems (Gilbert 2002, Gilbert and Webb 2007, Parker et al. 2015). Yet we found limited empirical evidence for foliar fungal pathogens generating negative frequency dependence, thereby maintaining plant species diversity (Fig. 1).

Consistent with grassland seed pathogens (Beckman et al. 2014), foliar pathogen damage did not strongly respond to host relative abundance (Fig. 2) in a way that would promote negative frequency dependence (Condition 1). This is perhaps unsurprising given that the grass species extensively shared foliar pathogens, and that most of the common pathogens infected several species (Table 2; Fig. 3; in contrast to Condition 1A). Many diseases are specific to a genus or family (Gilbert and Webb 2007, Barrett et al. 2009); thus, for the study grasses (all Poaceae), pathogen damage may be decoupled from the relative abundance of a single host species. It is also possible that pathogen damage responds to host relative abundance at the regional, rather than local, scale (Mitchell et al. 2002).

Despite pathogen sharing among the grass species (Table S6; Fig. 3), fungal community composition subtly varied across grass species (partially supporting Condition 1B; Table 1; Figs.
Moreover, several common fungi were relatively specialized (partially supporting Condition 1A). *Pyrenophora tritici-repentis* (E) occurred mainly on the native perennial *E. glaucus* and was the most common fungus infecting that species. The only other occurrence of this pathogen in our surveys was on a single native *S. pulchra* individual located within an *E. glaucus* stand, suggesting a potential pathogen spillover event. Moreover, the recently invading perennial grass *P. aquatica* ([http://jrbp.stanford.edu/content/oakmead-herbarium-arrivals-weeds#Arrival](http://jrbp.stanford.edu/content/oakmead-herbarium-arrivals-weeds#Arrival)) had low pathogen damage and a relatively distinct pathogen community, including two fungi exhibiting host preference, *Pyrenophora cf. dactylidis* (L) and *Parastagonospora caricis* (AM). Taken together, these results suggest that although this Northern California grassland fungal pathogen community is dominated by multi-host pathogens, they vary in their affinity for each host species (Condition 1A) and pathogen communities may be structured, at least in part, by host species identity (Condition 1B).

The low pathogen damage and relatively distinct pathogen community of *P. aquatica* suggest that only a few of the resident pathogens have been able to make the ecological or evolutionary jump to infect *P. aquatica* since its introduction. Exotics that are slow to accumulate pathogens in their naturalized range tend to be more noxious (Mitchell and Power 2003). In JRBP, *P. aquatica* is an aggressive species that forms monotypic stands that suppress native plant species. By contrast, the other exotic species are all annuals that co-occur with native perennial species and have been naturalized in the area for over one hundred years, potentially allowing more time for pathogen host switches to occur.

Although higher pathogen damage was weakly associated with lower seed production (Fig. 5), foliar pathogens, which reduce photosynthetic function, did not appear to dramatically affect fitness (Condition 2). Nonetheless, the ability to detect any relationship between percent
damage and seed production is notable given the range of other sources of variation, including individual size, competition with the surrounding community, and microhabitat suitability. However, a fuller assessment would require measuring fitness effects across life stages in experimental infections. Although some of the fungal species exhibited strong host affinity (Condition 1A; Fig. 3; Table 2) and pathogen community composition subtly varied across plant species (Condition 1B; Table 1; Figs. 4 and S5), supporting effective specialization (Hersh et al. 2012, Benítez et al. 2013), the foliar pathogens in this system are not likely to respond strongly enough to host relative abundance (Condition 1) or to have large enough fitness impacts to promote plant species coexistence by generating negative frequency-dependence (Condition 2).

More fully understanding the role of pathogens in shaping the outcome of competition between plant species requires measuring plant – pathogen interactions and their consequences across demographic rates, and incorporating those measurements into population growth models to understand emergent outcomes. For example, seedling damping off pathogens, root pathogens, and pathogens that castrate plants by infecting flowers and/or seeds may prove to be more important players (Gilbert 2005). Studies manipulating fungal communities with fungicides and soil sterilization have suggested that pathogens promote plant community diversity (Allan et al. 2010, Mangan et al. 2010, Bagchi et al. 2014).

In addition to considering all plant demographic rates, it is necessary to investigate the entire pathogen community. We focused on local lesion diseases of leaves and on culturable foliar fungi, which may be disproportionately host generalist due to their necrotrophic lifestyle and ability to grow on malt extract agar. Our study did not identify potentially important biotrophic fungi, viruses, bacteria, or parasitic nematodes that may occur in roots or stems. Biotrophic, obligate plant pathogens are generally expected to exhibit higher host specificity than
facultative pathogens (Gilbert 2005), making them more likely to respond to host density and thereby maintain plant community diversity. Further, our study did not determine whether a given multi-host pathogen exerts host-specific impacts (Condition 1C), which has been observed in other systems (Sarmiento et al. 2017) and could contribute to the maintenance of local diversity.

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

The generalist strategy of broad host ranges and minimal host impacts is well suited to persistence and spread in seasonal, high-density mixed species grasslands like our study system. Particularly in annual-dominated stands, pathogens must quickly recolonize and spread during
the limited winter and spring growing season, giving a selective advantage to flexible, multi-host infection strategies. Despite extensive theoretical and empirical work on host-specialist pathogens and their impacts on host plant communities, there is little theoretical or empirical framework for understanding the impact of diverse communities of generalist pathogens on host communities (but see Mordecai 2011, Hersh et al. 2012, Benítez et al. 2013, Sarmiento et al. 2017). Developing empirically testable theory for how diverse, generalist pathogens with subtle impacts on host fitness may impact the outcome of competition in host communities is a key direction for future research.

Conclusions

Much of the research suggesting that host-specific pathogens maintain the local diversity of plants is based on spatial and temporal patterns of conspecific negative density-dependent mortality (e.g., Packer and Clay 2000, Klironomos 2002, Bell et al. 2006, Petermann et al. 2008, Bagchi et al. 2010, Mangan et al. 2010, Comita et al. 2010) and there is a dearth of information about the identities and host affinities of the fungal pathogens in natural systems (but see Parker and Gilbert 2007, Gilbert and Webb 2007, Hersh et al. 2012, Schweizer et al. 2013, Spear 2017). By examining 290 fungal isolates from 280 individual symptomatic plants, we present one of the largest surveys of California grassland fungal pathogen composition. Additionally, few studies have concurrently identified the pathogen communities, described the amount of pathogen-mediated damage, and linked that damage to host species relative abundance and fitness costs (seed production) across host species in common habitat (Parker and Gilbert 2007, Roy et al. 2014).
Our holistic approach illustrated that foliar pathogens in this system are multi-host, do not strongly respond to host species relative abundance, and have minor impacts on host fitness. Together, these conditions make it unlikely that foliar pathogens promote coexistence via negative frequency-dependence in this system. More broadly, this and previous studies suggest that foliar pathogens may not maintain grassland diversity (Peters and Shaw 1996, Mitchell 2003; but see Allan et al. 2010). However, given conflicting results, more work is required to clarify the role of foliar pathogens in plant species coexistence, particularly relative to other potentially important factors.

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

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Table 1. Collection details, richness and diversity of fungal species, and specialization for the grass species sampled. The grass species were not evenly sampled (10 to 223 tissue pieces per host, median = 61). The isolation frequency (percent of tissue pieces with fungal growth) also differed among hosts (11.9% to 63.2%, median = 28.8%). As a result, 50% of the 290 successfully sequenced 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’) | No. of transects from which tissue pieces were collected (and sequenced isolates originated) |
|----------------------------|--------------------------------------|---------------------|---------------------------|---------------------------------------------|-----------------------------------------------|--------------------------------------|---------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| *Avena barbata*†            | 116                                  | 28.4%               | 30‡                       | 7 (43%)                                     | 9.9                                           | 2.87                                 | 0.462                     | 16 (12)                                                                                                                          |
| *Avena fatua*               | 59                                   | 28.8%               | 16‡                       | 10 (80%)                                    | 40                                            | 11.41                               | NA                        | 7 (5)                                                                                 |
| *Brachypodium distachyon*   | 10                                   | 40%                 | 4                         | 1 (0%)                                      | 1                                             | 0.43                                 | NA                        | 1 (1)                                                                                 |
| *Bromus diandrus*†          | 107                                  | 19.6%               | 22                        | 7 (57%)                                     | 12.73                                         | 3.54                                 | 0.302                     | 17 (8)                                                                                 |
| Species                  | Count | Percent | Damage | n (% of total species) | d' (CI) | d' (CI) | d' (CI) | d' (CI) | d' (CI) | d' (CI) |
|--------------------------|-------|---------|--------|------------------------|---------|---------|---------|---------|---------|---------|
| *Bromus hordeaceus*†     | 101   | 11.9%   | 11‡    | 7 (57%)                | 11.39   | 8.29    | 0.2     | 11.39   | 8.29    | 0.2     |
|                          |       |         |        |                        | (7.76, 32.25) | (5.5) | (7.76, 32.25) | (5.5) | (7.76, 32.25) | (5.5) |
| *Elymus glaucus*†        | 53    | 60.4%   | 33‡    | 9 (44%)                | 12.21   | 4.08    | 0.665   | 12.21   | 4.08    | 0.665 |
|                          |       |         |        |                        | (9.59, 26.52) | (1.76) | (1.76) | (1.76) | (1.76) | (1.76) |
| *Festuca perennis*       | 42    | 26.2%   | 11     | 8 (88%)                | 27.09   | 13.19   | NA      | 27.09   | 13.19   | NA      |
|                          |       |         |        |                        | (12.68, 85.87) | (9.33) | (9.33) | (9.33) | (9.33) | (9.33) |
| *Phalaris aquatica*†     | 61    | 31.1%   | 18‡    | 9 (67%)                | 24.67   | 7.16    | NA      | 24.67   | 7.16    | NA      |
|                          |       |         |        |                        | (13.21, 67.25) | (3.64) | (3.64) | (3.64) | (3.64) | (3.64) |
| *Stipa pulchra*†         | 223   | 63.2%   | 145‡   | 26 (42%)               | 43.27   | 9.23    | 0.327   | 43.27   | 9.23    | 0.327 |
|                          |       |         |        |                        | (32.35, 72.95) | (2.22) | (2.22) | (2.22) | (2.22) | (2.22) |
| All                      | 772   | 37.6%   | 290‡   | 41 (54%)               | 285.78  | 13.03   | NA      | 285.78  | 13.03   | NA      |
|                          |       |         |        |                        | (85.91, 1375.24) | (2.44) | (2.44) | (2.44) | (2.44) | (2.44) |

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.

*The specialization index d' ranges from 0 (perfect generalist) to 1 (perfect specialist) (Dormann 2011).
Table 2. Host specialization of the 17 non-singleton fungal species from symptomatic leaves of five grass species with independent estimates of relative abundance in JRBP. Fungal species are sorted by abundance from common to rare. Thirteen (76%) of the non-singleton fungal species were isolated from multiple hosts. The specialization index $d'$ represents the extent to which the grass species attacked deviated from random given the availability of the grass species, varying 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* *allounisepeta*               | 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                                  |
Figure 1. Mechanism by which host-specific pathogen species or communities can promote diversity via the Janzen-Connell hypothesis. The necessary conditions for pathogens to promote host community diversity are: (1) pathogen damage must increase with host species relative abundance, and (2) pathogen damage must reduce fitness, and in turn population growth. Frequency-dependent pathogen damage (Condition 1) may occur because: (A) pathogen species are host-specialized, (B) pathogen communities differentiate among host species, or (C) multi-host pathogens cause host-specific impacts. When these conditions are met, pathogens cause per-capita population growth rates to decline with relative abundance, stabilizing species coexistence. If pathogens were removed, per-capita growth rates would decline less steeply with relative abundance, making stable coexistence less likely.

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

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

Figure 4. Non-metric multidimensional scaling plot depicting differences in fungal pathogen community composition ($N = 99$ isolates, 21 fungal species) among five grass species (stress = 0.101). Data points represent grass species-by-transect combinations, with 95% confidence.
ellipses around the centroid of each grass species. The point sizes represent the goodness of fit for each point (i.e., the correlation between the plotted distances and the observed community dissimilarities [distances]), with smaller points indicating a better fit.

**Figure 5.** Relationships between pathogen damage in March and April, species frequency, and seed production, represented as z-scores. Z-scores were calculated by subtracting the species-level mean and dividing the difference by the species-level standard deviation, for ease of comparison across species. Mean percent damage in March versus seed production (top left), mean percent damage in April versus seed production (bottom left), mean percent damage in March versus April (top right), and species frequency in the community versus seed production (bottom right). Focal species are abbreviated as *Avena barbata* (AB; light blue), *Bromus diandrus* (BD; dark blue), *Bromus hordeaceus* (BH; light green), *Elymus glaucus* (EG; dark green), and *Stipa pulchra* (SP; red) (seed production was not measured on *Phalaris aquatica*).
Figure 1

A. Host-specific pathogen species
B. Host-specific pathogen communities
C. Multi-host pathogens exert host-specific effects

1. Pathogen damage
   - Relative abundance

2. Fitness
   - Pathogen load

Pathogens removed
Pathogens present

Per-capita growth rate
Relative abundance
Figure 2

Frequency

March percent damage

Elymus glaucus

Avena barbata

Bromus diandrus

Bromus hordeaceus

Phalaris aquatica

Stipa pulchra
Figure 3
Figure 4
Figure 5