Native perennial and non-native annual grasses shape pathogen community composition and disease severity in a California grassland

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Abstract

1. The densities of highly competent plant hosts (i.e., those that are susceptible to and successfully transmit a pathogen) may shape pathogen community composition and disease severity, altering disease risk and impacts. Life history and evolutionary history influence host competence: longer-lived species tend to be better defended than shorter-lived species and pathogens adapt to infect species with which they have longer evolutionary histories. It is unclear, however, how the densities of species that differ in competence due to life and evolutionary histories affect plant pathogen community composition and disease severity.
2. We examined foliar fungal pathogens of two host groups in a California grassland: native perennial and non-native annual grasses. We first characterized pathogen community composition and disease severity of the two host groups to approximate differences in competence. We then used observational and manipulated gradients of native perennial and non-native annual grass densities to assess the effects of each host group on pathogen community composition and disease severity in 1-m^2 plots.
3. Native perennial and non-native annual grasses hosted distinct pathogen communities but shared generalist pathogens. Native perennial grasses experienced 26% higher disease severity than non-native annuals. Only the observational gradient of native perennial grass density affected disease severity; there were no other significant relationships between host group density and either disease severity or pathogen community composition.
4. *Synthesis.* The life and evolutionary histories of grasses likely influence their competence for different pathogen species, exemplified by distinct pathogen communities and

differences in disease severity. However, there was limited evidence that the density of either host group affected pathogen community composition or disease severity. Therefore, competence for different pathogens likely shapes pathogen community composition and disease severity but may not interact with host density to alter disease risk and impacts at small scales.

KEYWORDS

plant-pathogen interactions, disease severity, pathogen community, host competence, life history, non-native species, grassland, fungi

1 | INTRODUCTION

Plant community composition can affect infectious disease risk and impacts (Mundt, 2002; Rohr et al., 2020). The competence (i.e., susceptibility to and ability to transmit a pathogen; Stewart Merrill & Johnson, 2020) and density of hosts can affect pathogen persistence and incidence (Burdon & Chilvers, 1982; Fenton, Streicker, Petchey, & Pedersen, 2015). Therefore, communities with higher densities of competent hosts are expected to experience greater disease risk (Joseph, Mihaljevic, Orlofske, & Paull, 2013; Young, Parker, Gilbert, Sofia Guerra, & Nunn, 2017). Because the relationship between community composition and disease can inform biodiversity conservation (Rohr et al., 2020), empirical studies of natural communities tend to focus on species richness more than host density (i.e., hosts per unit area; Wojdak, Edman, Wyderko, Zemmer, & Belden, 2014) or abundance (e.g., percent cover of plant hosts; Mitchell, Tilman, & Groth, 2002; Parker et al., 2015; Schmidt et al., 2020). It is therefore unclear how the densities of hosts that differ in competence drive disease risk.

Disease incidence (i.e., the proportion of hosts with symptoms in a given sampling period; Nutter, Esker, & Netto, 2006) and severity (i.e., the intensity of symptoms per sampling unit, such as a leaf or individual; Nutter et al., 2006) are indicators of disease risk (Rohr et al., 2020). Typically, multiple pathogens circulate within host communities, driving disease incidence and severity (Halliday, Umbanhowar, & Mitchell, 2017; Vasco, Wearing, & Rohani, 2007). Variation among hosts in competence for different pathogens, which can arise through variation in traits and evolutionary histories with pathogens (Barrett, Kniskern, Bodenhausen, Zhang, & Bergelson, 2009; Joseph et al., 2013; Parker & Gilbert, 2004), can promote diversity in pathogen communities (Johnson et al., 2016). Therefore, variation in life history and evolutionary history may alter disease risk through pathogen community composition.

Shorter-lived species, such as annual plants, are expected to be less well-defended against pathogens and experience greater disease severity than longer-lived species because they allocate more resources to reproduction than survival (Cronin, Welsh, Dekkers, Abercrombie, & Mitchell, 2010; Joseph et al., 2013; Miller, White, & Boots, 2007). Plant species with longer evolutionary histories with pathogens in a given location may be more susceptible to attack by specialists that have overcome specific plant defenses (Parker & Gilbert, 2004; Telfer & Bown, 2012). In addition, species introduced to a new geographic area are likely to leave their specialist pathogens behind, as predicted by the enemy release hypothesis (Keane & Crawley, 2002). However, non-native plants tend to be annual species (Sutherland, 2004) and can accumulate pathogens and disease symptoms comparable to native congeners within centuries (Hawkes, 2007; Mitchell, Blumenthal, Jarošík, Puckett, & Pyšek, 2010), suggesting that long-established non-native plants may have overlapping pathogen communities with native species.

In general, higher densities of plant assemblages result in more contacts between susceptible host tissue and pathogen propagules (Burdon & Chilvers, 1982) and increase the negative effects of infection (Lively, Johnson, Delph, & Clay, 1995). Changes in the density of a single host type are more likely to affect specialist pathogens than generalist pathogens (Alexander & Holt, 1998), but specific plant traits may interact with plant density to mediate infection by generalist pathogens. For example, non-native annual grasses in California grasslands may increase pathogen transmission by filling in gaps between native perennial bunchgrasses (Parker, Seabloom, & Schimel, 2012) and native perennial grasses may grow later into the growing season than non-native annuals (Chiariello, 1989), providing additional opportunities for transmission (Thrall, Biere, & Antonovics, 1993). Thus, pathogen communities may shift, and disease severity may increase with increasing density of either non-native annuals or native perennials, but to a greater extent with increasing density of the more competent group.

Here we assess how the densities of native perennial and non-native annual grasses affect foliar fungal pathogen community composition and disease severity in a California grassland. California grasslands are dominated by non-native annual grasses, which differ in life history and local residence time from native perennial bunchgrasses (Heady, 1977). Non-native annual grasses have been established in California for more than a century (Heady, 1977) and, with native perennials, serve as hosts for a diversity of foliar fungal pathogens (Spear & Mordecai, 2018) that are transmitted through density-dependent mechanisms (McCartney, Fitt, & West, 2006). We collected data from ten studies within the grassland to answer the question: 1. *How do (a) pathogen community composition and (b) disease severity differ between native perennial and non-native annual grass hosts?* Pathogen community composition and disease severity depend on, among other factors, host competence and can indicate propensity for transmission

(Barrett et al., 2009). We hypothesized that native perennials would host more specialized pathogens due to longer evolutionary history with local pathogens, that non-native annuals would experience higher disease severity due to lower allocation to defenses, and that the two groups would host overlapping pathogen communities due to the long residence time of non-native annuals (>100 generations). We then evaluated the effects of host density on disease risk in an observational study and a manipulated experiment, answering the question: 2. *How do native perennial and non-native annual grass densities affect (a) pathogen community composition and (b) disease severity?* We hypothesized that increasing densities of either native perennials or non-native annuals would shift pathogen communities and increase disease severity, and that the density of the more competent group (suggested by the results of *Question 1*) would have a larger effect. We hypothesized that the relationship between host density and disease risk would be stronger in the manipulated experiment, where plots contained more extreme values of host density and had fewer plant species than the observational study.

2 | MATERIALS AND METHODS

2.1 | Study system

We evaluated the pathogen community composition and disease severity of native perennial and non-native annual grasses at Stanford University's Jasper Ridge Biological Preserve (JRBP) in San Mateo County, California, USA. California grasslands are dominated by non-native Mediterranean annual grasses that rapidly established during European settlement, replacing dominant perennial bunchgrass species, such as *Stipa pulchra* (Heady, 1977). Grasslands at JRBP occur on sedimentary-derived soil, which we focus on in this study, and serpentine soil (McNaughton, 1968). Plant growth at JRBP begins with the onset of precipitation in late fall, progresses through cool, wet winters into spring, and ends in warm, dry summers (Chiariello, 1989). The cumulative precipitation in San Mateo county between September and April was 579 mm (2014-2015), 728 mm (2015-2016), and 1139 mm (2016-2017), ranging on both sides of the 100-year average of 683 mm (NOAA, 2020). Such temporal variation in precipitation is typical of California grasslands, can impact plant community composition (Fernandez-Going, Anacker, & Harrison, 2012), and may also affect plant-pathogen interactions (Thompson, Levin, & Rodriguez-Iturbe, 2013).

A study at JRBP in 2015 demonstrated that unique pathogen communities were associated with several grass species, but that generalist pathogens were shared among them (Spear & Mordecai, 2018). The data from that study are included here, along with data collected in the next two years. We assessed foliar fungal disease associated with four non-native annual grass species (*Avena barbata, Bromus diandrus, Bromus hordeaceus*, and in some cases, *Avena fatua*, Table 1) and two native perennial grass species (*S. pulchra* and *Elymus glaucus*). While other non-native annual grasses, including *Brachypodium distachyon, Bromus sterilis, Festuca myuros,* and *Gastridium phleoides*, were locally common (Table S1), we focused on the four *Avena* and *Bromus* species because they are widespread at JRBP, have spatially variable densities, and are the primary competitors of native grasses (McNaughton, 1968; Uricchio, Daws, Spear, & Mordecai, 2019). All of the non-native species except *B. sterilis* and *G. phleoides* are considered invasive in California (Cal-IPC, 2020).

2.2 | JRBP compilation

To evaluate the differences between foliar fungal pathogen communities (*Question 1a*) and disease severity (*Question 1b*) of native perennial and non-native annual grasses, we ~~used plants located within ten~~compiled data from two previous sampling efforts ~~studies~~ at JRBP in

2015 and 2016 and collected additional data in 2017 (i.e., JRBP compilation, Fig. S1). The sampling efforts~~se studies~~ were ~~established~~ conducted to characterize variation in the host ranges and fitness impacts of pathogens (observational study and additional sampling across JRBP in 2015; Spear & Mordecai, 2018) and to~~for multiple purposes beyond the questions addressed here, including~~ measure~~ing~~ plant demographic responses to competition and pathogen infection ~~(obervational study, manipulated experiment, and germinant study in 2016; Uricchio et al., 2019)~~(obervational study, manipulated experiment, and germinant study in 2016; Uricchio et al., 2019).~~,~~ In 2017, we repeated sampling in the observational and germinant studies and collected samples from plants grown in pots and growing medium and placed in areas around JRBP (i.e., “sentinel plants”, Fig. S1). ~~characterizing natural variation in disease severity and pathogen community composition (Spear & Mordecai, 2018), and assessing the effects of global change factors on grasslands (Shaw et al., 2002).~~ The o~~Two of the studies (o~~bservational study and manipulated experiment (described below) contained gradients of native perennial to non-native annual grasses and were therefore used to answer *Questions 2a* and *2b* (sections 2.3 and 2.4). ~~Depending on the study goals, plants were either planted at JRBP by researchers, grown in pots and growing medium (i.e., “sentinel plants”), or occurred at JRBP without known human involvement.~~ The plants sampled received no experimental treatment besides, in some cases, manipulation of plant community composition.

To characterize the pathogen community composition of native perennial and non-native annual grasses (*Question 1a*), we collected one leaf with disease symptoms per plant from grasses ~~in ten studies~~ at JRBP between March and June in ~~2015, 2016, and 2017~~each year (Table 1). We isolated fungi from the lesions, assigned each an operational taxonomic unit (OTU), and estimated the species identity (section 2.5). We defined a community as the fungal isolates associated with a grass species in a particular year and omitted communities with fewer than four isolates, leading to six communities associated with native perennials and ten communities associated with non-native annuals (Table S2).

To characterize the disease severity of native perennial and non-native annual grasses (*Question 1b*), we selected plants without a priori knowledge of their infection status. Disease severity was assessed in a subset of the JRBP compilation locations ~~from three studies at JRBP (Table 1)~~: four transects (T11-T14, Fig. S1) in March and April 2015, the observational study in March and April 2015 and May 2016, and the manipulated experiment in May 2016 (Table 1). The assessments in March and April of 2015 used many of the same plants, so we analyzed these data separately. For each plant, we haphazardly selected up to six leaves, based on availability, and visually approximated the proportion of leaf surface area with lesions. Disease severity was measured as the proportion of leaf surface area with lesions, including of leaves lacking lesions, which, when averaged over all leaves of a plant, combines the proportion of leaves with lesions and the proportion of leaf surface area with lesions.

2.3 | Observational study

Together with the manipulated experiment (section 2.4), the observational study was designed to measure plant demographic responses to ~~competition and~~ pathogen infection (Spear & Mordecai, 2018; Uricchio et al., 2019). Both studies contained gradients of native perennial and non-native annual grass densities. Five plant species were included in both studies: seedlings of *A. barbata*, *B. diandrus*, *B. hordeaceus*, *E. glaucus*, and *S. pulchra* and adults of *E. glaucus* and *S. pulchra*. We included both seedlings and adults of perennial species because the demographic responses of both life stages contributed to the original study goals (Uricchio et al., 2019). In spring 2015, we established ten transects across visually-assessed gradients of perennial grass–dominated to annual grass–dominated areas. Transects consisted of four to five 1-m^2 plots (Fig. S1) and were sampled over two years. Following the first year, we supplemented transects lacking the five plant species to allow for better comparison with the manipulated experiment by adding approximately 20 seeds of each species and one adult of each perennial species into every other plot (i.e., plots 1, 3, and 5 of a transect with 5 plots). To characterize the density of native perennial and non-native annual grasses, we counted the number of individuals per species within subplots of 47 and 18 plots during April 2015 and late June/early July 2016, respectively, and scaled the counts up to 1-m^2 (Table S1). We did not include forb density in our analyses even though forbs were present in the plots because foliar fungal pathogens often exhibit a phylogenetic signal (Gilbert & Webb, 2007; Parker et al., 2015), so forbs are less likely to share pathogens with the sampled grasses than are other grass species.

To evaluate the effects of native perennial and non-native annual grass densities on pathogen community composition (*Question 2a*), we collected one leaf with disease symptoms per plant from grasses in 31 plots in 2015 and six plots in 2016 (Table 1). We isolated and identified fungi from the lesions (section 2.5) and evaluated changes in isolation frequencies of the most common OTUs over the host group density gradients (section 2.6.3).

To evaluate the effects of native perennial and non-native annual grass densities on disease severity (*Question 2b*), we conducted disease severity assessments of grasses in 46 plots in March 2015, 25 plots in April 2015, and 18 plots in 2016 following the methods described for the JRBP compilation (section 2.2). We evaluated changes in disease severity over the host group density gradients (section 2.6.4).

2.4 | Manipulated experiment

In fall 2015, we established 210 1-m^2 plots in a 35 m x 35 m area of JRBP where weed matting had been placed in the preceding spring to suppress background plant recruitment (Fig. S2; Uricchio et al., 2019). Within the 1-m^2 plots, we manipulated the densities of the five plant species described in section 2.3 to 10%, 20%, 40%, 80%, or 100% of the density of each in monoculture by sowing seeds of each species or transplanting adult perennial species. In addition, 30 2 x 2 m plots were cleared and planted with one seed of each species and one adult of each perennial species. In January 2016, we added "focal" individuals to the 1-m^2 plots by planting ten seeds of each species and one adult of each perennial species. We also reseeded 18 plots with 75%–100% of their original added seed weight to account for low germination. Two-thirds of the plots received either fungicide application or liquid fungal inoculum and one-third received an equivalent volume of water (ambient). However, we only used the ambient plots in this analysis (70 1-m^2 plots and 10 4-m^2 plots, Fig. S2). During June 2016, we counted up to 50 individual grasses in each plot, identified them to species, and scaled the densities to 1-m^2 (Table S1). We weeded non-planted species throughout the growing season, but some survived and we included their densities in our analyses.

To evaluate the effects native perennial and non-native annual grass densities on pathogen community composition (*Question 2a*), we collected one leaf with disease symptoms per plant from grasses in 28 plots (Table 1). Because destructive sampling could interfere with assessing plant competition in low density plots (one of the goals of the experiment; Uricchio et al., 2019)~~ and because we expected the largest effect of background plants to occur at higher densities~~, we only sampled from plots planted at 80% and 100% density. These two planting treatments still produced a range of realized native perennial and non-native annual densities (Fig. S3B, D) because of variation in survival of intentional and unintentional plants. We isolated and identified fungi from the lesions (section 2.5) and evaluated changes in isolation frequencies of the most common OTUs over the host group density gradients (section 2.6.3).

To evaluate the effects of native perennial and non-native annual grass densities on disease severity (*Question 2b*), we assessed grasses across all of the ambient plots (Table 1) following the methods described for the JRBP compilation (section 2.2). We evaluated changes in disease severity on native perennial and non-native annual grasses over the host group density gradients (section 2.6.4).

2.5 | Identifying foliar fungi

We isolated fungi associated with foliar lesions and estimated the species identity to address questions pertaining to pathogen community composition (*Questions 1a* and *2a*). As described by Spear and Mordecai (2018), we excised 2 mm x 2 mm segments of symptomatic tissue from the edge of foliar lesions and surface-sterilized (sequential immersion for 60 s each in 70% ethanol and 10% household bleach) and plated each tissue piece on 2% malt extract agar (MEA) with the antibacterial agent chloramphenicol. For each tissue piece, morphologically distinct hyphae (i.e., morphotypes) were isolated into pure culture on 2% MEA plates. The Mordecai lab maintains reference strains (California Department of Food and Agriculture permit 3160). For each fungal isolate, we extracted genomic DNA and amplified the internal transcribed spacer (ITS) regions 1 and 2, the 5.8S rRNA gene, and part of the rRNA LSU as detailed in Spear and Mordecai (2018). However, in 2017, we modified our protocol to produce longer consensus reads. Specifically, we paired the forward primer ITS1-F (Gardes & Bruns, 1993) with the reverse primer LR3 (Vilgalys & Hester, 1990), rather than ITS4-B (Gardes & Bruns, 1993).

We processed the Sanger sequencing reads from MCLAB (San Francisco, California, USA) with Geneious 7.1.9 (Kearse et al., 2012). We trimmed and automatically assembled reads when possible; when not possible, we manually assembled reads or selected the longest trimmed individual read over 100 bp. We clustered all consensus sequences into OTUs based on 97% sequence similarity using USEARCH 10.0.240 (Edgar, 2010). If different morphotypes from the same tissue piece were clustered into the same OTU, we assumed they represented the same isolate. We estimated the taxonomic placement of the ITS OTUs with the UNITE database 01.12.2017 (Nilsson et al., 2019) and assigned taxonomy in mothur 1.40.5 (Schloss et al., 2009) using the naïve Bayesian classifier (Wang, Garrity, Tiedje, & Cole, 2007) with a bootstrapping confidence score of at least 80% for species name and at least 60% for any other taxonomic rank.

2.6 | Statistical analyses

Statistical analyses were completed in R version 3.5.2 (R Core Team, 2018) using vegan (Oksanen et al., 2019), rusda (Krah et al., 2018), glmmTMB (Brooks et al., 2017), MuMIN (Bartoń, 2019), and tidyverse (Wickham, 2017) packages.

2.6.1 | *Question 1a*: Pathogen community differences between host groups

We evaluated dissimilarities among pathogen communities (section 2.2) with a permutational multivariate analysis of variance (PERMANOVA) using the Chao method, which accounts for unobserved species and is robust to differences in sample sizes (Chao, Chazdon, & Shen, 2005). We used a community matrix (each community as a row, each OTU as a column, isolate abundances as entries) as the response variable and the "grass species", "year", and "host group" as predictor variables. We visualized results with non-metric multidimensional scaling (NMDS), also using the Chao method.

We estimated the host ranges of pathogens associated with each host group to evaluate whether escape of non-native annual grasses from specialist pathogens could explain differences in pathogen community composition (Keane & Crawley, 2002). We used two methods to estimate host range: (1) we searched the U.S. National Fungus Collections Database (hereafter, “database”; Farr & Rossman, 2019) for host species associated with the estimated fungal species (Schmidt et al., 2020) and (2) we compiled the host species from which each OTU was isolated across ten studies at JRBP (section 2.2). Host species sampled (sample sizes in parentheses) included *A. barbata* (210), *A. fatua* (12), *B. diandrus* (120), *B. distachyon* (4), *B. hordeaceus* (85), *E. glaucus* (242), *Festuca perennis* (a non-native perennial, 21), *Phalaris aquatica* (a non-native perennial, 19), and *S. pulchra* (436). To test whether pathogens associated with native perennial and non-native annuals differed in their host ranges, we performed Welch two sample t-tests for each of the two host range sources. By using each fungal isolate as a replicate, species or OTUs that were isolated more frequently contributed more to the average host range. Note that the database may provide more information, and potentially larger host range estimates, for fungi of crops and economically important plants, fungi intercepted at ports of entry, common fungi, and invasive or emerging fungal pathogens (Farr & Rossman, 2019).

2.6.2 | *Question 1b*: Disease severity differences between host groups

To evaluate the differences in disease severity between native perennial and non-native annual grasses, we fit a generalized linear mixed effect model with a logit-link beta error distribution to the proportion of leaf surface area with lesions (section 2.2). Because our data contained many zeros, which cannot be included in a beta regression, we transformed disease severity using the equation $t = (s \times (n - 1) + 0.5) / n$, where t is the transformed value, s is the original value, and n is the size of the dataset (Douma & Weedon, 2019). The predictor variable was “~~the~~ host group” and the random effect intercepts were “plant ID” nested within “plot” nested within “study” and crossed with “year”. We removed study from the random effects (variance < $2x10^{-22}$) for model convergence; the random effect "plot" still accounted for spatial heterogeneity.

2.6.3 | *Question 2a*: Effects of host density on pathogen communities

To evaluate the effects of native perennial and non-native annual density on pathogen community composition, we analyzed the isolation frequencies of the most common OTUs. To select the most common OTUs, we ranked all of the OTUs from ~~ten~~ the JRBP ~~studies~~ compilation (section 2.2) by the number of isolates obtained in each year (i.e., abundance). We evaluated the differences in abundance between consecutive ranks and found relatively large differences between the fifth and sixth most common OTUs in 2015 and 2016 and between the fourth and fifth most common OTUs in 2017 (Fig. S4~~3~~). Therefore, we selected the top five, five, and four most common OTUs in 2015, 2016, and 2017, respectively, which resulted in seven focal OTUs. The fungal species associated with these OTUs were *Alternaria infectoria*, *Parastagonospora avenae*, *Pyrenophora chaetomioides*, *Pyrenophora lolii*, *Pyrenophora tritici-repentis*, a ~~n unidentified~~ *Pyrenophora* species identified only to genus level ("*Pyrenophora* sp."), and *Ramularia proteae*. Note that we refer to the OTUs by their estimated species names in the results, but these same species names may be associated with less common OTUs as well.

We fit generalized linear mixed effect models with logit-link binomial error distributions to the presence/absence of each focal OTU for each isolate collected from the observational study and manipulated experiment. The predictor variables were ~~the~~ "host group" (from which the isolate was collected) and plot-level densities of "native perennial grasses", "non-native annual grasses", and, when present, grasses that were either unidentified or not included in either host group ("other grasses", Table S1). The fixed effects also included interactions between "host group" and each of the grass density measurements. Random effect intercepts were "plot" crossed with "year" for the observational study and "plot" for the manipulated experiment (which had only one year of data). Exceptions to the general model formulation were made to aid in model convergence (Methods S1). We performed model selection by fitting models with all subsets of the fixed effects. The Akaike information criterion with a correction for small sample sizes (AICc) was calculated for each model and we extracted the subset of the models for which the cumulative sum of the normalized model likelihoods was greater than or equal to 0.95 (i.e., the 95% confidence set of models). We report coefficient estimates from the average of the 95% confidence set.

2.6.4 | *Question 2b*: Effects of host density on disease severity

To evaluate the effects of native perennial and non-native annual grass densities on the disease severity of each host group, we fit generalized linear mixed effect models with logit-link beta error distributions to the scaled proportion of leaf surface area with lesions (section 2.6.2). The fixed effects were the same as those described for the pathogen isolate models (section 2.6.3). The random effect intercepts were "plant ID" nested within "plot" and crossed with "year" for the observational study and "plant ID" nested within "plot" for the manipulated experiment. We did not perform model selection as sub-models could not converge during model averaging.

3 | RESULTS

3.1 | *Question 1a*: Pathogen community differences between host groups

We identified 83 unique OTUs from the 961 foliar fungal isolates collected from six grass species across JRBP (Fig. 1A). Forty-one OTUs were isolated from only native perennial grasses, 18 were isolated from only non-native annual grasses, and 24 were isolated from both host groups. The host groups explained 25% of the variance in pathogen community composition (Table 2) and the pathogen communities associated with the two groups were distinct (Fig. 1B). However, the 24 OTUs isolated from both host groups made up 78% and 96% of the isolates from native perennial grasses and non-native annual grasses, respectively, leading to overlap between the pathogen communities associated with the two host groups (Fig. 1A). Fungal species names (29 total) were estimated for 282 native perennial grass isolates (53%) and 266 non-native annual grass isolates (62%). Based on the database, the estimated fungal species isolated from non-native annual grasses had, on average, smaller host ranges than those isolated from native perennial grasses (Fig. 1C, $t = 4.53$, $df = 480$, $P < 0.001$). However, within JRBP, the OTUs isolated from non-native annual grasses had, on average, larger host ranges than those isolated from native perennial grasses (Fig. 1C, $t = -7.97$, $df = 944$, $P < 0.001$).

3.2 | *Question 1b*: Disease severity differences between host groups

Based on assessments collected from three studies at JRBP, native perennial grasses had 26% higher disease severity than non-native annual grasses ($P < 0.001$, Table S3). However, disease severity was generally low: an average of 1.5% and 1.1% of leaf surface area was covered with lesions for native perennials and non-native annuals, respectively. These patterns were maintained when data collected in April 2015 from the observational study were substituted for data collected in March 2015 (Table S3).

3.3 | *Question 2a*: Effects of host density on pathogen communities

The seven most common OTUs from the foliar fungal isolates collected at JRBP (i.e., the focal OTUs) comprised 66% and 77% of the isolates from native perennial and non-native annual grasses, respectively, across the density gradients (Table 3). In both the observational study and manipulated experiment, the grass communities included either high densities of one host group and low densities of the other or low densities of both (~~Fig. S4~~Fig. S3A–B). The densities in the manipulated experiment exceeded those in the observational study. The majority of the plots had more non-native annuals than native perennial grasses (~~Fig. S4~~Fig. S3C–D).

None of the focal OTUs significantly increased in relative abundance with native perennial or non-native annual grass density (Tables S4–S5). We calculated the predicted change in relative abundance of each pathogen on each host group with the addition of 50 native perennial grasses m^{-2} (Fig. 2A–B) or 5000 non-native annual grasses m^{-2} (Fig. 2C–D) to bare plots—reflecting the difference in naturally occurring densities of these species groups. Such increases in density exceed those recorded in the observational study (~~Fig. S4~~Fig. S3A), but they still had small predicted impacts on the relative abundances of most pathogens (Fig. 2). Although not statistically significant, *P. lolii* relative abundance decreased with 50 additional native perennial grasses (Fig. 2A–B), the relative abundance of ~~*the unidentified*~~ *Pyrenophora* ~~*species*~~ sp. increased with 5000 additional non-native annual grasses (Fig. 2C–D, Fig. S5), and *P. chaetomioides* relative abundance on non-native annuals decreased with 5000 additional non-native annual grasses in the manipulated experiment (Fig. 2D).

3.4 | *Question 2b*: Effects of host density on disease severity

Disease severity was generally low across both host groups and grass density ranges (Fig. 3) and did not significantly change with non-native annual grass density (Tables S6–S7). Native perennial grass density significantly increased disease severity in the observational study (Table S6), particularly on native perennial hosts (Fig. 3A), but this effect was lost later in the season (Fig. S6A). Disease severity was higher on native perennials than non-native annuals across grass densities (Tables S6–S7).

4 | DISCUSSION

To evaluate the effects of host competence and density on disease risk, we characterized pathogen communities and disease severity of native perennial and non-native annual grasses in a California grassland. Consistent with two of our hypotheses, the host groups shared generalist pathogens and native perennials hosted more specialized pathogens based on data collected from JRBP (*Question 1a*). However, non-native annuals hosted more specialized pathogens based on the U.S. National Fungus Collections Database (*Question 1a*). Both host groups experienced low disease severity, but native perennials had higher disease severity than non-native annuals—the opposite of what we had expected (*Question 1b*). Despite distinct pathogen community compositions between the host groups and differences in disease severity, we did not find substantial effects of host group density on pathogen community composition (*Question 2a*) or disease severity (*Question 2b*). Our findings suggest that native perennial and non-native annual grasses differ in competence, shaping their own pathogen communities, but that their densities do not amplify their pathogen communities, or foliar fungal disease in general, at least at the 1-m^2 plot scale and over the time span of our study.

4.1 | *Question 1a*: Pathogen community differences between host groups

Plant species vary in susceptibility to different pathogens (Barrett et al., 2009), in part due to life history (Cronin et al., 2010) and evolutionary history (Parker & Gilbert, 2004). Accordingly, native perennial and non-native annual grasses had distinct foliar fungal pathogen communities. Plant–pathogen interactions can also depend on environmental conditions (Barrett et al., 2009). Sampling year explained 42% of variation in pathogen community composition, suggesting the influence of temporally variable factors such as precipitation (Thompson et al., 2013) and temperature (Liu et al., 2019). Our study included two (*Questions 1b, 2a, 2b*) to three

(*Question 1a*) years of data from a single grassland, but longer time series could help link climate variation to pathogen community composition.

Hosts are frequently infected by multiple pathogens and many pathogens can circulate among different hosts within the same community (Halliday et al., 2017; Schmidt et al., 2020). Our study is unique, however, in seeking to understand how hosts that differ in life and evolutionary histories shape aboveground pathogen community composition (but see Seabloom, Borer, Lacroix, Mitchell, & Power, 2013). A pathogen community perspective demonstrated that high relative susceptibility of one group to one pathogen (e.g., native perennial grasses to *P. tritici-repentis*) can be balanced by high relative susceptibility of another group to another pathogen (e.g., non-native annual grasses to *P. chaetomioides*). This insight is likely to be general given that variation in evolutionary history also shapes soil microbial community composition (Kourtev, Ehrenfeld, & Häggblom, 2002; Wolfe, Rodgers, Stinson, & Pringle, 2008) and cautions against conclusions about disease risk that focus on a single pathogen (Lloyd-Smith, 2013).

The enemy release hypothesis posits that native plants will experience greater disease pressure than non-native plants because the latter will lose specialist enemies during transport to a new region and resident specialist enemies will be slow to attack non-native plants (Keane & Crawley, 2002). We isolated more unique OTUs from native perennials than non-native annuals, which supports this hypothesis, but the average host range of pathogens associated with non-native annuals was more specialized than that of pathogens associated with native perennials based on the database. The latter result should be interpreted with caution, however, because many pathogens did not have host range information available in the database, some estimates of host range were smaller than those characterized at JRBP (e.g., *R. proteae*), and we found the opposite relationship from nine host species at JRBP. Nonetheless, non-native annual grasses may host more specialized pathogens because the grass species have been in JRBP or nearby counties since 1893 or earlier (JRBP, 2020), allowing more than 120 annual plant generations for resident pathogens to adapt to the novel hosts (Carroll et al., 2005; Hawkes, 2007) and for repeated introductions of pathogens from their native geographic ranges to occur (Dutech et al., 2012). Some of the identified pathogens are globally-distributed (Aboukhaddour, Cloutier, Lamari, & Strelkov, 2011; Stukenbrock, Banke, & McDonald, 2006), suggesting that repeated introductions may be plausible.

4.2 | *Question 1b*: Disease severity differences between host groups

Native perennial grasses experienced higher disease severity than non-native annual grasses. While this finding contradicts our expectation that non-native annuals would have higher disease severity because of life history, it is consistent with multiple studies demonstrating higher disease severity on native than non-native plants (Chun, van Kleunen, & Dawson, 2010; Han, Dendy, Garrett, Fang, & Smith, 2008; Hawkes, Douglas, & Fitter, 2010; but see Parker & Gilbert, 2007). Native perennials may be more exposed to transmission and/or more susceptible to infection than non-native annuals. Exposure may be partially explained by the long-lived life-history of native perennials and their role as long-term pathogen reservoirs (Thrall et al., 1993). Indeed, the difference in disease severity between native perennials and non-native annuals was greater in the observational study than in the manipulated experiment, where plant communities had been recently assembled. In addition, non-native annual grasses may shed leaves with fungal lesions more frequently than native perennials, creating the appearance of lower disease severity (Vloutoglou & Kalogerakis, 2000). In general, disease severity was low, suggesting that both host groups have low competence for foliar fungal pathogens.

4.3 | *Question 2a*: Effects of host density on pathogen communities

Changes in density of native perennial and non-native annual grasses had limited effects on the relative abundance of foliar fungal pathogens. Therefore, even though the two host groups had different pathogen communities (*Question 1a*), they may not amplify the transmission of pathogens with which they are frequently infected. For example, *P. chaetomioides* was isolated frequently from the two *Avena* species in the non-native annual group but was predicted to decrease in relative abundance on non-native annuals with increasing density of non-native annuals. Changes in plant community composition within the host group, such as an increase in *Bromus* spp. and a decrease in *Avena* spp., along the density gradient or generally low disease severity and limited capacity for transmission may have contributed to this pattern.

Our results indicate that shifts in the densities of hosts that have similar life history strategies and local residence times do not necessarily shape the assembly of pathogen communities. While interpretations of biodiversity–disease risk relationships often invoke a strong role for host density (e.g., Young et al., 2017), plant pathogen communities may be more influenced by other factors, such as microbial interactions. For example, priority effects can influence the assembly of yeast communities in flower nectar (Peay, Belisle, & Fukami, 2012) and foliar fungal communities on grasses (Halliday et al., 2017). One limitation to evaluating disease risk by particular pathogens in our study is that we lack data on the absence of infection. In addition, transmission events may occur at a scale greater than study plots, causing the plot-level density of grasses to be an inaccurate estimate of transmission pressure.

4.4 | *Question 2b*: Effects of host density on disease severity

Disease severity increased with increasing native perennial grass density in the observational study, but not with increasing non-native annual grass density or in the manipulated experiment. Not only was the manipulated experiment more recently established, but the experimental designed differed in multiple ways that could affect pathogen transmission: the total area of the experiment was smaller, there were open corridors between the plots instead of continuous grassland, and plant community composition varied randomly in space instead of gradually shifting between the two host groups. Deviations of our results from strong positive relationships between plant abundance and disease severity (Mitchell et al., 2002; Parker et al., 2015) may be explained by low average disease severity and high plant diversity. High host diversity can hinder foliar fungal pathogen adaptation to specific host defenses (Mundt, 2002) or otherwise prevent specialized pathogens from becoming common (i.e., the dilution effect; Rohr et al., 2020), making pathogens less capable of exploiting locally-abundant hosts, and in turn less sensitive to changes in the density of any particular host group. Indeed, host abundance in diverse old fields did not affect disease severity caused by aboveground pathogens (Schmidt et al., 2020). In addition, density effects may be transient, as exemplified by dampened density–disease severity relationships later in the growing season.

5 | CONCLUSIONS

This study of foliar fungal pathogen communities and disease severity on native perennial and non-native annual grasses suggests that differences in life history or local residence time may contribute to disease risk through differences in competence, but not through changes in density. We could not parse out the independent effects of life history and local residence time, but previous studies of plant diseases suggest that both life history (Cronin et al., 2010) and evolutionary history (Parker & Gilbert, 2004) are strong drivers of competence. Our results are likely to be generally relevant, however, because non-native plants are likely to be annuals (Sutherland, 2004). These findings have implications for understanding the impacts of invasive species. For example, when species initially invade a community, they can affect total host density, altering the proportion of hosts infected (Searle et al., 2016). The invasive species we evaluated, however, are well-established, suggesting that the expected impacts of invasive species on disease risk may be greater earlier in invasions. Our study demonstrates that host community composition can affect pathogen community composition and disease severity through variation in competence among hosts.

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Authors' contributions

EAM and ERS designed the research, ERS and SCD conducted the field work and laboratory work, AEK conducted the analyses and wrote the first draft of the manuscript, all authors contributed to manuscript revisions and approved the final version.

Data availability

The data and code will be available on the Github repository https://github.com/aekendig/invasion-pathogen-communities and archived with Zenodo.

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Tables

Table 1. Data types, sources, years, and sample sizes used to address each question. All data sources included two native perennial species (*E. glaucus* and *S. pulchra*) and three non-native annual species (*A. barbata*, *B. diandrus*, and *B. hordeaceus*). Some also included the non-native annual species *A. fatua* (indicated with †). Sample sizes in parentheses represent an additional sampling period in the same year (analyzed separately).

Question	Data type	Data source	Year	Native perennials	Non-native annuals
1a	isolates	~~JRBP compilation~~obs. and additional† ~~(10 studies)~~	2015	91	75
		obs., man., and additional	2016	261	242
		obs. and additional	2017	182	110
1b	severity	~~JRBP compilation (3 studies)~~obs. and additional	2015	101 (71)	292 (129)
		obs. and man.	2016	338	172
2a	isolates	~~observational study~~obs.†	2015	69	55
		obs.	2016	22	17
		~~manipulated experiment~~man.	2016	135	76
2b	severity	~~observational study~~obs.	2015	101 (56)	292 (79)
		obs.	2016	69	11
		~~manipulated experiment~~man.	2016	269	161

Notes: obs. = observational study, man. = manipulated experiment, additional = additional sampling (see Fig. S1 for details)

Table 2. PERMANOVA describing the effects of host group (native perennial versus non-native annual), grass species, and sampling year on pathogen community composition (n = 961 isolates).

Variable	df	Sums of sqs.	Mean sqs.	F	R^2	*P*
Host group	1	0.516	0.516	23.506	0.250	**0.001**
Grass species	4	0.510	0.127	5.801	0.247	**0.004**
Year	2	0.862	0.431	19.606	0.417	**0.001**
Residuals	8	0.176	0.022	NA	0.085	NA
Total	15	2.064	NA	NA	1	NA

df = degrees of freedom, sqs. = squares, NA = not applicable; *P*-values indicating statistical significance ($P < 0.05$) are in bold.

Table 3. Pathogen species assigned to the focal OTUs, their host ranges based on the number of host species found in the database and identified at JRBP (out of nine species), and their abundances (the number of isolates, and proportion in parentheses) from each host group collected across the observational and manipulated density gradients.

Pathogen	Database host range	JRBP host range	Native perennial abundance	Non-native annual abundance
Alternaria infectoria	50	8	27 (0.12)	35 (0.24)
Parastagonospora avenae	54	5	32 (0.14)	4 (0.03)
Pyrenophora chaetomioides	7	7	0 (0)	19 (0.13)
Pyrenophora lolii	11	7	12 (0.05)	19 (0.13)
Pyrenophora tritici-repentis	63	2	23 (0.1)	0 (0)
Pyrenophora sp.	NA	6	44 (0.19)	31 (0.21)
Ramularia proteae	1	7	11 (0.05)	6 (0.04)

Figure legends

Figure 1. Pathogen communities associated with native perennial and non-native annual grasses. (A) Composition of fungal pathogen isolates for each grass species. Each OTU is represented by a different color (including varying shades of brown) and the legend is provided for the seven focal OTUs: *Alternaria infectoria* (*A. inf.*), *Parastagonospora avenae* (*P. ave.*), *Pyrenophora chaetomioides* (*P. cha.*), *Pyrenophora lolii* (*P. lol.*), *Pyrenophora tritici-repentis* (*P. tri.*), *Pyrenophora* sp. (*Pyr.* sp.), and *Ramularia proteae* (*R. pro.*). The number of isolates per grass species is to the right of the bars. (B) Non-metric multidimensional scaling (NMDS) plot of pathogen communities associated with the two host groups. A community is defined as all of the foliar fungal isolates from one grass species in a year. Ellipses represent 95% confidence regions for the host group centroids (means). (C) The average number of host species in the database (top panel) and the JRBP compilation (bottom panel) for fungal pathogens isolated from each host group (mean ± 1SE). Averages comprise all fungal isolates with estimated species names and available data (n = 548, top panel) or all OTUs (n = 961, bottom panel).

Figure 2. The average predicted effect (± 1SE) of adding (A–B) 50 native perennial grass individuals m^{-2} or (C–D) 5000 non-native annual grass individuals m^{-2} to bare plots on the relative abundance of each of the seven focal OTUs on each host group (x-axes) based on regressions fit to the (A and C) observational (n = 163 isolates) and (B and D) manipulated (n = 211 isolates) studies (Tables S4–S5). Pathogen abbreviations are in Fig. 1.

Figure 3. The effect of (A–B) native perennial and (C–D) non-native annual grass density on scaled disease severity of native perennial and non-native annual hosts in the (A and C) observational study (n = 1847 leaves) and (B and D) manipulated experiment (n = 1177 leaves).

The average scaled disease severity is plotted at each density value (points). Lines and shaded regions represent linear regression fits (mean ± 1SE, Tables S6–S7).