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Evaluating the impacts of multiple generalist fungal pathogens on temperate tree seedling survival

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Abstract. Host-specific mortality driven by natural enemies is a widely discussed mechanism for explaining plant diversity. In principle, populations of plant species can be regulated by distinct host-specific natural enemies that have weak or nonexistent effects on heterospecific competitors, preventing any single species from becoming dominant and thus promoting diversity. Two of the first steps in exploring the role of natural enemies in diversity regulation are to (1) identify potential enemies and (2) evaluate their levels of host specificity by determining if interactions between any one host and its enemy have equivalent survival impacts on co-occurring host species. We developed a bioinformatics framework to evaluate impacts of potential pathogens on seedling survival, for both single and multiple infections. Importantly, we consider scenarios not only if there are specialist pathogens for each plant, but also when generalist pathogens have differential effects on multiple host species, and when co-infection has species-specific effects. We then applied this analytical framework to a field experiment using molecular techniques to detect potential fungal pathogens on co-occurring tree seedling hosts. Combinatorial complexity created by 160 plant–fungus interactions was reduced to eight combinations that affect seedling survival. Potential fungal pathogens had broad host ranges, but seedling species were each regulated by different combinations of fungi or by generalist fungi that had differential effects on multiple plant species. Soil moisture can have the potential to shift the nature of the interactions in some plant–fungal combinations from neutral to detrimental. Reassessing the assumption of single-enemy–single-host interactions broadens the mechanisms through which natural enemies can influence plant diversity.

Key words: Bayesian analysis; co-infection; Duke Forest, North Carolina, USA; forest diversity; fungi; host specificity; Janzen-Connell hypothesis; pathogens; plant–fungal interactions; seedling mortality; seedling recruitment.

Introduction

Ecologists have long hypothesized that specialist pathogens and other host-specific natural enemies play an important role in influencing plant demographic patterns and maintaining plant diversity (Gillett 1962, Janzen 1970, Connell 1971, Burdon 1987). Natural enemies contribute to diversity maintenance when interactions between plants and their enemies are host-specific, in that each host plant species is negatively impacted by enemies that suppress conspecific but not heterospecific recruitment, preventing dominance by any one plant species. It follows that each plant harbors its own unique enemy or group of enemies that are detrimental to that particular plant and not to co-occurring species. Understanding of the level of host specificity in existing pools of natural enemies has been identified as a critical research need to elucidate the role of natural enemies in diversity maintenance (Gilbert 2005, Freckleton and Lewis 2006)

Microbial plant pathogens, including fungi and oomycetes (fungus-like protozoa), may be responsible for host-specific seedling mortality and, in some cases, demographic patterns of negative density dependence. Negative interactions between plants and their host-specific pathogens impact plant population sizes, competitive interactions, and community structure (Gilbert 2002). Several studies involving biocides or sterilization treatments to remove soil-borne pathogens have demonstrated that these treatments can increase seedling survival (Mangan et al. 2010) or competitive ability (Petermann et al. 2008) when seedlings are grown close to conspecific adults or in soils previously occupied by conspecific plants under controlled conditions. Others have not found analogous negative biotic effects (McCarthy-Neumann and Kobe 2008, 2010*a*, *b*). In some cases, negative biotic effects of conspecifics only become apparent when seedlings are grown at high densities

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(Packer and Clay 2000, Bell et al. 2006, Bagchi et al. 2010).

Much attention in the ecological literature has been focused on identifying patterns of negative density dependence or the "Janzen-Connell effect" (Janzen 1970, Connell 1971). Under this hypothesis, host-specific enemies impact plant survival only when that specific host is abundant, such as when seedlings are grown at high densities or recruit close to conspecific adults. Numerous studies have documented plant demographic responses consistent with this pattern, including studies examining spatial relationships between juveniles and conspecific adults and/or correlations between conspecific and heterospecific seed, seedling, or sapling densities and juvenile survival and recruitment (e.g., Augspurger 1984, Harms et al. 2000, HilleRisLambers et al. 2002, Comita et al. 2010, Gonzalez et al. 2010, Martin and Canham 2010, Metz et al. 2010, Swamy et al. 2011; but see also Condit et al. 1992, Uriarte et al. 2010). Although pathogens are often discussed as probable causes of host-specific mortality, the natural enemies that create these demographic patterns are rarely identified. This combination of evidence for both host-specific negative impacts of the soil microbial community and negative density-dependent seedling mortality has been interpreted as supporting Janzen-Connell models, but this support should be considered provisional unless host-specific pathogens causing mortality are found.

The high diversity of fungi living in plant tissue and soils (Vandenkoornhuyse et al. 2002, O'Brien et al. 2005, Buee et al. 2009) provides a pool of potential host-specific enemies. However, determining if host-specific fungal pathogens explain coexistence of tens to hundreds of tree species requires inference on the effects of a large suite of potential pathogens on the survival of all potential hosts. Plant–fungal interactions resulting in disease must be distinguished from the many benign associations involving plants and fungi (Schulz and Boyle 2005), and it must be confirmed that any deleterious effects are host-specific. This inference is substantially more challenging than has previously been appreciated given the following three factors: (1) the prevalence of generalist fungi that may not have equivalent effects on all hosts; (2) potential interactions between fungi or emergent effects of co-infection; and (3) the influence of the environment on shaping the outcome of plant–fungal interactions.

Many plant-associated fungi infect multiple hosts and can span the continuum of specialization (Gilbert and Webb 2007, Barrett et al. 2009). The ability of a fungus to infect multiple hosts does not necessarily imply that the effects of infection are equal on each host. For example, pathogenic *Pythium* isolates from tropical and temperate soils could infect seeds and/or seedlings of multiple plant hosts but had unequal effects on mortality (Augspurger and Wilkinson 2007). The virulence and aggressiveness of a pathogen may show patterns at levels deeper than species; for example, in a cross-inoculation study of 53 necrotrophic fungi in Panama, most fungi had multiple hosts, but their ability to infect other species decreased with phylogenetic distance (Gilbert and Webb 2007). Even severe pathogens can have broad host ranges with a variety of effects on different hosts, such as *Phytophthora ramorum*, the oomycete causal agent of Sudden Oak Death (Rizzo et al. 2005). Some of the most common genera of seedling pathogens (e.g., *Pythium, Phytophthora, Rhizoctonia*) have long been classified as relatively unspecialized (Burdon 1987, Jarosz and Davelos 1995). But if pathogens with wide host ranges have differential effects on mortality depending on host species, they still may contribute to diversity maintenance by causing measurable harm in some hosts and having minimal effects on others. Thus, we include in our working definition of host-specific enemies both strict specialists with narrow host ranges and generalists with broad host ranges that disproportionally affect a single or limited number of hosts.

The level of complexity in disentangling plant–fungal interactions is amplified when hosts are co-infected by multiple fungi simultaneously. Although previous work has emphasized single pathogen infections in a given host, individual plants host diverse microbial communities (Vandenkoornhuyse et al. 2002, Rodriguez et al. 2009), and multiple pathogen infections are common (Barrett et al. 2009, Seabloom et al. 2010). In theory, pathogens could directly or indirectly compete for resources, facilitate or thwart one another via immune-mediated responses, or have no interactions at all (Pedersen and Fenton 2007). Considering the impacts of multiple infections vastly increases the dimensionality of the problem from an analytical standpoint. This can be represented by combinations of potential pathogens attacking diverse tree communities, given by $H \times 2^K$ for H host species and K potential pathogens. For example, 10 pathogens and 10 hosts yield more than 10 000 host–pathogen combinations.

In addition, both hosts and fungi each have their own responses to the environment, as does their interaction (Barrett et al. 2009). For example, moisture availability can influence the extent to which the symbiotic fungus *Discula quericina* is parasitic or mutualistic on its host *Quercus cerris* (Moricca and Ragazzi 2008). Dispersal of seedlings into light gaps and experimental growth under high-light treatments decreased the amount of damping-off in seedlings of several tropical tree species (Augspurger and Kelly 1984). Conversely, the fungus *Diplodia mutila* is more pathogenic in seedlings of its tropical palm host, *Iriartea deltoidea,* under high-light than low-light conditions (Alvarez-Loayza et al. 2011). Environmental conditions, such as light and soil moisture, need to be taken into account when assessing the nature of each interaction between host and fungus.

Given this complexity, it is not surprising that few cases of host-specific seedling pathogens have been

identified (but see Packer and Clay 2000). Mortality is often attributed to diseases that can have multiple causal agents, such as damping-off (Augspurger 1984). Teasing apart which plant-associated fungi are affecting seedling survival requires a synthetic analysis that can address the interactions between communities of fungi and plants, along with environmental conditions that may alter the nature of these interactions. A hierarchical Bayesian approach allows us to integrate information from a number of sources, including knowledge from separate analyses of detection probabilities and conditional relationships involving environmental variables, fungal incidence, fungal infection, fungal detection, and seedling survival.

To determine if biodiversity regulation could result from differential consequences of infection with generalist fungi or co-infection, we identified potential fungal pathogens on seedlings of five tree species in a temperate mixed hardwood forest (Duke Forest, Orange County, North Carolina, USA) using culture-based methods and subsequent DNA sequencing. We developed a modeling framework for analysis of their high-dimensional interactions (Clark and Hersh 2009) that quantifies the efficacy of all host–fungus combinations for five putative fungal pathogens, integrating fungal incidence, infection, and survival probabilities and their dependence on environmental variables. Our goal was to assess the importance of host specificity, co-infection, and environmental conditions when considering the effects of potential fungal pathogens on tree seedling survival. These first steps are crucial to the eventual evaluation of the role that fungal plant pathogens play in the regulation of tree diversity.

Materials and Methods

Field studies

Field experiments were conducted in the Duke Forest (Orange County, North Carolina, USA) a temperate, mixed hardwood forest. Fourteen common southeastern tree species were initially planted from seed in May 2006 and December 2006; host species were selected to include a range of life history strategies. Seeds were planted in 60 herbivore exclosures ($1 \times 0.9 \times 0.46$ m; henceforth "plots") constructed from 1.27-cm mesh hardware cloth. Planted seeds were randomly arranged. Three plots were installed at each of 20 locations in the Eno and Blackwood divisions of the Duke Forest, for a total of 60 plots. At each location, two plots contained 90 seeds (five per species), and one plot contained 38 seeds (two per species). Pre-emergence (seed) pathogens affecting germination are known to have some host affinity and decrease seedling emergence in other systems (Gallery et al. 2007, 2010); however, assaying for these pathogens was outside the scope of this study. Seeds were washed with a mild surfactant and rinsed thoroughly before planting. Data from five tree species with sufficiently high rates of emergence and mortality were used in subsequent analyses: *Acer barbatum* (southern sugar maple), *Diospyros virginiana* (persimmon), *Liquidambar styraciflua* (sweetgum), *Nyssa sylvatica* (black tupelo), and *Pinus taeda* (loblolly pine).

Seedling mortality was assessed approximately every seven days from March through November 2007; survival data were collected on 601 individuals of the five target species. Dead and dying seedlings were collected throughout the growing season to recover potential fungal pathogens; additional samples of live seedlings were collected during the 2007 growing season and in November 2008 to distinguish between fungi present only in dead seedlings and fungi found in both live and dead individuals. As seedlings were collected throughout the growing season, collections included new germinants through fully lignified, established seedlings. Our ability to collect dead seedlings for fungal isolation was limited by unpredictable factors, most notably the location and positive identification of dying or recently dead seedlings, and our sampling effort represents the maximum number of collections we were able to obtain for each species. Fungi isolated from 237 seedlings of the five target species were used in this analysis; sample sizes for target species are listed in Appendix A. Hemispherical canopy photos were taken at all plots to measure canopy openness in September 2006. Photos were analyzed using Hemiview (Delta-T Devices, Cambridge, UK; detailed methods are available in Clark et al. [2003]). Soil moisture was measured monthly at each location using time-domain reflectometry (Tektronic 1502B; Tektronix, Beaverton, Oregon, USA).

Fungal identification

All seedlings were rinsed thoroughly for 1 h under running tap water to remove soil particles. Duplicate fragments of tissue from each component (root, stem, leaf if available) of each seedling were treated additionally with 30 s in 70% ethanol followed by 30 s in 10% bleach, and cultured on the same media. Fungi were initially cultured from fragments of root, stem, and leaves from both surface treatments for each seedling on both on alkaline water agar (AWA) and pimaricin–ampicillin–rifampicin–pentachloronitrobenzene (PARP) media, and subcultured onto potato dextrose agar (PDA30) and corn meal agar (CMA), respectively. Cultures from both surface treatments were pooled in a single analysis, as there was substantial overlap in community composition (Morisita-Horn similarity index, calculated using EstimateS [Colwell 2006], comparing surface treatments = 0.851 for all five plant species combined; values for individual plant species ranged from 0.547 to 0.914). Ribosomal DNA was amplified directly from fragments of fungal mycelia using primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990), cleaned using a Qiaquick PCR purification kit (Qiagen, Valencia, California, USA), and sequenced on an ABI 3730 Autosequencer (Applied Biosystems, Carlsbad, California, USA). ITS (internal transcribed spacer) sequences were trimmed and edited by hand and

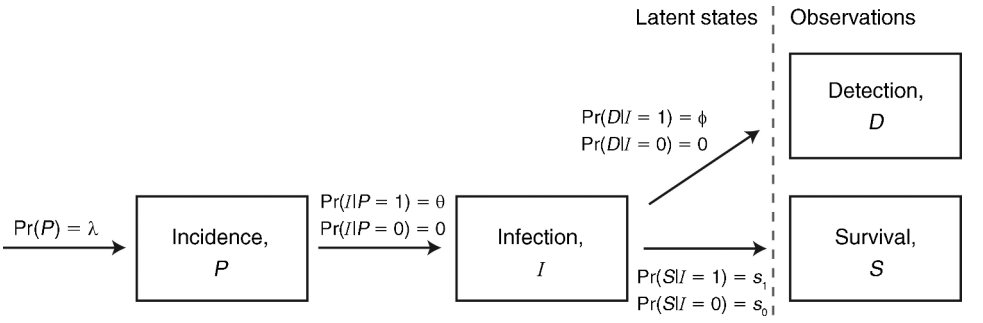

FIG. 1. Schematic depiction of the graphical framework of the model of fungal incidence (P), infection (I), survival (S), and detection (D). Equations adjacent to arrows describe model parameters: incidence probability (λ), infection probability (θ), detection probability (φ), and survival probabilities with and without infections (s_1 and s_0, respectively). The figure is adapted from Clark and Hersh (2009).

clustered into operational taxonomic units (OTUs) based on 96% sequence similarity using the program Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Names were assigned to OTUs based on results of NCBI BLAST searches of GenBank using the blastn algorithm accessed on 16 June 2009 (Altschul et al. 1990). OTUs were assigned a genus or species name if the top named fungal sequence had an E-value of 0.0 and maximum identity greater than or equal to 97%. The Expect-Value (E-value) is used as a threshold of statistical significance for matches produced by the NCBI BLAST algorithm. It is a metric of Type I error specific to a sequence and its BLAST match given the size of the database. Representative sequences from each of the 130 OTUs identified were deposited in GenBank under accession numbers GQ996064–GQ996193.

Data analysis

We developed a hierarchical Bayesian model of fungal effects on seedling survival and their dependence on fungal incidence (P), host infection (I), host survival (S), and fungal detection (D) (Fig. 1; Clark and Hersh 2009). Infection and incidence are inferred as latent states (Fig. 1). We define *incidence* as presence of the fungus at the 0.9-m^2 plot level and *infection* as presence of the fungus within an individual plant. Information enters the model in the form of observations of survival, detection, and environmental covariates (light and soil moisture). We define *detection* as the observation of infection, when a fungus is successfully cultured from a given host plant. The model includes causal relationships (arrows in Fig. 1) for fungal incidence at host locations, host infection by each fungus, fungal detection on hosts, and host survival (with and without infection). Environmental covariates affect fungal incidence (soil moisture) and host survival (light, soil moisture). We did not observe plot-level differences in residual survival effects. Information on incidence of fungi within plots is shared across individuals of all host species within plots, but otherwise all parameters were estimated at the individual level.

The challenge the model addresses concerns the many potential combinations of infection by different fungal taxa and their impacts on host survival, individually and in combination. Combinations of hosts and fungi are evaluated as a network of interactions, using a reversible jump Markov chain Monte Carlo (RJMCMC) algorithm to reduce the model space of all potential host–fungus combinations to those having consequential impacts on host survival. The RJMCMC algorithm allows for model selection within a hierarchical Bayesian framework (Green 1995). Testing of such complex models is done through simulation (Gelman and Hill 2007, Clark et al. 2010). Simulation studies are used to determine, given the study design (sample sizes, distributions of input variables across host taxa and environmental variables), if known parameter values can be recovered. Clark and Hersh (2009) summarize a large simulation study demonstrating that relationships between infection and survival are confidently estimated and that false negative (failure to find a fungal effect when it exists) and false positive (attributing an effect when it does not exist) rates are below 5%.

Five of the most common fungi identified (complete list available in Appendix B) that are known to be pathogens or members of genera containing pathogens were selected for analysis, including (with acronyms) *Colletotrichum acutatum* (COLA), *Cylindrocarpon* sp. A (CYLA), *Phomopsis* sp. A (PHMA), *Phomopsis* sp. B (PHMB), and *Phomopsis* sp. D (PHMD). Data on detection of fungi (237 seedlings) were then combined with seedling survival data (601 seedlings; Appendix A) and covariates to predict the full effects of each host–fungus combination. We tested 160 combinations of hosts and fungi, including each host infected with all possible combinations of 0, 1, 2, 3, 4, and 5 fungi. We implemented the full model with the five hosts and five fungi using a MCMC algorithm based on Metropolis-within-Gibbs with a reversible jump component. For results presented here, the Gibbs sampler was initially run for 500 000 iterations until convergence was observed graphically in all parameters. These initial iterations were discarded, and the model was run for an

additional 500 000 Gibbs steps. Results from previous studies in the Duke Forest (Clark et al. 2004) were used to specify host species-specific prior densities for the effects of light and soil moisture on seedling survival. Prior densities for fungal effects on survival were truncated as fungi selected for analysis in the model were from genera known to contain plant pathogens. A uniform prior density on the logit scale, Unif(−10, 10), allows that fungi might have negative or positive effects on survival but will not be arbitrarily close to 0 or 1. The prior density Unif(−10, 0) allows that fungal effects are non-positive. We implemented the model using both prior distributions, and found that both analyses predicted the same combinations of fungal taxa having negative effects on survival of particular hosts. For computational efficiency, we performed the final analysis using the second prior density.

Results

Distinct fungi or combinations of fungi impact seedling survival for each plant host, which is quantified using posterior model probabilities for each combination of host and fungus/fungi. The Bayesian posterior model probability (P_M) is the probability that infection with fungus or fungal combination M decreases host survival, and $1 - P_M$ is the probability that it does not (Scott and Berger 2006). Fig. 2 shows posterior model probabilities for combinations of fungal infections (rows) on seedling hosts (columns). Posterior model probabilities greater than 0.5 (warm colors, shaded boxes in Fig. 2) indicate infections or co-infections that reduce survival. Probabilities less than 0.5 (cool colors, unshaded boxes) indicate no effect. Empty cells represent infection or co-infection combinations that do not occur in the data set or were rarely detected. Each host plant species was infected by 2–5 fungal taxa, and four of the five fungi tested were associated with at least three host plants (Fig. 2). No fungus isolated more than 5 times was restricted to a single plant species.

Although many of these fungi infect multiple hosts, their effects on seedling survival differ between hosts and depend on the co-infection combinations in which they occur (Fig. 2, rows 1–5). For example, infection with *Cylindrocarpon* alone decreased survival of *Pinus taeda*, but not any of the other four species that it can infect. In addition, effects of co-infection can differ from infection by individual fungi. In some hosts, fungi reduce survival only in combination with others (Fig. 2). For example, none of the five fungi alone decreased survival of *Nyssa sylvatica*, yet the combination of *Colletotrichum* and *Cylindrocarpon* did. On the other hand, *P. taeda* survival was reduced by both the combination of *Colletotrichum* and *Cylindrocarpon* and by *Cylindrocarpon* alone.

In addition to identifying co-infections that reduce survival, we evaluated the magnitude of the change in seedling survival probabilities as a result of fungal infection (Fig. 3). First, we compared the differences in

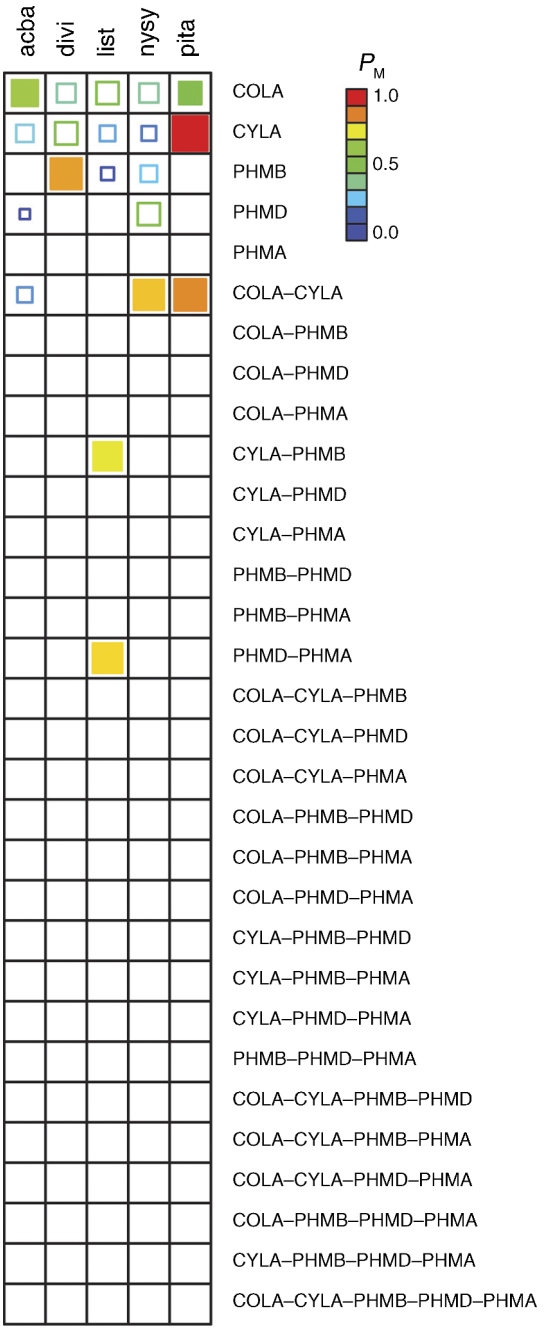

FIG. 2. Posterior model probabilities (P_M) of effects of different combinations of potentially pathogenic fungi on survival of seedling hosts. The rows are fungi, individually and in combination, and the columns are seedling hosts, so that each square on the grid represents a specific host–fungus combination. Box sizes are scaled according to posterior model probabilities (P_M); shading in boxes is filled when $P_M \geq 0.5$, and open when $P_M < 0.5$. Empty cells represent combinations that do not occur or rarely occur in the data set. Fungal species key: COLA, *Colletotrichum acutatum*; CYLA, *Cylindrocarpon* sp. A; PHMA, *Phomopsis* sp. A; PHMB, *Phomopsis* sp. B; and PHMD, *Phomopsis* sp. D. Host tree species key: acba, *Acer barbatum*; divi, *Diospyros virginianan*; list, *Liquidambar styraciflua*; nysy, *Nyssa sylvatica*; pita, *Pinus taeda*.

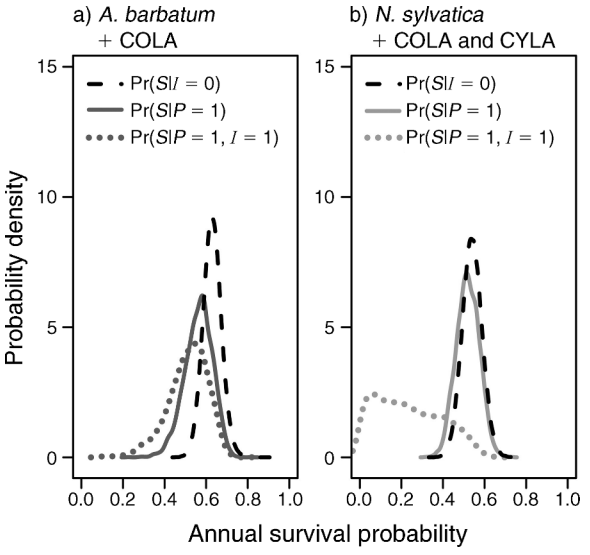

FIG. 3. Predictive distributions of survival (S) probabilities of hosts under different infection (I) scenarios. Survival probabilities for uninfected hosts [$\Pr(S \mid I = 0)$, dashed black lines], infected hosts [$\Pr(S \mid I = \mathrm{I})$, dotted gray lines], and survival probabilities given fungal incidence (P) [$\Pr(S \mid P = 1)$, solid gray lines] are shown for (a) *Acer barbatum* and (b) *Nyssa sylvatica*. Only combinations of fungi and hosts with $P_{\mathrm{M}} \geq 0.5$ are included. For abbreviations, see Fig. 2 legend; for all hosts, see Appendix C.

seedling survival, S, in locations where a given fungus is present, ($\Pr(S \mid P = 1)$; solid gray lines) or absent ($\Pr(S \mid P = 0)$; dashed black lines). Survival given incidence marginalizes over infection probabilities, taking into account the fact that even where the fungus is present, not all individuals are infected. Comparing the probability of survival given infection, I, ($\Pr(S \mid I = 1)$; dotted gray lines), the probability of seedling survival is reduced further. In the eight combinations with a posterior model probability ≥ 0.5, fungal infection reduces survival, but the magnitude of these effects depends on infection rates (Appendix C).

Incidence probability of all five fungi is also greater with increasing soil moisture (Appendix D). High soil moisture improves host survival (Fig. 4; black lines, 10th percentile of observed soil moisture values; gray lines, 90th percentile of observed soil moisture values). To evaluate the combined effects of soil moisture and fungi, we compare survival of uninfected seedlings, $\Pr(S \mid I = 0)$ (Fig. 4, dashed lines) with survival probabilities marginalized over infection and incidence, $\Pr(S)$ (Fig. 4, solid lines). In the case of *Colletotrichum* and *Acer barbatum* (Fig. 4a), differences between survival probabilities with and without the fungus are similar under dry and wet conditions. However, in the case of *Phomopsis* sp. B and *Diospyros virginiana* (Fig. 4b), soil moisture shifts the nature of the interaction. At low soil moisture, survival probability is not influenced by infection, but at high soil moisture, the fungus decreases survival. The nature of these soil moisture effects varies among the eight host–fungus combinations with $P_{\mathrm{M}} \geq 0.5$ (Appendix E). All posterior estimates for incidence, infection, and detection probabilities, along with covariate effects on incidence and survival, are provided in Appendix F.

DISCUSSION

In this study we sought to identify plant–fungal interactions that could play a role in maintaining temperate forest diversity via differential seedling mortality. Using a Bayesian hierarchical model, we tested all possible combinations of five temperate tree seedlings and five putative fungal pathogens to determine if fungal infection reduced seedling survival. The model space was reduced from all 160 potential host–fungus combinations to those eight combinations having

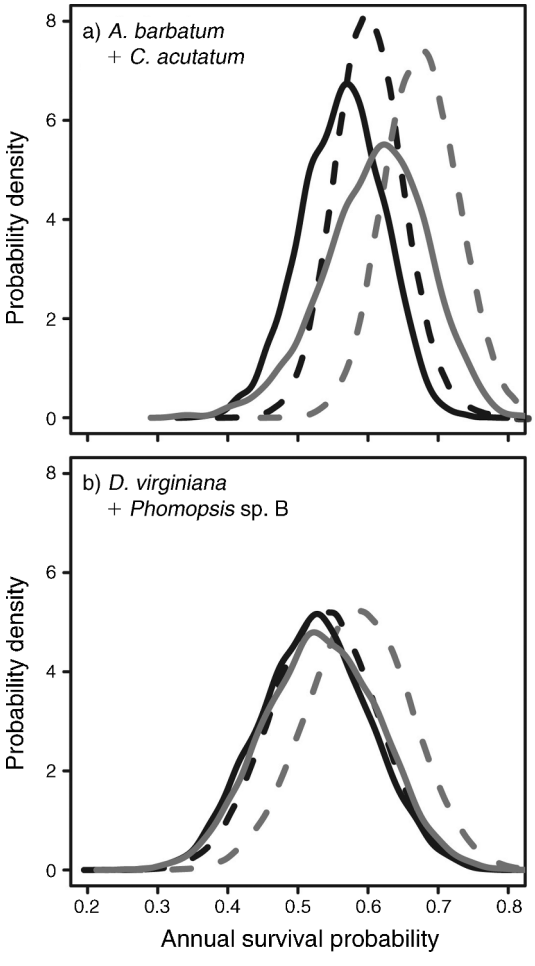

FIG. 4. Predictive distributions of survival (S) probabilities of hosts under different scenarios of infection (I) and levels of soil moisture. Each panel includes uninfected plants [$\Pr(S \mid I = 0)$, dashed lines] and survival probabilities marginalized over infection and incidence [$\Pr(S)$, solid lines]. Black lines show survival at low soil moisture (10th percentile of observed values); gray lines show survival at high soil moisture (90th percentile). For species key, see Fig. 2 legend; for all hosts, see Appendix E.

consequential impacts on host survival. We emphasize three key results of this study that highlight aspects of seedling disease ecology relevant to biodiversity maintenance. First, although most of the fungi we isolated were found on multiple hosts, effects on seedling survival were unequal depending on plant species identity. Second, some plant species were only negatively impacted by combinations of fungal infections. Finally, differences in soil moisture have the potential to alter the nature of some of these plant–fungus interactions, indicating that these interactions can be environmentally mediated.

Janzen-Connell effects and negative density dependence are reported frequently in natural systems. Patterns of plant demography consistent with Janzen-Connell effects are found in tropical forests (Metz et al. 2010), temperate forests (Martin and Canham 2010), and grasslands (Petermann et al. 2008, Allan et al. 2010), and throughout the early life stages of trees, from seeds (Kotanen 2007) to seedlings (Comita et al. 2010) to saplings (Gonzalez et al. 2010). Experimental evidence from sterilization or biocide treatments (Bell et al. 2006), reciprocal transplants (Mangan et al. 2010), and herbivore exclosures (Swamy and Terborgh 2010) frequently points towards microbial plant pathogens as driving such demographic patterns. To play an important role in maintaining plant diversity, however, theory contends that pathogens must be specific to high-density hosts and suppress them sufficiently to foster survival of heterospecific individuals. Few studies have documented pathogen–host associations sufficiently to address host specificity and conditions driving pathogen virulence. Thus, the mechanism through which diversity is maintained remains unclear.

In order to determine which pathogens, if any, affect plant diversity, it is necessary to assess not only the ability of any given potential seedling pathogen to infect different plant hosts, but also its relative effect on all species in its host range. Our results showing differential effects of multi-host fungi are consistent with current knowledge that plant-associated fungi can display multiple lifestyles, depending on host identity (Barrett et al. 2009). For example, several fungal species within the genus *Colletotrichum* can exhibit different lifestyles, from mutualism to parasitism, depending on which host species they infect (Redman et al. 2001). This work corroborates other studies showing that necrotrophic fungal seed and seedling pathogens (Augspurger and Wilkinson 2007) and foliar pathogens (Gilbert and Webb 2007) can infect multiple hosts, but have differing impacts on host health depending on host identity. This could be driven by any one of a suite of different mechanisms throughout disease development, involving the physiology and defense responses of a particular plant host and the aggressiveness of its particular fungal symbiont. Although we chose to examine effects of fungi at the putative fungal species level, variation in lifestyles within species may also be caused by host specificity at the fungal strain level (Burdon 1987). Our modeling framework allows us to infer differential effects of fungal taxa on seedling survival, and creates targeted hypotheses of specific plant–fungal combinations for future inoculation experiments or other empirical tests.

Differential mortality caused by sets of co-infecting pathogens is an understudied mechanism for biodiversity maintenance. Although infections by multiple pathogens or potential pathogens are common and multiple studies have examined co-infection in managed systems (see Morris et al. 2007 for a meta-analysis including several such studies), relatively few ecological studies have examined co-infection in unmanaged plant communities (but see Seabloom et al. 2010). In our present study, we found that two of the five tree species we analyzed are negatively affected only by combinations of fungi. The effects of combined infections on a plant may not be additive because of potential interactions between infecting fungi. Within the host, fungi could compete directly or indirectly for resources, compete indirectly by stimulating plant immune responses, or even facilitate one another (e.g., Pedersen and Fenton 2007). Nonadditive interactions between pathogens within plants have been documented in multiple studies. For example, in a mesocosm study of four species of *Brassica*, two pathogens (one bacterial, one fungal) had more positive impacts on plant diversity singly than in combination (Bradley et al. 2008). Other studies show evidence of competition between fungal pathogens (Al-Naimi et al. 2005) and viruses (Power 1996) in plants. The extent of localization of infections with different pathogens in a single plant may limit direct interactions, but not indirect or immune-mediated interactions (Pedersen and Fenton 2007). The methods we used to identify fungi did not determine the order of infection or quantify the relative amounts of each fungus in a given seedling, both of which could impact the outcome of interactions between fungi (Adee et al. 1990, Al-Naimi et al. 2005). Similarly, we did not consider interactions between potential pathogens and mutualistic mycorrhizal fungi, which may alter interactions between plants and pathogens via multiple mechanisms (Bennett et al. 2006). Our modeling approach can be used to assess the net effect of combinations of infections when the number of potential combinations is large and the nature of these interactions is unknown.

Finally, any pathogen effects influencing biodiversity need to be considered in the context of relevant abiotic factors (Fig. 4; Appendix E). In particular, our present study highlights the potential importance of soil moisture in determining the effects of fungi on seedling survival. Adequate soil moisture can improve spore dispersal and germination, and invasion into plant tissue (Colhoun 1973), but it can also improve plant growth and survival. In the case of *Diospyros virginiana* and *Phomopsis* sp. B, both host and fungus respond positively to soil moisture, but effects of the fungus offset the survival benefits to the host at high soil

moisture. Improved spore dispersal and tissue infection under wet conditions are two possible explanations. We found few changes in plant–fungal interactions under differing light conditions (data not shown); however, as this study did not include plots in canopy gaps or otherwise outside of closed-canopy conditions, the range of variation in light levels may not have been large enough to induce responses.

This study represents a first attempt to assess the level of host specificity and ecological functions of five potential fungal pathogens in the context of environmental variation, and helps to begin to elucidate their potential roles in diversity maintenance. We acknowledge that a study such as this has several limitations. First, given the complexity described above, it is challenging to distinguish fungi that elicit disease symptoms from endophytes and opportunistic saprophytes. The traditional method to establish causality is through experimental inoculations to demonstrate that Koch's postulates have been met (e.g., Packer and Clay 2000). To thoroughly perform a study at the community level with so many potential combinations of plants, fungi, and environmental conditions without any a priori hypotheses as to which interactions are important would require considerable resources. Using model-based inference can distinguish particular combinations of plants and fungi with high probabilities of affecting seedling survival for future empirical testing to conclusively demonstrate pathogenicity. The effects on seedling survival of the fungi analyzed in this study and their subsequent impacts on plant community structure will depend on if they compound annually, as trees can remain in the seedling stage for years (Streng et al. 1989). In addition, input of non-local fungal inoculum from seed coats, as described in *Materials and methods: Field studies*, choice of surface sterilization methods, and asynchrony of collection between live and dead seedlings may have potentially biased the assessment of fungal community composition. Finally, application of the model we apply here is limited computationally in that model runs with five seedling hosts and five fungi took approximately three days to run on a remote server.

By accounting for the interactions among potential fungal pathogens, their hosts, and environmental variation, we show evidence of specialization in multi-host fungi. A single fungal infection could have differential impacts on the survival of each of its hosts. Each additional fungus could help support the coexistence of multiple hosts, through its 2^K potential interactions with K other fungi, each of which can be modified by the environment. High soil moisture tends to increase the probability of fungal incidence, but it may also improve the capacity of a vigorous host to defend itself in some cases. Thus, interactions among seedlings, fungi, and the environment must be considered for a full evaluation of how fungi affect seedling dynamics.

In this study we applied a novel analytical approach to field-collected data on fungal infection to evaluate the role of fungi on forest diversity in the context of environmental variability. Our model selection framework helps distill the complexity of interactions between many hosts and fungi, creating focused hypotheses of which host–fungus interactions could affect survival for further testing. A synthetic study that incorporates the effects of multiple types of host-specific enemies (fungi, oomycetes, bacteria, herbivores, seed predators, and pathogens) could provide further insight. This analytical method makes large-scale efforts to incorporate the effects of multiple fungi possible, and begins to shed light on the complex web of interactions that underlies forest diversity.

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Supplemental Material

Appendix A

A table of survival rates and sample sizes of tree seedlings used in this study (*Ecological Archives* E093-047-A1).

Appendix B

A tabular list of all fungal taxa isolated more than five times and their top NCBI BLAST matches (*Ecological Archives* E093-047-A2).

Appendix C

A figure presenting predictive distributions of survival probabilities for all host–fungus combinations with posterior model probability (P_M) ≥ 0.5 (*Ecological Archives* E093-047-A3).

Appendix D

A figure presenting predictive distributions of fungal incidence probabilities, Pr(P), at the range of soil moisture levels observed (*Ecological Archives* E093-047-A4).

Appendix E

A figure presenting predictive distributions of host survival probabilities under different scenarios of infection and levels of soil moisture for all host–fungus combinations with $P_M \geq 0.5$ (*Ecological Archives* E093-047-A5).

Appendix F

Four tables with posterior median parameter estimates of incidence, infection, and detection probabilities and covariate effects on fungal incidence and seedling survival (*Ecological Archives* E093-047-A6).