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RESEARCH ARTICLE

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Closely related tree species support distinct communities of seed-associated fungi in a lowland tropical forest

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Abstract

1. Previous theoretical work has highlighted the potential for natural enemies to mediate the coexistence of species with similar life histories via density-dependent effects on survivorship. For plant pathogens to play this role, they must differ in their ability to infect or induce disease in different host plant species. In tropical forests characterized by high diversity, these effects must extend to phylogenetically closely related species pairs. Mortality at the seed and seedling stage strongly influences the abundance and distribution of tropical tree species, but the host preferences and spatial distributions of fungi are rarely determined.
2. We examined how host species identity, relatedness and seed viability influence the composition of fungal communities associated with seeds of four co-occurring pioneer trees (*Cecropia insignis*, *C. longipes*, *C. peltata* and *Jacaranda copaia*). Seeds were buried in mesh bags in five common gardens in the understorey of a lowland tropical forest in Panama and retrieved at intervals from 1 to 30 months. A subset of the seeds in each bag was used to determine germination success. One half of each remaining seed was tested for viability; and the other half was used to culture and identify seed-infecting fungi.
3. Seeds were infected by fungi after burial. Although fungal communities differed in viable versus dead seeds, and across burial locations, community composition primarily varied as a function of plant species identity (30.7% of variation in community composition vs. 4.5% for viability and location together), even for congeneric *Cecropia* species. Phylogenetic reconstruction showed that relatedness of fungi mostly reflected differences between *Jacaranda* (Bignoniaceae) and *Cecropia* (Urticaceae).
4. Although the proportion of germinable seeds decreased gradually over time for all species, intraspecific variation in survival was high at the same location (e.g. ranging from 0% to 100% for *C. peltata*) suggesting variable exposure or susceptibility to seed pathogens.

5. *Synthesis*. Our study provides evidence under field conditions that congeneric tree species with similar life history traits differ markedly in seed-associated fungal communities when exposed to the same soil-borne fungi. This is a critical first step supporting pathogen-mediated coexistence of closely related tree species.

KEYWORDS

Cecropia, *Jacaranda*, lowland tropical forests, pioneer trees, seed pathogens, seed persistence, seed-associated fungi, soil seed bank

1 | INTRODUCTION

Pathogens are hypothesized to play a fundamental role in regulating the diversity and distribution of plant species in tropical forests (Bagchi et al., 2014; Mangan et al., 2010). These community-level effects arise from density-dependent regulation of populations of individual host species, facilitating recruitment of locally less abundant, heterospecific taxa (Bagchi et al., 2014; Comita et al., 2014; Fine et al., 2004). Empirical support for this hypothesis comes from diverse studies, including experiments that compare seed and seedling survival in forest soil with and without fungicide treatments. These studies strongly suggest that pathogens increase mortality rates for species that occur at high densities, thus increasing species diversity (Bagchi et al., 2014; Bell et al., 2006; Dalling et al., 1998). In general, experimental studies that manipulate pathogen pressure are consistent with the role of pathogens in mediating coexistence in species-rich plant communities, but they often focus on species representing different genera, with distinctive life-history and functional traits (e.g. Bagchi et al., 2014). An underlying tenet of the hypothesis—that even closely related host species will differ in their susceptibility to infection from the same pathogen source—has not been tested previously.

Seed mortality due to fungal infection varies widely within plant communities (Blaney & Kotanen, 2001; Gallery et al., 2010), with susceptibility to pathogens varying among species (see Augspurger & Wilkinson, 2007; Mommer et al., 2018). Despite the importance of pathogenic fungi as primary agents of mortality, our understanding of the spatial distribution, host range and impact of fungi on tropical forest species remains limited. One possible explanation for this knowledge gap is the difficulty in developing experimental systems that are amenable to characterizing pathogen communities across spatial scales and host ranges. Seed-infecting fungal communities can provide a model system due to their manageable diversity, the ease of manipulation and replication of seed studies (Sarmiento et al., 2017), and because fungi play a preeminent role in seed survival in tropical forests, especially for species that form persistent soil seed banks (Dalling et al., 1998; Gallery et al., 2010).

The soil seed bank represents a key life-history stage for tropical pioneer species, equivalent to the seedling stage for shade-tolerant species. Unlike the seeds of shade-tolerant species that typically germinate within weeks of dispersal, seeds of pioneers can survive in the soil seed bank for years to decades until a canopy gap creates favourable conditions for germination (Dalling & Brown, 2009). Seeds persist in a dormant state, or—in some species—remain in a quiescent state, wherein seeds lack any intrinsic barrier to germination (Thompson, 2000). Zalamea et al. (2018) showed that quiescent seeds lack many of the chemical and physical defences that characterize dormant seeds. Such weak defences leave quiescent seeds susceptible to microbial infection before germination occurs (Gallery et al., 2010), and in general are associated with seed persistence times that are shorter than those of dormant seeds (Zalamea et al., 2018). However, our understanding of how quiescent seeds interact with soil-borne microbes is limited.

Using a phylogenetically diverse group of common pioneer tree species from lowland tropical forests in Panama (nine species from six families), Sarmiento et al. (2017) found that the composition of fungal communities that infect seeds buried in the soil for up to 12 months is strongly determined by plant species rather than by soil type, forest characteristics or duration of burial. This suggests a high degree of compositional dissimilarity of seed-infecting fungal communities across taxa at the family and higher taxonomic levels. However, if the pathogen-mediated coexistence hypothesis is to provide a general mechanism for the maintenance of diversity, then it needs to account for the co-occurrence of species-rich genera that are a product of recent divergence, and where traits that influence susceptibility to natural enemies are likely to show strong phylogenetic signal (Queenborough et al., 2009). A previous study of foliar pathogens has indeed shown that closely related taxa are more likely to be susceptible to attack by the same pathogens (Gilbert & Webb, 2007).

Here we report the structure and phylogenetic relationships of seed-associated fungal communities of three *Cecropia* species (Urticaceae), and a more distantly related, co-occurring species *Jacaranda copaia* (Bignoniaceae). These four species lack seed dormancy. In the lowland tropical forest of Barro Colorado Island (BCI), Panama, two species are locally common (*C. insignis* and *J. copaia*) and the other two (*C. longipes* and *C. peltata*) are locally rare. The majority of fungi that infect seeds in this forest are acquired directly from soil (Sarmiento et al., 2017). We placed seeds of these species into common gardens in the forest understorey at BCI that together spanned multiple soil types and forest ages. We measured seed germination (equivalent to seed survival) for seeds

buried for up to 30 months, and cultured and identified fungi from the interior of surface-sterilized seeds to assess the importance of host species, burial location and time course of infection in shaping the communities of fungi that naturally colonized seeds in the soil. By combining information on fungal infection with data on seed germination, we explore how fungi were associated with seed survival.

In this context, we tested five main predictions. First, we predicted that seed infection rates would increase through the 30-month time course of our experiment, consistent with fungi encountering and colonizing seed interiors over time. Second, we anticipated that seed infection rates would be higher on dead seeds than live seeds given the potential for soil saprotrophs to colonize decaying material. Third, we predicted that locally abundant tree species would harbour a greater diversity of seed-associated fungi than rare species, as the proximity or higher regional density of conspecific individuals would increase dispersal among susceptible hosts, fostering populations of host-specialist fungi (as observed for wood decay fungi; Gilbert et al., 2002). Fourth, we anticipated that congeneric *Cecropia* species with similar seed morphology and dispersal traits would harbour distinct fungal communities from one another, and that these communities would differ in community composition from those of a more distantly related species (*J. copaia*). Finally, we predicted that species with higher infection rates should have lower germination rates, consistent with the expectation that seed-infecting fungi are primarily antagonistic.

2 | MATERIALS AND METHODS

2.1 | Study site, species selection and common garden experiment

We carried out this study in a seasonally moist tropical forest at BCI, Panama (9°10′N, 79°51′W). Rainfall on BCI averages 2,600 mm per year, with a pronounced dry season starting in late December or early January and continuing until late April (Windsor, 1990).

We selected three (*Cecropia insignis* Liebm., *C. longipes* Pittier and *C. peltata* L.) of the four species of *Cecropia* Loefl. (Urticaceae, Rosales) that naturally occur on Barro Colorado Nature Monument (Croat, 1978). *Cecropia* are dioecious canopy trees with animal-dispersed seeds (birds, bats and primates) that recruit into gaps and other canopy openings from seed rain and the soil seed bank (Berg & Franco-Rosselli, 2005). The genus includes 61 species distributed across a wide range of climates from southern Mexico to northern Argentina (Berg & Franco-Rosselli, 2005). We also included *Jacaranda copaia* Aubl. (Bignoniaceae; Lamiales), a widely distributed pioneer tree in Central and South America with wind-dispersed seeds that is distantly related to *Cecropia* but that has similar regeneration requirements (Croat, 1978).

Seeds of all species were collected from at least five different maternal sources in 2012–2014 in accordance with seasonal variation in fruiting phenology. Mature infructescences of *Cecropia* spp. were collected directly from trees or from freshly fallen material beneath their crowns. *Jacaranda copaia* trees can attain 35 m in height when mature, precluding collection of ripe fruits directly from the crown. For this species, we used recently fallen seeds estimated to have been on the soil surface for a short time (from minutes to a few hours).

Seeds of *J. copaia* were separated from dehiscent woody seed pods and the wings were cut from each seed. Seeds of *Cecropia* (technically fruits; Lobova et al., 2003) were removed from whole infructescences. Immediately after collection seeds were cleaned with tap water and 0.7% sodium hypochlorite for 2 min to remove small pieces of pulp. Clean seeds of all species were air-dried at room temperature (~22°C) for several days under low red:far red irradiance.

We established five common garden plots (9 m × 15 m each) in lowland forest at BCI. Common gardens were at least 350 m apart (average distance 800 m) and were located under closed-canopy forest to limit seed germination (see Table S1 for a summary of common garden characteristics; see also Sarmiento et al., 2017; Ruzi et al., 2017; Zalamea et al., 2015). They were placed in multiple forest and soil types that varied in slope and aspect (Baillie et al., 2006). No adults of the study species occurred within 20 m of the garden edges.

Dry seeds from all maternal sources of each species were thoroughly mixed, pooled and placed into seed bags. Each seed bag contained 45 seeds of one species mixed with 10 g of sterile forest soil (previously autoclaved at 121°C for 2 hr). Each set of soil and seeds was enclosed in a nylon mesh bag (pore size = 0.2 mm) and covered with aluminium mesh (pore size = 2 mm) to exclude seed predators but not microorganisms.

In all, 28 bags per plant species were distributed among four replicate subplots per garden, where they were buried 2 cm below the soil surface and 40 cm apart. A total of 140 seed bags and a total of 6,300 seeds per species were included in the burial experiment. Seeds of each species were buried less than a month after seed collection, matching the natural phenology of fruit production and seed dispersal. We evaluated initial infection rates and confirmed the viability status of the seed lot before burial by processing 400 fresh seeds (i.e. seeds that were not buried) per species (200 seeds for germination; 200 seeds for fungal culture—see below for details).

2.2 | Seed processing and retrieval after burial

Four seed bags for each species in each garden were collected at each of seven time points: 1, 3, 6, 12, 18, 24 and 30 months after burial. Seeds were retrieved by rinsing the contents of each bag with tap water over a sieve. Seeds were partitioned for tests of (a) germination (10 seeds per seed bag) and (b) seed viability and identification of seed-associated fungi (10 seeds per seed bag). In general, seeds were recovered intact, precluding lethal germination as a source of seed mortality. In 2% of cases, we recovered fewer than

20 seeds per bag, suggesting that small invertebrate seed predators penetrated or chewed the nylon mesh. In these cases, the recovered seeds were partitioned evenly among germination and culturing approaches. We did not find 28 (i.e. 5%) of the bags that were buried, likely reflecting displacement by small mammals or rain events.

To record the time series of seed germination for each species, 10 seeds from each seed bag were placed in a Petri dish lined with paper towel, moistened with sterile distilled water and sealed with two layers of Parafilm® (Zalamea et al., 2015, 2018). Seeds were incubated in a shade house on BCI under *c*. 30% sunlight, high red:far-red irradiance (*c*. 1.4) and ambient temperature (23–30°C). Germination (i.e. radicle protrusion) was scored over 6 weeks. The maximum temperature recorded on the germination bench was *c*. 38°C, similar to the temperature near the soil surface in large treefall gaps on BCI (Marthews et al., 2008).

Fungal isolation and seed viability assessment followed Sarmiento et al. (2017). Briefly, 10 intact seeds were haphazardly selected from each bag, surface sterilized (95% ethanol, 10 s; 0.7% NaClO, 2 min; 70% ethanol, 2 min) and allowed to surface-dry under sterile conditions. Surface sterilizing the seeds allowed us to isolate fungi that had penetrated the seed coat while eliminating fungi that were restricted to the seed surface.

After surface-drying briefly, surface-sterilized seeds were cut in half under sterile conditions. Half of each seed was placed on 2% malt extract agar (MEA) in an individual 1.5 ml microcentrifuge tube for fungal isolation (see below). The other half was used to score seed viability (i.e. viable or inviable) via tetrazolium staining (TZ; 2, 3, 5-triphenyl tetrazolium chloride) such that both infection status and viability were scored for each seed. Prior to TZ staining, *Cecropia* seed halves were placed in a Petri dish lined with wet filter paper and incubated for 48 hr in a shade house under *c*. 30% sunlight at ambient temperature. After 48 hr of incubation, seed halves were saturated with 0.5% TZ and kept in the dark at *c*. 22°C for 24 hr after which seed viability was scored using a stereomicroscope. Seed halves of *J. copaia* were saturated with 0.5% TZ immediately after cutting and kept in the dark at *c*. 22°C for 24 hr prior to scoring seed viability (Zalamea et al., 2018). Dishes were sealed with Parafilm® throughout incubation.

2.3 | Vouchering and molecular analyses of fungi

Cultures were incubated at room temperature (*c*. 22°C) with natural light–dark cycles for at least 12 months. If fungal growth was observed, we excised a small piece of fresh mycelium from each microcentrifuge tube under sterile conditions. The excised pieces were cut in half. One half was used for DNA extraction and Sanger sequencing (see below). The other half was plated in a 60 mm Petri dish with MEA to obtain a pure culture. Pure cultures of all fungi were vouchered in sterile water and deposited at the Robert L. Gilbertson Mycological Herbarium, University of Arizona (MYCO-ARIZ; Table S2).

Total genomic DNA was extracted from ground mycelium of each sample with the REDExtract-N-Amp Plant PCR kit (Sigma-Aldrich). Primers ITS1F and LR3 were used to PCR amplify a (*c*. 1,000–1,200 basepair [bp]) fragment of nuclear ribosomal DNA from each extraction. Amplicons consisted of the nuclear internal transcribed spacers and 5.8S gene (ITS rDNA) and an adjacent portion (*c*. 600 bp) of the large subunit (LSU rDNA). If amplification failed with these primers, PCR was repeated with primers ITS5 and LR3. PCR mixture and cycling reactions followed Sarmiento et al. (2017). PCR products were verified by staining with SYBR Green I (Molecular Probes) followed by electrophoresis on a 1% agarose gel. All products that yielded single bands were cleaned using 1 μl of the USB ExoSAP-IT reagent (Affymetrix, Inc.), quantified, normalized and sequenced bidirectionally at the University of Arizona Genetics Core with the original sequencing primers. We assembled contigs and verified base calls from chromatograms following Sarmiento et al. (2017).

2.4 | Analyses of seed-associated fungal communities

Operational taxonomic units (OTUs) were defined by 97% similarity in the Sanger clustering workflow of the Mobyle SNAP Workbench (Monacell & Carbone, 2014) based on previously published methods for clustering ITS-LSU rDNA sequence data (U'Ren et al., 2009, 2012). Results described below generally were consistent when analysed with more stringent OTU delimitation (e.g. at 99% similarity; data not shown). Diversity was measured as Fisher's alpha, which is robust to variation in sample size and is appropriate given the relative abundance of OTU (see Sarmiento et al., 2017). Isolation frequency was calculated as the number of isolates divided by the number of examined seeds.

We obtained 1,322 sequences from 1,594 cultures (83%; Table 1). Of these, 1,264 sequenced isolates came from buried seeds and 58 came from fresh seeds (Table 1). Overall, 165 OTUs were defined at 97% of sequence similarity. All OTUs were included in measures of diversity. In most cases, only one fungal OTU emerged from each infected seed in culture. Two OTUs were successfully sequenced from six seeds, and three OTUs were successfully sequenced from one seed.

Seed-associated fungal communities were defined as fungal isolates from seeds of a given plant species, burial duration, burial location and viability classification. By definition, rare OTUs could not be present in all plant species or all communities; thus, we excluded OTUs that had fewer than four isolates and fungal communities with fewer than four isolates from analyses of community structure, with a resulting pool of 39 OTUs and 98 communities.

We used a multivariate GLM to test the extent to which each factor (i.e. plant species, burial duration, burial location or seed viability) was relevant to fungal community structure. We used a negative-binomial GLM based on sequence count data and a binomial GLM

TABLE 1 Number of maternal seed sources, cultivable fungi and OTU of seed-associated fungi obtained from fresh and buried seeds of three species of *Cecropia* and *Jacaranda copaia* in common gardens at Barro Colorado Island, Panama. Maternal sources were pooled at the outset of the experiment

Plant species	Maternal sources	Number of seeds	Number of cultivable fungi	% of seeds with cultivable fungi	Number of sequences obtained	Total number of OTU	Number of singletons	% of singletons	Fisher's alpha	Phylogenetic diversity
Fresh seeds										
Cecropia insignis	7	200	0	0	0	0	0	—	—	—
Cecropia longipes	5	200	7	3.5	7	4	3	75	3.9	—
Cecropia peltata	5	200	0	0	0	0	0	—	—	—
Jacaranda copaia	5	200	52	26	51	15	8	53.3	7.2	—
Buried seeds										
Cecropia insignis	—	1,290	194	15	97	36	21	58	20.7	3.1
Cecropia longipes	—	1,425	427	30	339	63	37	59	22.8	4.7
Cecropia peltata	—	1,316	565	43	504	51	30	59	14.2	4.5
Jacaranda copaia	—	1,254	349	28	324	62	31	50	22.8	4.7

based on presence–absence data, performed with the R package MVABUND (Wang et al., 2012). We complemented the results from the multivariate GLM with non-metric multidimensional scaling (NMDS) analysis and variation-partitioning analyses that were performed with the R package VEGAN (Oksanen et al., 2019). The NMDS analysis was performed using the function *metaMDS* with the dissimilarity matrix calculated using the Morisita–Horn index and the parameter *k* set to three dimensions (for graphical representations, we only included the first two dimensions). Variance-partitioning analyses were performed using the function *varpart*, with plant species, burial duration, burial location and seed viability coded as explanatory factors.

2.5 | Taxonomic placement and phylogenetic relationships of seed-associated fungi

We complemented OTU-level analyses of fungal communities with a phylogenetic approach that contextualized OTUs in an evolutionary framework and provided a basis for preliminary taxonomic identification. A kingdom level phylogenetic tree for fungi was constructed with scaffolding sequences downloaded from T-BAS v. 2.1 (Carbone et al., 2019), which included all major classes of Ascomycota (the most common phylum among seed-associated fungi; Sarmiento et al., 2017). We integrated one representative sequence for each OTU and aligned the resulting dataset in PASTA with the following settings: aligner (MAFFT), merger (OPAL), tree estimator (FastTree), model (GTR + G20), max.subproblem (default size), decomposition (centroid) and iteration limit (3) (Mirarab et al., 2015). The best alignment was again optimized by eye and manually corrected using Seaview (Version 4.6). Maximum likelihood (ML) analyses were used to estimate phylogenetic relationships and were performed with RAxML-HPC2 on XSEDE (8.2.8) through the CIPRES Science Gateway v. 3.3 (http://www.phylo.org) with a GTRCAT approximation. Nodal support was determined by rapid bootstrapping (BS) with 1,000 bootstrap iterations. Graphical representation was completed in EvolView (He et al., 2016). Taxonomic annotation of the sequences was based on the NCBI BLASTN suite and T-BAS v. 2.1 (Carbone et al., 2019) and are reported up to the genus level in the Supporting Information (Table S2) to avoid mistakenly assigning species names based on limited evidence (see the disclaimer at the T-BAS portal, indicating that the results are not to be used for species-level identification: https://vclv99-239.hpc.ncsu.edu/tbas2_1/pages/tbas.php). We chose not to categorize fungi into ecological modes (e.g. pathogens, saprotrophs) based on barcode sequences because in several groups represented here, barcode sequences can be the same for fungal isolates with positive, negative or neutral impacts on seed germination (see Sarmiento et al., 2017; Shaffer et al., 2018), and many of our fungi represent novel genotypes not present yet in databases such as FunGUILD.

The diversity of OTUs detected here represent taxa with varying degrees of phylogenetic relatedness. We used the R package APE (Paradis & Schliep, 2019) to analyse fungal communities in a phylogenetic framework. We restricted the analyses to the Ascomycota, which comprised 88.5% of observed OTUs. We used the R package PEZ (Pearse et al., 2015) to estimate the Faith's

diversity index (PD) for Ascomycota from each plant species, defined as the sum of branch lengths of the phylogeny connecting all species in the target community. We used the R packages GUNIFRAC (Chen et al., 2012) and VEGAN (Oksanen et al., 2019) to calculate phylogenetic beta-diversity between seed-associated fungal communities via the *adonis* function (PERMANOVA) based on the generalized Unifrac distance matrix with $d = 0.5$ and 1,000 permutations.

2.6 | Analyses of seed germination

We analysed the change in the proportion of seeds that survived (and therefore germinated) as a function of species, burial duration and garden location, using generalized linear mixed-effects models. We used binomial error distributions to fit the models and coded replicate as a random effect. We used species, burial duration and garden location as fixed effects. We used likelihood ratio tests based on χ^2 tests to compare models with and without species, burial duration and garden location as explanatory variables. Analyses were performed with the R package NLME (Pinheiro et al., 2020).

Although the gold standard for inferring impacts of fungi on seeds would be to perform inoculation experiments to satisfy Koch's postulates (e.g. Sarmiento et al., 2017), the 165 OTUs found here made that impractical. Therefore, we examined the relationship of particular fungal OTUs to seed germination. While not establishing causality with regard to the impacts of each strain on germination, these analyses provide data that can guide future inoculation trials. We used two approaches: generalized linear mixed-effects models and partial least squares regression (PLSR) models. We used generalized linear mixed-effects models for each of the species separately to characterize the time series in germination and included burial duration nested within burial location as a fixed effect. For each of the species, we then included the rate of seed viability loss (represented by the slope of the generalized linear mixed-effects models) for each of the burial locations as the dependent variable in the PLSR models where OTU abundance (number of isolates belonging to each OTU per burial location) was the independent variable. For this analysis, we also excluded OTUs represented by fewer than four isolates. To perform the PLSR models, we used the *plsr* function from the PLS R package (Mevik et al., 2019). We used the 5th percentile of the PLSR loadings as the threshold to identify OTUs that had positive or negative associations with seed germination.

To explore the potential effect of OTUs identified by PLSR on germination of each species, we calculated the relative abundance of these fungal OTUs recovered from seed bags with low (<30%) and high (>70%) germination. If OTUs are important drivers of seed mortality (or seed survival), then we expected that the relative abundance of these OTUs in bags with high and low germination would differ from that expected by chance alone. The relative abundance of any given OTU was calculated as the observed abundance of OTUs coming from low- and high-germination bags, respectively, minus the expected abundance (calculated as expected values in a χ^2 test) which predicts that any given OTU will be equally distributed in low- and high-germination bags.

3 | RESULTS

When adjusted for the number of seeds examined, detectable fungal infections were over 20 times more common in seeds that were buried than in fresh seeds of *Cecropia* species (Table 1), confirming that buried seeds were colonized naturally by soil-borne fungi. Detectable fungal infections were similar in frequency for buried and fresh seeds of *J. copaia*, reflecting colonization of seeds in fruits retrieved from the forest floor prior to our experiment as well as colonization after burial (Table 1). Seed-associated fungal communities did not differ meaningfully between fresh and buried seeds of *J. copaia* (Figure S1). This result suggests that colonization of fresh seeds occurred at the soil surface and lower variation among seed-associated fungal communities of fresh seeds reflects lower sampling or that pre-burial fungi are different from post-burial but were retained after burial.

In contrast to our first prediction, we did not find that infection frequencies of seeds increased over the time course of our experiment. After burial, detectable fungal infections in seeds differed in frequency among plant species (Table 1; generalized linear model (GLM): $df_{3,265}$, Dev = 2.81, $p < 0.0001$); decreased as a function of burial duration (Figure 1; GLM: $df_{6,259}$, Dev = 4.62, $p < 0.0001$) and did not vary among burial locations or as a function of seed viability status (Figure S2; GLM: location, $df_{4,255}$, Dev = 0.35, $p = 0.071$; viability, $df_{1,254}$, Dev = 0.001, $p = 0.847$; Table S3). These analyses also show that in contrast to our second prediction, dead seeds did not have higher infection frequencies than live seeds. While isolation frequency remained constant for *J. copaia*, it decreased over time for *C. peltata*, *C. longipes* and to a lesser extent for *C. insignis* (GLM: plant species × duration, $df_{18,236}$, Dev = 2.91, $p < 0.0001$; Figure 1).

The diversity of fungi associated with fresh seeds was lower than the diversity of fungi associated with buried seeds (Table 1). The diversity of seed-associated fungi did not differ significantly among tree species ($df_{3,119}$, Dev = 137.7, $p = 0.062$), nor among burial locations ($df_{4,109}$, Dev = 138.1, $p = 0.114$) or burial durations ($df_{6,113}$, Dev = 88.4, $p = 0.559$); nor as a function of seed viability status (Figure S3; $df_{1,108}$, Dev = 8.9, $p = 0.483$; Table S4). In contrast to our third prediction, we did not observe a higher diversity of seed-associated fungi in seeds of the more common tree species (Table 1; Figure S3).

Consistent with our fourth prediction, seed-associated fungal communities differed among plant species (negative binomial GLM; $df_{3,94}$, Dev = 652.5, $p < 0.001$); among burial locations ($df_{4,84}$, Dev = 266.8, $p < 0.001$) and burial durations ($df_{6,88}$, Dev = 353.3, $p < 0.001$); and as a function of seed viability ($df_{1,83}$, Dev = 91, $p < 0.001$). Non-metric multidimensional scaling (NMDS) revealed the importance of plant species in defining fungal community composition, with additional but minor contributions from burial

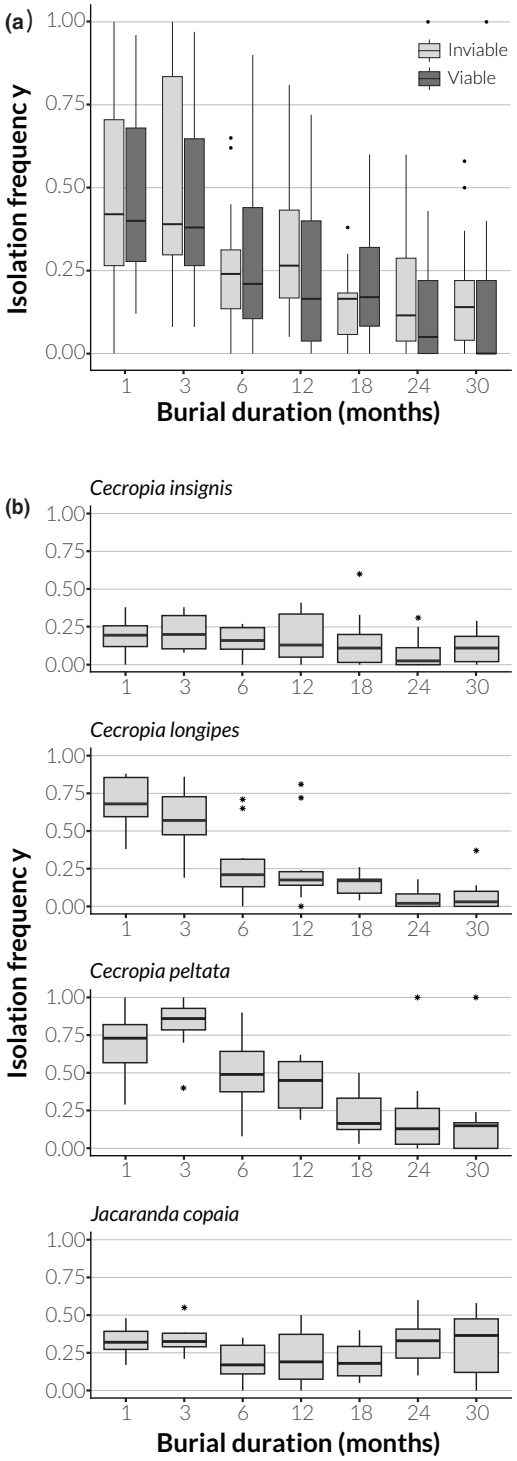

FIGURE 1 Boxplot representation of isolation frequency of fungal isolates in quiescent seeds of tropical trees after burial in common gardens in a lowland tropical forest in Panama. (a) Frequency was calculated as the number of isolates divided by the number of seeds processed for each burial duration, and seed viability class including all the species together. (b) Frequency was calculated as the number of isolates divided by the number of seeds processed for each burial duration and each species separately. For each boxplot, the boxes represent the lower and upper quartiles (25% and 75% of the observations). The bold line represents the median and whiskers represent minimum and maximum values, excluding the outliers that are represented as stars

duration, burial location and seed viability (Figure 2a–d). Plant species alone explained 30.7% of the total variance in OTU abundance while the other three variables together only explained 5.7% of the total variance (Figure 2e). Analyses of seed-associated fungal communities defined by presence–absence data showed similar results (Figure S4a).

Pairwise comparisons showed that fungal community similarity was greater among conspecific seeds than heterospecific seeds (df_1, $F = 3{,}783.4$, $p < 0.001$), seeds of the same genus than seeds of the other genus (df_1, $F = 552.3$, $p < 0.001$), seeds buried for the same duration than different durations (df_1, $F = 46.9$, $p < 0.001$) and between seeds of the same versus different viability classes (df_1, $F = 5.4$, $p = 0.02$; Figure 2f). A dendrogram of Morisita–Horn similarities among fungal communities isolated from seeds of *Cecropia* and *Jacaranda* showed that communities isolated from *Cecropia* are more similar to each other than when compared to communities isolated from *Jacaranda* (Figure S4b).

3.1 | Phylogenetic structure and taxonomy of seed-associated fungi

As for the OTU-level analyses, phylogenetic community structure of fungi representing the Ascomycota differed among tree species ($F = 8.64$, $R^2 = 0.02$, $p = 0.001$), indicating that fungal communities from the same species of seeds were more closely related than expected by chance. This result was influenced by fungi isolated from *J. copaia*, for which we found that the standardized effect size of mean phylogenetic distance among OTUs was higher than the null expectation (MPD = 0.41, $p = 0.001$), in contrast to the other species (Table S5). The three species of *Cecropia* did not differ in terms of the phylogenetic community structure of fungi that colonized seeds ($F = 0.69$, $R^2 = 0.001$, $p = 0.63$). Faith's diversity index (PD) ranged from 3.1 in *C. insignis* to 4.7 in *J. copaia* (Table 1).

Fungi isolated from seeds represented a broad range of phylogenetic lineages (Figure 3a). The most abundant classes of Ascomycota were Sordariomycetes (83.4% of isolates), Dothideomycetes (9.6%) and Eurotiomycetes (0.9%). Basidiomycota were isolated less frequently and were mainly represented by Agaricomycetes (4.6% of isolates; Figure 3, Figure S5, Table S2). The prevalence of taxonomic classes and orders differed across plant species (Figure 3b,c). Hypocreales (50% of isolates) and Xylariales (15%) were the most abundant orders and *Trichoderma* (39%) and *Fusarium* (9%) were the most abundant genera.

3.2 | Seed germination and seed-associated fungi

For all species, the proportion of seeds that germinated was strongly and positively correlated with seed viability (as determined via TZ staining). In all cases, slopes were close to 1, ranging from 0.85 for *C. insignis* to 1.05 for *C. peltata*. All correlations were highly significant ($p < 0.001$) and R^2 values ranged from 0.65 for *C. longipes* to 0.80 for

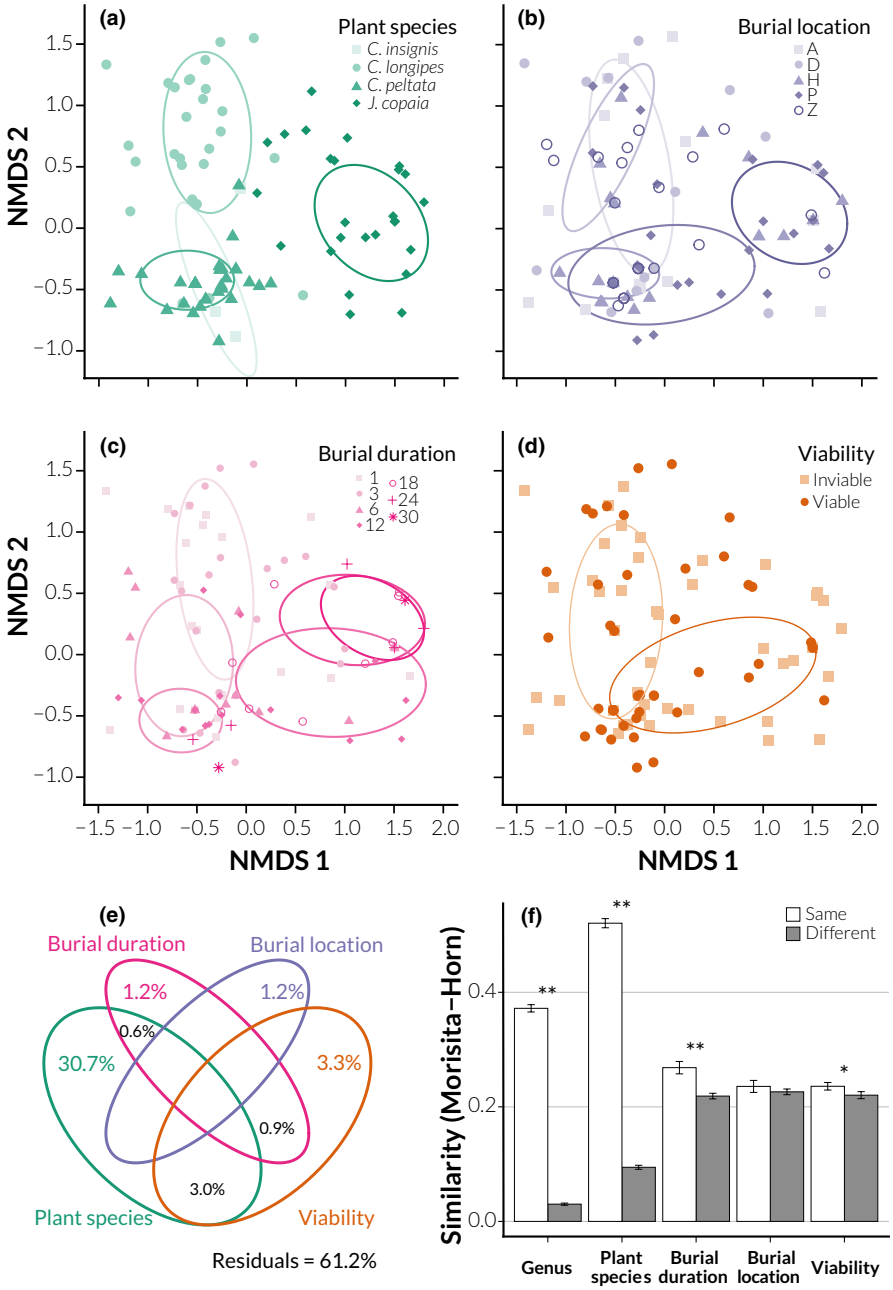

FIGURE 2 Structure of seed-associated fungal communities. Non-metric multidimensional scaling (NMDS) analysis representing similarity among fungal communities (symbols) isolated from seeds of three different species of *Cecropia* and *Jacaranda copaia* (a–d). Panels (a)–(d) depict the same ordination but are colour-coded to represent plant species (a), garden location (b), burial duration (c) and seed viability (d); stress = 0.107. Ellipses represent a standard deviation of point scores relative to their centroid. Venn diagram represents the percent of variation in fungal community composition explained by plant species, burial duration, garden location and seed viability, based on abundance data (e). Pairwise comparisons of fungal community similarity from abundance data for within versus across comparisons of taxonomic, site, burial time and viability classes, $^{**}p < 0.001$, $^{*}p < 0.05$ (f)

C. peltata. Initial germinability of fresh seeds was higher than 50% for all species, varying from 51% in *C. longipes* to 81.5% in *C. peltata*.

In contrast to our prediction, seed viability was not negatively associated with seed infection frequency. After burial, the proportion of germinable seeds differed among species (df_3, $\chi^2 = 2{,}205$, $p < 0.001$) and decreased gradually over time for all species (df_1, $\chi^2 = 7{,}281$, $p < 0.001$; Figure 4). For *C. insignis*, germination ranged from 63% after 1 month to 15% after 30 months of burial (Figure 4a). Similar results were found for *C. longipes* (Figure 4b), *C. peltata* (Figure 4c) and *J. copaia* (Figure 4d). There was also a significant effect of common garden location on germination (df_4, $\chi^2 = 729$, $p < 0.001$), but locational effects on seed germination were not consistent across species (Figure 4e–h). After 30 months of burial, average seed germination for *C. longipes* ranged from 0% at the 25 ha site to 40% at the Pearson site (Figure 4f). Similar variation was also observed for *C. insignis* (Figure 4e), *C. peltata* (Figure 4g) and *J. copaia* (Figure 4h).

Partial least square regressions identified individual fungal OTUs associated with seed germination. For each of the species, we implemented a two-component PLSR model. The root mean square error of prediction (RMSEP) was 0.07 for *C. insignis*, 0.15 for *C. peltata*, 0.03 for *C. longipes* and 0.02 for *J. copaia* (Table S6). High RMSEP values for *C. insignis* and *C. peltata* indicate that the predictive power of the two-component PLSR models had low accuracy for these species. For *C. longipes* and *J. copaia*, respectively, 5 of 19 OTUs and 3 of 23 OTUs represented by at least four isolates had positive or negative associations with seed germination. For these eight OTUs, the absolute value of the loadings was 0.4 or higher. The highlighted OTUs were OTU 7 (*Trichoderma* sp. 1), OTU 9 (*Trichoderma* sp. 2), OTU 17

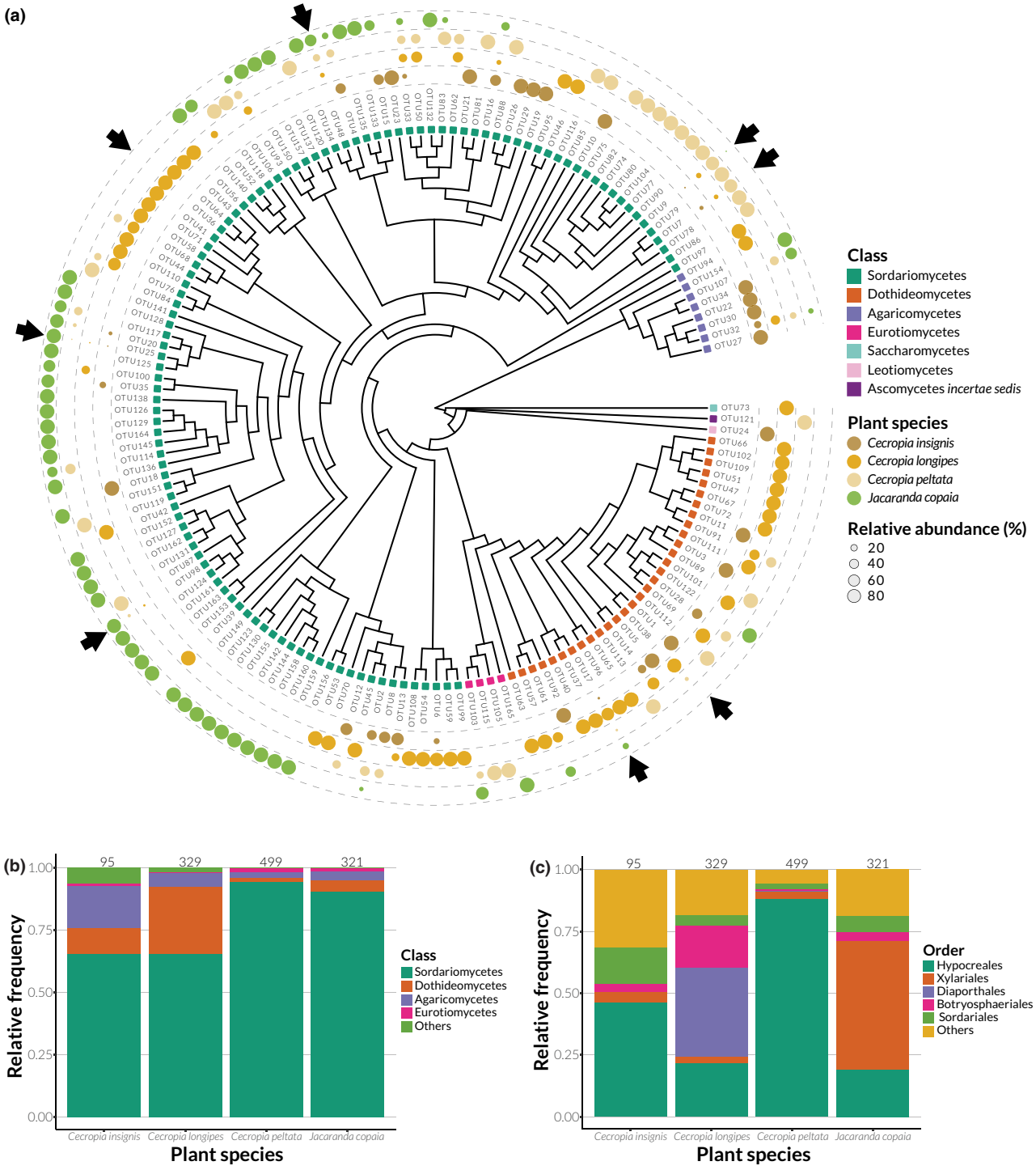

FIGURE 3 Cladogram and taxonomic composition of seed-associated fungi isolated from *Cecropia insignis*, *C. longipes*, *C. peltata* and *Jacaranda copaia*. (a) Circular cladogram including 156 fungal OTUs (i.e. all OTUs of Ascomycota observed in the present study) created in EvolView from an RAxML maximum likelihood analysis based on partial LSU rDNA sequences. Species from T-BAS were used to construct the backbone, then filtered to only include seed-associated fungi via the R package APE v.5.3. Each outer circle represents a host species. The size of the bubbles indicates the relative abundance for each OTU across host species. Arrows indicate the eight OTUs highlighted by the PLS regression analysis and squares represent fungal classes sensu *lato*. The stacked bars represent the relative frequency and taxonomic composition of seed-associated fungi at the class (b) and order (c) levels. Numbers on top of each bar represent the number of isolates for which we obtained sequences

(*Lasiodiplodia* sp.), OTU 25 (*Xylaria* sp. 1), OTU 36 (*Diaporthe* sp.), OTU 38 (*Pleospora* sp.), OTU 48 (*Fusarium* sp.) and OTU 98 (*Xylaria* sp. 2).

Overall, four OTUs were isolated preferentially from seed bags associated with high germination success and one OTU was associated with seed bags with low germination success (Figure 5). These OTUs were not the same in all species of seeds (Figure 5).

Collectively, eight OTUs highlighted by the PLSR analysis (4.8% of the total OTUs) accounted for 65.6% of the fungal collections.

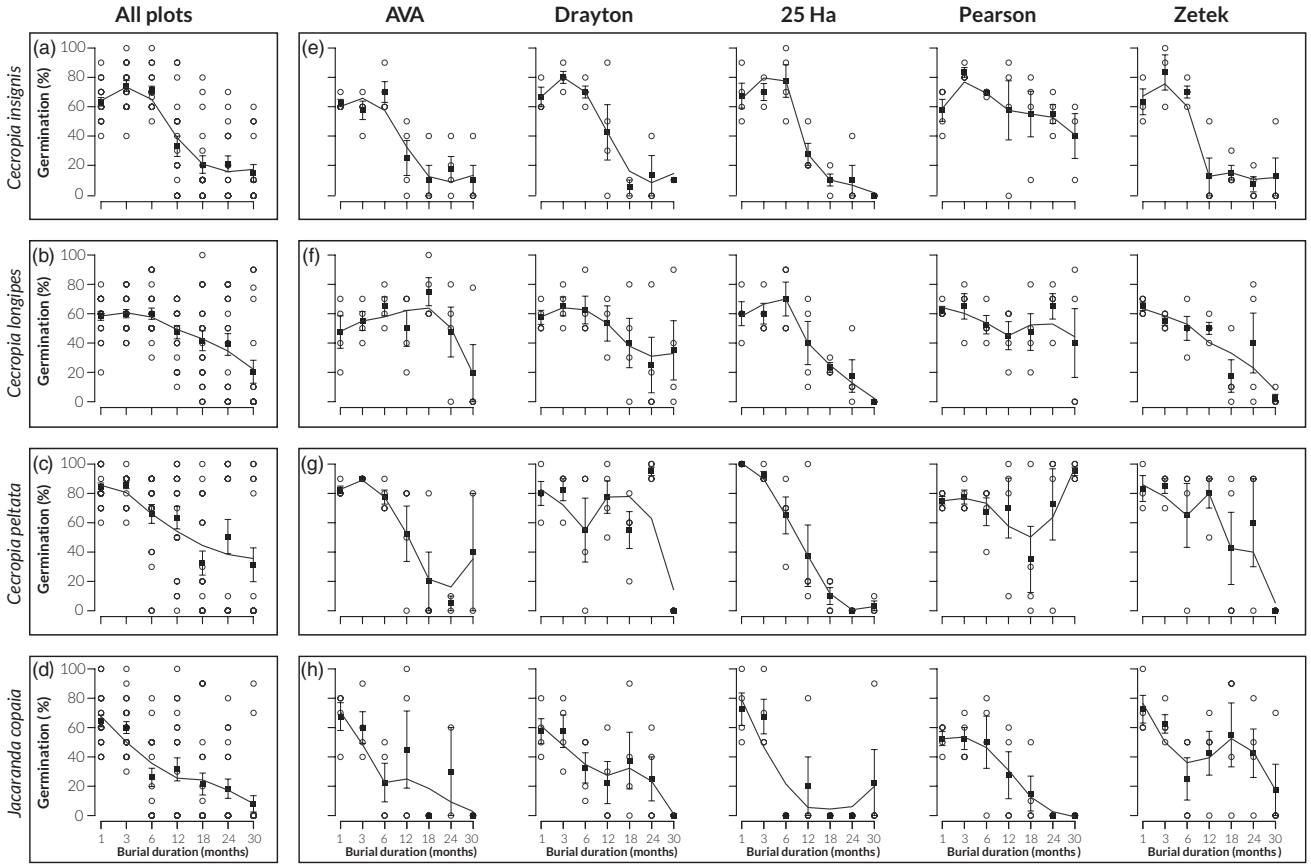

FIGURE 4 Percentage of germinable seeds of (a) *Cecropia insignis*, (b) *C. longipes*, (c) *C. peltata* and (d) *Jacaranda copaia* recovered at seven time intervals after burial (i.e. 1, 3, 6, 12, 18, 24 and 30 months) for all plots combined (a–d), and for each of the five burial locations separately (Ava, Drayton, 25 Ha, Pearson, and Zetek; e–h). Open circles show the percentage of germinable seeds recovered from a single bag; black squares show the mean and bars the standard error. The black line represents the loess regression using a smoothing span of 0.75

For example, the two most abundant OTUs in the dataset, OTU 7 and OTU 9, represented *Trichoderma* and each accounted for 18.5% of the fungal collections. These OTUs were isolated from three and four species of seeds, respectively, but they were only commonly associated with seeds of *C. peltata* and *C. longipes* (Figure S6; Table S7).

4 | DISCUSSION

Plant pathogens are a major source of seed and seedling mortality in tropical forests (Augspurger, 1984; Dalling et al., 1998), and are increasingly recognized for playing a fundamental role in the maintenance of diversity (Bagchi et al., 2014). In this study, we found strong evidence that *Cecropia* species with similar seed traits and overall life histories (Dalling et al., 1997; Zalamea et al., 2018) acquire distinct fungal communities when exposed to the same soils in common-garden settings. This finding provides strong support for the common assumption of host differentiation of fungal communities, a prerequisite for the density-dependent regulation of population densities via fungal pathogens.

Cecropia seeds are common in the soil seed bank at BCI (Dalling et al., 1997). Reports on the ability of seeds to persist are contradictory and vary dramatically from transient (Alvarez-Buylla & Martínez-Ramos, 1990; Dalling et al., 1997; Zalamea et al., 2018) to long-lived (Holthuijzen & Boerboom, 1982). However, in the case where seeds were found to be long-lived, they were protected from pathogens and predators by earthenware pots (Holthuijzen & Boerboom, 1982), suggesting that exposure to enemies, including fungal pathogens, may be a key factor determining seed longevity in *Cecropia*.

We found that after burying the same batches of seeds for each of the four species in five common gardens in the forest understorey, the proportion of germinable seeds decreased gradually over time for *Cecropia* spp. and *J. copaia*. We infer that pathogens had a primary role in explaining variation in seed mortality among seed bags that were buried in close proximity because (a) seeds were protected from insect predators; (b) we found no evidence for fatal germination (germinated seeds in bags); (c) within species, although seed germination decreased gradually over time (suggesting a natural process of seed aging), variance in seed survival among bags of buried seeds remained high at the end of the experiment (e.g. ranging by as much as 0%–100% for *C. peltata*) and (d) previous experiments at BCI using the same species (*C. insignis*) and the same methodology (seeds enclosed in mesh bags) has shown that application of fungicide significantly increased seed survival (Dalling et al., 1998). We explore this in further detail below.

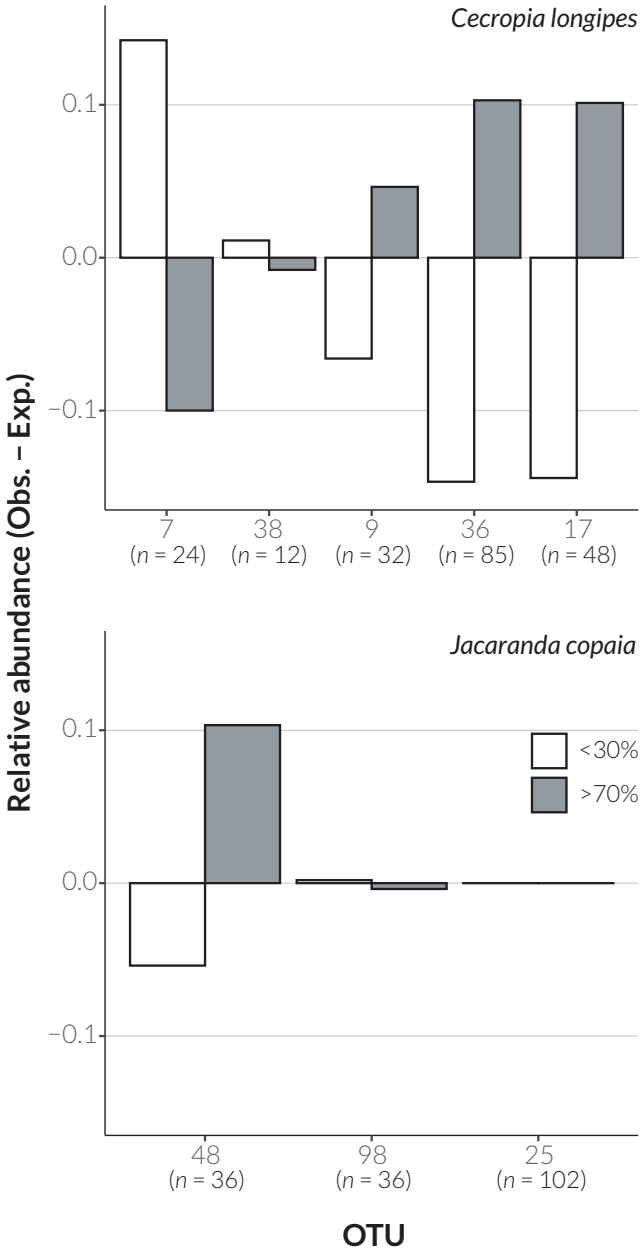

FIGURE 5 Eight fungal OTUs from seed bags of *Cecropia longipes* and *Jacaranda copaia* with either low (<30%) or high (>70%) seed germination were identified as influential by PLSR. For any given OTU, the observed frequency reflects the proportion of isolates belonging to that OTU that came from low- and high-germination bags, respectively. The expected abundance is based on the prediction that isolates of a given OTU will be equally distributed in low- and high-germination bags. The n represents the number of isolates recovered for each particular OTU and seed species. OTU 7: *Trichoderma* sp. 1; 9: *Trichoderma* sp. 2; 17: *Lasiodiplodia* sp.; 25: *Xylaria* sp. 1; 36: *Diaporthe* sp.; 38: *Pleospora* sp.; 48: *Fusarium* sp.; 98: *Xylaria* sp. 2

4.1 | Seed fungal infection rates and seed survival

We predicted that as fungi encounter seeds in the soil, the proportion of infected seeds would increase over time, and that if most culturable fungi are pathogens and saprotrophs, then infection rates would be higher for dead seeds than live seeds. Instead, we found that the isolation frequency of fungi remained constant over time for *J. copaia*, and decreased for *C. peltata*, *C. longipes* and to a lesser extent for *C. insignis*. For all species, infection rates were similar between viable and inviable seeds. We also found that the two species with the highest infection rates had the highest survival after 30 months of burial. These results together suggest that high germinability in some bags is not due to a lack of fungal colonization, but due to associations with non-lethal fungi (also see the section below: '*Seed-associated fungi and their consequences for seed survival*', in which we describe the concept of *functional specificity*). Declines over time in the proportion of seeds yielding culturable fungi cannot be attributed to the selective loss of infected seeds because the number of seeds that we retrieved per bag did not decline over time. Instead, either fungi became more difficult to culture out of older seeds or some seeds lost infections over time.

4.2 | Local tree species abundance, seed survival and fungal diversity

Variation in seed mortality may reflect intrinsic seed traits or local tree species abundance. High host local abundance could enhance pathogen transmission among hosts, resulting in an increased pathogen inoculum and decreased seed survival. Alternatively, host relative abundance itself may be conditioned by pathogens. In temperate grassland, and in the BCI forest, host relative abundance is negatively correlated with susceptibility to soil-borne pathogens that reduce seedling growth (Klironomos, 2002; Mangan et al., 2010). In contrast, the two locally abundant species in this study, *C. insignis* and *J. copaia*, had relatively low seed survival after 30 months of burial (14.6% and 8.0%, respectively), compared to the locally rare species *C. longipes* (20.4%) and *C. peltata* (31.3%). Although the proportion of germinable seeds decreased over time, particularly over the first year, strong intraspecific variation in seed survival was evident among seed bags. Seeds that were buried only 40 cm apart therefore encountered markedly different fates. After 30 months of burial, a few seed bags consistently had >60% seed viability while others reached zero. Without inoculation experiments, we cannot definitively attribute variation in seed survival to variation in fungal community composition. Nonetheless, strong spatial structure in soil microbial communities is consistent with this pattern (Barberán et al., 2014), and in the absence of other sources of mortality, suggests that seed survival patterns are likely influenced by the frequency with which seeds encounter fungal pathogens to which they are susceptible.

We predicted that locally abundant species would be infected by more diverse fungal communities, reflecting greater opportunities for host specialization. However, while the phylogenetic diversity and to a lesser extent alpha diversity of fungi associated with buried seeds varied across species, local abundance was not a strong predictor of fungal diversity. *Jacaranda copaia* and *C. longipes*, the most abundant and least abundant species in our study, harboured the most diverse communities of seed-associated fungi measured as by both

Fisher's alpha and Faith's phylogenetic diversity. Fungal diversity also was unrelated to the frequency with which culturable fungi were isolated from seeds: *C. peltata* had the least diverse community of seed-associated fungi, but the highest isolation frequency of the four species. Thus, although our study was restricted to only four species, these results suggest that local abundance of host species is not associated with the diversity of culturable fungi associated with seeds.

4.3 | Phylogenetic structure and taxonomy of seed-associated fungal communities

The fungi isolated from seeds represent a broad range of phylogenetic lineages. We predicted that congeneric *Cecropia* species would harbour distinct fungal communities from one another, and that these communities differ from the one associated with the more distantly related *J. copaia*, resulting in clusters of related fungi which are capable of infecting a particular host. Our results support our prediction. We found that phylogenetic composition of Ascomycota differed among tree species and show that fungal communities infecting a particular host were more closely related than expected by chance. However, this result is largely driven by *J. copaia*, where clustering tended to occur deeper in the phylogeny, with higher than expected mean phylogenetic distance and higher Faith's phylogenetic diversity. Studies of plant–microbial associations in tropical forests often reveal associations at the family level or genus level. For instance, Liu et al. (2016) found that phylogenies between tree hosts and their potential fungal pathogens were congruent, consistent with our observation of distinctive fungal communities at the OTU level and in a phylogenetic context for *J. copaia* versus the *Cecropia* species considered here.

The diversity of seed-associated fungi represents taxa with varying degrees of phylogenetic relatedness. Foliar endophytes are primarily filamentous Ascomycota (mainly Pezizomycotina), including many species within the classes Sordariomycetes and Dothideomycetes (Arnold, 2008; U'Ren et al., 2009). In common with previous studies of seed fungi, we found that these two classes also accounted for more than 90% of the seed-infecting fungi (Nelson, 2018). Although seed microbiomes have been proposed to be less diverse than foliar microbiomes (Arnold et al., 2003; Newcombe et al., 2018), we found that common leaf endophyte genera such as *Xylaria*, *Fusarium* and *Trichoderma* (Arnold, 2008) were also well represented in seeds. The eight most influential fungal OTUs highlighted by the PLSR analysis also belonged to genera commonly associated with seeds in temperate and tropical ecosystems, including seeds studied previously at BCI (Gallery et al., 2010; Klaedtke et al., 2016; Kluger et al., 2008; Nelson, 2018; Sarmiento et al., 2017).

4.4 | Seed-associated fungi and their consequences for seed survival

To limit damage by herbivores and pathogens, plants have evolved an array of physical and chemical seed defences (Dalling et al., 2020; Gripenberg et al., 2017). Species with quiescent seeds, however, invest relatively little in chemical and physical defences compared to more persistent dormant seeds (Zalamea et al., 2018), which makes them especially susceptible to fungal infection (Sarmiento et al., 2017). While most pathogens show a degree of specificity and are unable to attack the majority of plant species (Parker & Gilbert, 2004), closely related taxa are more likely to share susceptibility to the same foliar pathogens (Gilbert & Webb, 2007) as a result of phylogenetically conserved defensive traits. In contrast, we found that even co-occurring congeneric plant species with overlapping habitat requirements differ in infections from a common soil fungal community. For example, OTU 9 (the second most abundant OTU in the dataset; *Trichoderma* sp.), which accounted for 39% of infections in *C. peltata* (isolated from all burial durations and locations), was rare on *C. insignis* (3% of infections), accounted for 9% of infections in *C. longipes*, and was almost absent on *J. copaia* (0.6% of infections).

While common OTUs in our fungal community tended to be associated with, or occur predominantly on, a single host, the majority of OTUs represented by more than four sequences (64%) were isolated from multiple hosts. Overall, 15% of OTUs were present in all three *Cecropia* species. A lack of strict host specificity among these OTUs does not preclude them from having species-specific demographic effects on their hosts that promote diversity in plant communities. Experimental work has shown that some fungal isolates can exhibit functional specificity, where the same fungal isolate infects but differentially impacts germination and seed viability of different plant species (Benítez et al., 2013; Sarmiento et al., 2017). For example, Sarmiento et al. (2017) found that species of *Lasiodiplodia* can colonize several species of seeds, with negative effects on seed viability of one host (e.g. *Ficus insipida*), but not others (e.g. *Trema micrantha*). We found also evidence to support earlier findings suggesting that colonization of seeds by particular fungi is associated with variation in seed survival and germination (Gallery et al., 2007). The PLSR analysis indicated that the abundance of eight fungal OTUs was either negatively or positively associated with seed germination in *C. longipes* and *J. copaia*. Moreover, five of the eight OTUs highlighted by the PLSR analysis were isolated more commonly than expected by chance from seed bags in which the germination was low (OTU 7) or high (OTUs 9, 17, 36 and 48). Although our results do not yet provide sufficient evidence to draw firm conclusions about the functional role of individual fungal OTUs on seed germination and survival, they provide the basis for taxon selection for future experiments.

4.5 | Implications for species coexistence

Cecropia species are distributed across a wide range of climates in the Neotropics but are particularly common in lowland forests (Berg & Franco-Rosselli, 2005). Very often pairs (or more) of *Cecropia* species are distributed in the same area (Zalamea et al., 2012), but differ in their habitats (e.g. stream-edges vs. landslides, gaps in old-growth vs. secondary forests, or high- vs. low-P soils; Dalling et al., 2009; Folgarait & Davidson, 1994; Zalamea et al., 2016). This habitat partitioning has been attributed to trade-offs between growth and

defence against herbivores (Folgarait & Davidson, 1994). For instance, Dalling et al. (2009) showed evidence that habitat partitioning of *Cecropia* species in Central Panama is due in part to browsing preferences of herbivores. We found a high degree of compositional and phylogenetic dissimilarity among seed-infecting fungal communities on congeneric *Cecropia* species that share similar reproductive traits and regeneration requirements. This result shows that natural enemies such as seed pathogens also discriminate among congeneric species, suggesting that they represent an additional factor relevant to species coexistence. Our results also highlight that most of the interactions between the seeds and their associated fungi were not necessarily lethal, showing a possible mechanism by which soil-borne seed-associated fungi can maintain population reservoirs by associating with hosts on which they are non-lethal at local and regional scales.

Seeds are often colonized by fungi, but the diversity of seed-infecting microbes is low when compared to leaves (Arnold et al., 2003; Newcombe et al., 2018; Sarmiento et al., 2017). Consistent with previous studies, we found that individual seeds are often colonized by a single fungus (Newcombe et al., 2018; Sarmiento et al., 2017). This low microbial richness compared to other plant tissues makes seeds and their microbes attractive model systems when compared to other plant–microbial interactions. When four species of *Cecropia* were buried under the crowns of *C. insignis*, Gallery et al. (2007) found a lower survival rate of *C. insignis* seeds when compared to other species. This result supports prior work suggesting that detrimental effect of fungal pathogens on seeds and seedlings is the highest beneath the crown of conspecific trees in natural settings (Dalling et al., 1998; reviewed in Bever et al., 2015). Our burial locations were selected to avoid conspecific adult neighbourhoods, but the results found in this study suggest that future work should be extended to neighbourhoods surrounding focal tree species, and thus determine whether there is a spatial aggregation of host-specific fungal taxa responsible for density-dependent seed mortality.

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AUTHORS' CONTRIBUTIONS

P.-C.Z., C.S., A.E.A., A.S.D. and J.W.D. conceived and designed the study; P.-C.Z. and C.S. collected the data; P.-C.Z., C.S. and A.F. analysed the data; P.-C.Z. drafted the manuscript with contributions from J.W.D. All authors contributed substantially to the revisions and gave final approval for publication.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/1365-2745.13611.

DATA AVAILABILITY STATEMENT

Sequences used in this study are deposited in NCBI GenBank under accession numbers: KU977742–KY775925 and MW529103–MW530356. Germination data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.qz612jmdq (Zalamea et al., 2021).

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REFERENCES

Alvarez-Buylla, E., & Martínez-Ramos, M. (1990). Seed bank versus seed rain in the regeneration of a tropical pioneer tree. *Oecologia*, *84*, 314–325. https://doi.org/10.1007/BF00329755

Arnold, A. E. (2008). Endophytic fungi: Hidden components of tropical community ecology. In W. P. Carson & S. A. Schnitzer (Eds.), *Tropical forest community ecology* (pp. 254–271). Wiley-Blackwell.

Arnold, A. E., Mejía, L. C., Kyllo, D., Rojas, E. I., Maynard, Z., Robbins, N., & Herre, E. A. (2003). Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 15649–15654. https://doi.org/10.1073/pnas.2533483100

Augspurger, C. K. (1984). Seedling survival of tropical tree species: Interactions of dispersal distance, light-gaps, and pathogens. *Ecology*, *65*, 1705–1712. https://doi.org/10.2307/1937766

Augspurger, C. K., & Wilkinson, H. T. (2007). Host specificity of pathogenic *Pythium* species: Implications for tree species diversity. *Biotropica*, *39*, 702–708. https://doi.org/10.1111/j.1744-7429.2007.00326.x

Bagchi, R., Gallery, R. E., Gripenberg, S., Gurr, S. J., Narayan, L., Addis, C. E., Freckleton, R. P., & Lewis, O. T. (2014). Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature*, *506*, 85–88. https://doi.org/10.1038/nature12911

Baillie, I., Elsenbeer, H., Barthold, F., Grimm, R., & Stallard, R. (2006). *A semi-detailed soil survey of Barro Colorado Island*. Smithsonian Tropical Research Institute.

Barberán, A., Ramirez, K. S., Leff, J. W., Bradford, M. A., Wall, D. H., & Fierer, N. (2014). Why are some microbes more ubiquitous than others? Predicting the habitat breadth of soil bacteria. *Ecology Letters*, *17*, 794–802. https://doi.org/10.1111/ele.12282

Bell, T., Freckleton, R. P., & Lewis, O. T. (2006). Plant pathogens drive density-dependent seedling mortality in a tropical tree. *Ecology Letters*, *9*, 569–574. https://doi.org/10.1111/j.1461-0248.2006.00905.x

Benítez, M.-S., Hersh, M. H., Vilgalys, R., & Clark, J. S. (2013). Pathogen regulation of plant diversity via effective specialization. *Trends in Ecology & Evolution*, *28*, 705–711. https://doi.org/10.1016/j.tree.2013.09.005

Berg, C., & Franco-Rosselli, P. (2005). *Cecropia. Flora Neotropica Monograph 94*. New York Botanical Garden Press.

Bever, J. D., Mangan, S. A., & Alexander, H. M. (2015). Maintenance of plant species diversity by pathogens. *Annual Review of Ecology, Evolution, and Systematics*, *46*, 305–325. https://doi.org/10.1146/annurev-ecolsys-112414-054306

Blaney, C. S., & Kotanen, P. M. (2001). Effects of fungal pathogens on seeds of native and exotic plants: A test using congeneric pairs. *Journal of Applied Ecology*, *38*, 1104–1113. https://doi.org/10.1046/j.1365-2664.2001.00663.x

Carbone, I., White, J. B., Miadlikowska, J., Arnold, A. E., Miller, M. A., Magain, N., U'Ren, J. M., & Lutzoni, F. (2019). T-BAS Version 2.1: Tree-based alignment selector toolkit for evolutionary placement of DNA sequences and viewing alignments and specimen metadata on curated and custom trees. *Microbiology Resource Announcements*, *8*, 1160–1165. https://doi.org/10.1128/MRA.00328-19

Chen, J., Bittinger, K., Charlson, E. S., Hoffmann, C., Lewis, J., Wu, G. D., Collman, R. G., Bushman, F. D., & Li, H. (2012). Associating microbiome composition with environmental covariates using generalized UniFrac distances. *Bioinformatics*, *28*, 2106–2113. https://doi.org/10.1093/bioinformatics/bts342

Comita, L. S., Queenborough, S. A., Murphy, S. J., Eck, J. L., Xu, K., Krishnadas, M., Beckman, N., & Zhu, Y. (2014). Testing predictions of the Janzen-Connell hypothesis: A meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. *Journal of Ecology*, *102*, 845–856. https://doi.org/10.1111/1365-2745.12232

Croat, T. B. (1978). *Flora of Barro Colorado Island*. Stanford University Press.

Dalling, J. W., Arnold, A. E., Davis, A. S., Sarmiento, C., & Zalamea, P. C. (2020). Extending plant defense theory to seeds. *Annual Review of Ecology, Evolution, and Systematics*, *51*, 123–141. https://doi.org/10.1146/annurev-ecolsys-012120-115156

Dalling, J. W., & Brown, T. A. (2009). Long-term persistence of pioneer species in tropical rain forest soil seed banks. *The American Naturalist*, *173*, 531–535. https://doi.org/10.1086/597221

Dalling, J. W., Pearson, T. R. H., Ballesteros, J., Sanchez, E., & Burslem, D. F. R. P. (2009). Habitat partitioning among neotropical pioneers: A consequence of differential susceptibility to browsing herbivores? *Oecologia*, *161*, 361–370. https://doi.org/10.1007/s00442-009-1385-y

Dalling, J. W., Swaine, M. D., & Garwood, N. C. (1997). Soil seed bank community dynamics in seasonally moist lowland tropical forest. *Panama. Journal of Tropical Ecology*, *13*, 659. https://doi.org/10.1017/S0266467400010853

Dalling, J. W., Swaine, M. D., & Garwood, N. C. (1998). Dispersal patterns and seed bank dynamics of pioneer trees in moist tropical forest. *Ecology*, *79*, 564–578. https://doi.org/10.1890/0012-9658(1998)079[0564:DPASBD]2.0.CO;2

Fine, P., Mesones, I., & Coley, P. (2004). Herbivores promote habitat specialization by trees in amazonian forests. *Science*, *305*, 663–665. https://doi.org/10.1126/science.1098982

Folgarait, P., & Davidson, D. (1994). Antiherbivore defenses of myrmecophytic *Cecropia* under different light regimes. *Oikos*, *71*, 305–320. https://doi.org/10.2307/3546279

Gallery, R. E., Dalling, J. W., & Arnold, A. E. (2007). Diversity, host affinity, and distribution of seed-infecting fungi: A case study with *Cecropia*. *Ecology*, *88*, 582–588. https://doi.org/10.1890/05-1207

Gallery, R. E., Moore, D. J. P., & Dalling, J. W. (2010). Interspecific variation in susceptibility to fungal pathogens in seeds of 10 tree species in the neotropical genus *Cecropia*. *Journal of Ecology*, *98*, 147–155. https://doi.org/10.1111/j.1365-2745.2009.01589.x

Gilbert, G. S., Ferrer, A., & Carranza, J. (2002). Polypore fungal diversity and host density in a moist tropical forest. *Biodiversity and Conservation*, *11*, 947–957. https://doi.org/10.1023/A:1015896204113

Gilbert, G. S., & Webb, C. O. (2007). Phylogenetic signal in plant pathogen-host range. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 4979–4983. https://doi.org/10.1073/pnas.0607968104

Gripenberg, S., Rota, J., Kim, J., Wright, S. J., Garwood, N. C., Fricke, E. C., Zalamea, P. C., & Salminen, J.-P. (2017). Seed polyphenols in a diverse tropical plant community. *Journal of Ecology*, *106*, 87–100. https://doi.org/10.1111/1365-2745.12814

He, Z., Zhang, H., Gao, S., Lercher, M. J., Chen, W.-H., & Hu, S. (2016). Evolview v2: An online visualization and management tool for customized and annotated phylogenetic trees. *Nucleic Acids Research*, *44*, W236–W241. https://doi.org/10.1093/nar/gkw370

Holthuijzen, A., & Boerboom, J. (1982). The *Cecropia* seedbank in the Surinam lowland rain forest. *Biotropica*, *14*, 62–68. https://doi.org/10.2307/2387761

Klaedtke, S., Jacques, M.-A., Raggi, L., Préveaux, A., Bonneau, S., Negri, V., Chable, V., & Barret, M. (2016). Terroir is a key driver of seed-associated microbial assemblages. *Environmental Microbiology*, *18*, 1792–1804. https://doi.org/10.1111/1462-2920.12977

Klironomos, J. N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, *417*, 67–70. https://doi.org/10.1038/417067a

Kluger, C. G., Dalling, J. W., Gallery, R. E., Sanchez, E., Weeks-Galindo, C., & Arnold, A. E. (2008). Host generalists dominate fungal communities associated with seeds of four neotropical pioneer species. *Journal of Tropical Ecology*, *24*, 351–354. https://doi.org/10.1017/S0266467408005026

Liu, X., Liang, M., Etienne, R. S., Gilbert, G. S., & Yu, S. (2016). Phylogenetic congruence between subtropical trees and their associated fungi. *Ecology and Evolution*, *6*, 8412–8422. https://doi.org/10.1002/ece3.2503

Lobova, T., Mori, S., Blanchard, F., Peckham, H., & Charles-Dominique, P. (2003). *Cecropia* as a food resource for bats in French Guiana and the significance of fruit structure in seed dispersal and longevity. *American Journal of Botany*, *90*, 388–403. https://doi.org/10.3732/ajb.90.3.388

Mangan, S. A., Schnitzer, S. A., Herre, E. A., Mack, K. M. L., Valencia, M. C., Sanchez, E. I., & Bever, J. D. (2010). Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature*, *466*, 752–755. https://doi.org/10.1038/nature09273

Marthews, T. R., Burslem, D. F. R. P., Paton, S. R., Yangüez, F., & Mullins, C. E. (2008). Soil drying in a tropical forest: Three distinct environments controlled by gap size. *Ecological Modelling*, *216*, 369–384. https://doi.org/10.1016/j.ecolmodel.2008.05.011

Mevik, B.-H., Wehrens, R., & Liland, K. H. (2019). *pls: Partial least squares and principal component regression*. R package version 2.7-1. Retrieved from https://CRAN.R-project.org/package=pls

Mirarab, S., Nguyen, N., Guo, S., Wang, L.-S., Kim, J., & Warnow, T. (2015). PASTA: Ultra-large multiple sequence alignment for nucleotide and amino-acid sequences. *Journal of Computational Biology*, *22*, 377–386. https://doi.org/10.1089/cmb.2014.0156

Mommer, L., Cotton, T. E. A., Raaijmakers, J. M., Termorshuizen, A. J., van Ruijven, J., Hendriks, M., van Rijssel, S. Q., van de Mortel, J. E., van der Paauw, J. W., Schijlen, E. G. W. M., Smit-Tiekstra, A. E., Berendse, F., de Kroon, H., & Dumbrell, A. J. (2018). Lost in diversity: The interactions between soil-borne fungi, biodiversity and plant productivity. *New Phytologist*, *218*, 542–553. https://doi.org/10.1111/nph.15036

Monacell, J. T., & Carbone, I. (2014). Mobyle SNAP Workbench: A web-based analysis portal for population genetics and evolutionary genomics. *Bioinformatics*, *30*, 1488–1490. https://doi.org/10.1093/bioinformatics/btu055

Nelson, E. B. (2018). The seed microbiome: Origins, interactions, and impacts. *Plant and Soil*, *422*, 7–34. https://doi.org/10.1007/s11104-017-3289-7

Newcombe, G., Harding, A., Ridout, M., & Busby, P. E. (2018). A hypothetical bottleneck in the plant microbiome. *Frontiers in Microbiology*, *9*, 1645. https://doi.org/10.3389/fmicb.2018.01645

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2019). *vegan: community ecology*

package. R package Version 2.3-4. Retrieved from https://CRAN.R-project.org/package=vegan

Paradis, E., & Schliep, K. (2019). ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, *35*, 526–528. https://doi.org/10.1093/bioinformatics/bty633

Parker, I. M., & Gilbert, G. S. (2004). The evolutionary ecology of novel plant-pathogen interactions. *Annual Review of Ecology, Evolution, and Systematics*, *35*, 675–700. https://doi.org/10.1146/annurev.ecolsys.34.011802.132339

Pearse, W. D., Cadotte, M. W., Cavender-Bares, J., Ives, A. R., Tucker, C. M., Walker, S. C., & Helmus, M. R. (2015). pez: Phylogenetics for the environmental sciences. *Bioinformatics*, *31*, 2888–2890. https://doi.org/10.1093/bioinformatics/btv277

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2020). *nlme: Linear and nonlinear mixed effects models*. R package version 3.1-149. Retrieved from https://CRAN.R-project.org/package=nlme

Queenborough, S. A., Burslem, D. F. R. P., Garwood, N. C., & Valencia, R. (2009). Taxonomic scale-dependence of habitat niche partitioning and biotic neighbourhood on survival of tropical tree seedlings. *Proceedings of the Royal Society B-Biological Sciences*, *276*, 4197–4205. https://doi.org/10.1098/rspb.2009.0921

Ruzi, S., Roche, D., Zalamea, P. C., Robison, A., & Dalling, J. W. (2017). Species identity influences secondary removal of seeds of Neotropical pioneer tree species. *Plant Ecology*, *218*, 983–995. https://doi.org/10.1007/s11258-017-0745-7

Sarmiento, C., Zalamea, P. C., Dalling, J. W., Davis, A. S., Stump, S. M., U'Ren, J. M., & Arnold, A. E. (2017). Soilborne fungi have host affinity and host-specific effects on seed germination and survival in a lowland tropical forest. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, 11458–11463. https://doi.org/10.1073/pnas.1706324114

Shaffer, J. P., Zalamea, P.-C., Sarmiento, C., Gallery, R. E., Dalling, J. W., Davis, A. S., Baltrus, D. A., & Arnold, A. E. (2018). Context-dependent and variable effects of endohyphal bacteria on interactions between fungi and seeds. *Fungal Ecology*, *36*, 117–127. https://doi.org/10.1016/j.funeco.2018.08.008

Thompson, K. (2000). The functional ecology of soil seed banks. In M. Fenner (Ed.), *Seeds: The ecology of regeneration in plant communities* (2nd ed., pp. 215–235). CABI.

U'Ren, J. M., Dalling, J. W., Gallery, R. E., Maddison, D. R., Davis, E. C., Gibson, C. M., & Arnold, A. E. (2009). Diversity and evolutionary origins of fungi associated with seeds of a neotropical pioneer tree: A case study for analysing fungal environmental samples. *Mycological Research*, *113*, 432–449. https://doi.org/10.1016/j.mycres.2008.11.015

U'Ren, J. M., Lutzoni, F., Miadlikowska, J., Laetsch, A. D., & Arnold, A. E. (2012). Host and geographic structure of endophytic and endolichenic fungi at a continental scale. *American Journal of Botany*, *99*, 898–914. https://doi.org/10.3732/ajb.1100459

Wang, Y., Naumann, U., Wright, S. T., & Warton, D. I. (2012). mvabund – An R package for model-based analysis of multivariate abundance data. *Methods in Ecology and Evolution*, *3*, 471–474. https://doi.org/10.1111/j.2041-210X.2012.00190.x

Windsor, D. M. (1990). Climate and moisture variability in a tropical forest: Long-term records from Barro Colorado Island, Panama. *Smithsonian Contributions to the Earth Sciences*, *29*, 1–145. https://doi.org/10.5479/si.00810274.29.1

Zalamea, P. C., Dalling, J. W., Sarmiento, C., Arnold, A. E., Delevich, C., Berhow, M. A., Ndobegang, A., Gripenberg, S., & Davis, A. S. (2018). Dormancy-defense syndromes and tradeoffs between physical and chemical defenses in seeds of pioneer species. *Ecology*, *99*, 1988–1998. https://doi.org/10.1002/ecy.2419

Zalamea, P. C., Heuret, P., Sarmiento, C., Rodríguez, M., Berthouly, A., Guitet, S., Nicolini, E., Delnatte, C., Barthelemy, D., & Stevenson, P. R. (2012). The genus *Cecropia*: A biological clock to estimate the age of recently disturbed areas in the Neotropics. *PLoS ONE*, *7*, e42643. https://doi.org/10.1371/journal.pone.0042643

Zalamea, P. C., Sarmiento, C., Arnold, A. E., Davis, A. S., & Dalling, J. W. (2015). Do soil microbes and abrasion by soil particles influence persistence and loss of physical dormancy in seeds of tropical pioneers? *Frontiers in Plant Science*, *5*, 799. https://doi.org/10.3389/fpls.2014.00799

Zalamea, P. C., Sarmiento, C., Arnold, A. E., Davis, A. S., Ferrer, A., & Dalling, J. W. (2021). Data from: Closely related tree species support distinct communities of seed-associated fungi in a lowland tropical forest. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.qz612jmdq

Zalamea, P. C., Turner, B. L., Winter, K., Jones, F. A., Sarmiento, C., & Dalling, J. W. (2016). Seedling growth responses to phosphorus reflect adult distribution patterns of tropical trees. *New Phytologist*, *212*, 400–408. https://doi.org/10.1111/nph.14045

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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