# Host-generalist fungal pathogens of seedlings may maintain forest diversity via host-specific impacts and differential susceptibility among tree species

Erin R. Spear[1*] and Kirk Broders[1]

1. Smithsonian Tropical Research Institute, Panama City, Panama, Republic of Panama.

[*]Corresponding author: erin.r.spear@gmail.com

**Summary**

- Host-specialized pathogens are credited with the maintenance of tropical forest diversity under the Janzen-Connell hypothesis (JCH). Yet, in diverse forests, selection should favor pathogens with broad host ranges given their passive dispersal and the relative rarity of tree species.
- We surveyed the host associations of potential pathogens isolated from symptomatic seedlings in the forests in Panama; and used inoculations to assess (i) the pathogenicity and host ranges of 27 fungal isolates, and (ii) differences among tree species in susceptibility.
- Thirty-one of the 33 non-singleton OTUs isolated from seedlings are multi-host. All 31 multi-host OTUs exhibit low to moderate specialization and phylogenetically dispersed host use, with no phylogenetic signal. The pathogenicity of 10 isolates was experimentally confirmed; nine caused disease in seedlings in multiple families. However, the outcome of infection differs among tree species susceptible to a given multi-host pathogen. Furthermore, some tree species were seemingly resistant to all fungi tested, while others were susceptible to multiple fungi. Tree species adapted to environments with lower disease pressure were most likely to exhibit disease symptoms.
- Our results suggest that generalist pathogens contribute to the maintenance of local forest diversity via host-specific impacts rather than the host specificity originally envisioned under JCH.

**Key words:** *Clonostachys*, host generalists, host-specific impacts, multi-host pathogens, *Mycoleptodiscus*, Nectriaceae, seedlings, tropical forests

## Introduction

The maintenance of local diversity is commonly attributed to host-specific natural enemies believed to prevent any single host species from becoming competitively dominant. Under the Janzen-Connell hypothesis (JCH), conspecific seeds and seedlings at high densities near conspecific adults suffer disproportionately higher mortality relative to heterospecific seeds and seedlings due to an accumulation of host-specialized enemies (Gillett, 1962; Janzen, 1970; Connell, 1971; Comita *et al*., 2014). However, support for the JCH has often been based on spatial and temporal patterns of conspecific negative density-dependent mortality (e.g., Harms *et al*., 2000; Comita *et al*., 2010), which cannot identify the mechanism(s) driving the observed patterns.

Experimental studies suggest that pathogens may contribute to density- and distance-dependent mortality of conspecific seedlings (Augspurger & Kelly, 1984; Bell *et al*., 2006; Mangan *et al*., 2010; Bagchi *et al*., 2014). However, the pathogens are rarely identified, and most studies fail to experimentally address a crucial assumption of the JCH, that the pathogens exhibit high host specificity with regard to locally available hosts (but see Packer & Clay, 2000; Y. Liu *et al*., 2012). Furthermore, the relative rarity of tree species in diverse tropical forests and the passive dispersal of fungi may select for pathogens with intermediate to broad host ranges (May, 1991; Coley & Barone, 1996). Indeed, mounting evidence from tropical and temperate forests suggests that multi-host pathogens are prevalent (Augspurger & Wilkinson, 2007; Gallery *et al*., 2007; Gilbert & Webb, 2007; Kluger *et al*., 2008; Hersh *et al*., 2012; Schweizer *et al*., 2013; Sarmiento *et al*., 2017; Chen *et al*., 2019).

Pathogens are classified as specialists or generalists based on the number of host species and the phylogenetic relatedness among hosts (Barrett *et al*., 2009). Host-specialized multi-host pathogens are predicted to exhibit a phylogenetic signal to their host range (Barrett *et al*., 2009). A variety of plant traits influence the outcome of plant-pathogen interactions, specifically susceptibility to infection, and resistance and tolerance post-infection (Coley & Barone, 1996; Bradley *et al*., 2003; Barrett *et al*., 2009). Plant defense traits are often, but not always, phylogenetically conserved (Fine *et al*., 2006; Agrawal, 2007; Kursar *et al.*, 2009). Under the assumption that closely related plants are more likely to possess similar defense traits, the likelihood of tree species sharing a

62 pathogen should decrease with decreasing phylogenetic relatedness (Barrett *et al.*., 2009).
63 Pathogens specialized at the clade-level could still maintain plant community diversity,
64 but at higher taxonomic levels (Parker *et al*., 2015).
65 While specialist pathogens are invoked under JCH, generalist pathogens may
66 contribute to the maintenance of host community diversity via effective specialization
67 (Benítez *et al*., 2013). One form is host-specific impacts (Sarmiento *et al*., 2017);
68 specifically, disease severity differs among the hosts of a given pathogen. Host-specific
69 impacts could serve as an equalizing mechanism that maintains plant community
70 diversity if the growth and survival of infected seedlings varies among host species
71 (Barrett *et al*., 2009); particularly, if differential impacts reflect ecological trade-offs
72 between allocation of resources to defense, competitive ability, and/or tolerance of
73 abiotic stressors. For example, for tree species adapted to environments with relatively
74 high and/or consistent disease pressure, the fitness benefits of physical and chemical
75 resistance and/or tolerance traits may outweigh the associated allocation costs of those
76 traits, while the costs may outweigh the benefits for tree species adapted to environments
77 with relatively low and/or inconsistent disease pressure (Haak *et al*., 2012). The risk of
78 pathogen attack, referred to here as pathogen pressure, varies with environmental
79 conditions that impact pathogen and plant fitness (Barrett *et al*., 2009). High pathogen
80 pressure has been observed in areas with low light, high soil moisture, and persistent fog
81 and dew (Augspurger & Kelly, 1984; Bradley *et al*., 2003; Mordecai, 2012; Spear *et al*.,
82 2015). Plants adapted to environments with high and/or consistent pathogen pressure may
83 be under selection for increased defenses, whereas plants adapted to low and/or
84 inconsistent pathogen pressure environments may be poorly defended (Coley & Barone,
85 1996, Talley *et al*., 2002).
86 Here we describe (i) pathogens infecting tree seedlings in the forests of Panama,
87 (ii) their host ranges, (iii) interspecific variability among tree species in pathogen
88 susceptibility, and (iv) the relationship between disease susceptibility and plant habitat
89 associations. We had four central hypotheses. First, in these diverse tropical forests,
90 seedling pathogens tend to be multi-host. Second, there is a phylogenetic signal to the
91 host ranges of these multi-host pathogens (i.e., pathogen sharing is positively correlated
92 with host relatedness). Third, tree species differ in their susceptibility to pathogens

because the cost-to-benefit ratio of defense traits differs among species (Strauss & Agrawal 1999; Endara & Coley, 2010). Fourth, plants adapted to environments with relatively low and/or inconsistent disease pressure are more susceptible to disease than those adapted to relatively high and/or consistent disease pressure.

## Materials and Methods

### *Isolation and identification of putative pathogens*

Potentially pathogenic fungi and oomycetes were isolated from 124 seedlings, representing 26 tree species in 13 families, with disease on the leaf, stem, and/or root (Supporting Information Fig. **S1;** Table **S1**). Symptomatic seedlings were collected from seven forest sites in 2007, 2010-2012, and 2019 by: (1) opportunistic collection of naturally occurring seedlings ($n$ = 59); (2) collection of seedlings germinated in a shadehouse and then transplanted to forest sites ($n$ = 22), and (3) collection of seedlings that germinated from surface-sterilized seeds planted directly in the forests ($n$ = 43) (Table **S2**). Most putative pathogens were isolated from leaves (52.13%), followed by stems (37.44%) and roots (10.43%). Molecular identification was completed by bidirectional Sanger sequencing of either the nuclear ribosomal Internal Transcribed Spacer (ITS) region (149 isolates) or the ITS plus an adjacent portion of the large subunit (ITS+LSU) (62 isolates). See Table **S2** and Spear (2017) for details.

All 211 unique isolates were identified and assigned to operational taxonomic units (OTUs) based on 99% sequence similarity (minimum overlap of 40 bp) in Sequencher v. 5.4.6. We used a stringent similarity threshold that splits rather than lumps, while accounting for ≤1% sequencing error, because we make statements about host specificity and to avoid masking strain-specific effects (Higgins *et al*., 2011). We estimated the taxonomic placement of the fungal isolates by querying all 209 edited sequences against the well-curated Full UNITE+INSD dataset for Fungi (v. 8.2, released 2020-02-04; Abarenkov *et al*., 2020) (detailed in Methods **S1**). For the two oomycetes, we queried their sequences against GenBank (accessed 8-Oct-2020; Benson *et al*., 2012) and the curated database Phytopthora-ID (v. 2.0, accessed 8-Oct-2020; Grünwald *et al.*, 2011). All assigned nomenclature should be interpreted as an estimate as: (i) the ITS region is an imprecise barcode for species discrimination for certain taxa; (ii) multi-locus phylogenies are required for accuracy; and (iii) precision is limited by incomplete

124 taxonomic and geographic coverage of databases (Kang *et al*., 2010; Hofstetter *et al*.,
125 2019; Lücking *et al*., 2020). Additionally, sequences in the UNITE database are clustered
126 into hypothesized species-level groups (Species Hypotheses, SHs) based on sequence
127 similarity thresholds ranging from 97–100% (Kõljalg *et al*., 2013; Robbertse *et al*.,
128 2017), while we used 99% sequence similarity to designate OTUs because 97-98.5% is
129 too relaxed for species delimitation for some taxa (Lücking *et al*., 2020). Because our
130 grouping strategy often differed from that of the UNITE database, multiple, distinct
131 OTUs in our study sometimes share the same nomenclature based on the SH of the best-
132 matching UNITE reference sequence (e.g., OTUs Z and AAG are both annotated as
133 *Colletotrichum brevisporum*). Finally, for certain groups of fungi, UNITE SHs may
134 erroneously include distinct species (Robbertse *et al*., 2017).
135 Sequences for the 90 previously published fungal isolates (Spear, 2017) were
136 submitted to the NCBI GenBank database (KY413686–KY413775). Sequence data for
137 the 121 isolates collected in 2019 will be submitted to GenBank (XXXXXXX–
138 XXXXXXX).
139 To characterize sampling efficacy and the relative abundance of OTUs, we
140 calculated the richness of observed OTUs and plotted the taxon accumulation curve
141 (vegan package; Oksanen *et al*., 2019) and rank-abundance distribution (BiodiversityR
142 package; Kindt & Coe, 2005) ($n$ = 211 isolates from 124 seedlings of 26 tree species).
143 We defined OTUs observed ≥10 times as abundant.
144 *Survey-based assessment of host associations and ranges of putative pathogens*
145 Our survey-based assessment of the host associations and ranges of putative
146 pathogens utilize our full dataset ($n$ = 211 isolates, representing 66 OTUs, from 26 tree
147 species) and three subsets to ameliorate the influence of undersampled taxa. Subset A
148 excludes singleton OTUs ($n$ = 178 isolates, representing 33 OTUs, from 24 tree species),
149 B excludes singleton and doubleton OTUs and tree species ($n$ = 144 isolates, representing
150 22 OTUs, from 13 tree species), and C excludes singleton OTUs and the two non-
151 singleton OTUs only isolated from one host species ($n$ = 174 isolates, representing 31
152 OTUs, from 24 tree species). All four datasets include isolates collected in 2007, 2010-
153 2012, and 2019.

154 We used four approaches to characterize host associations. We (1) summed and
155 plotted the number of observed host tree species (unweighted links) (full dataset: $n$ = 211
156 isolates), and (2) calculated Blüthgen's weighted specialization index d' for each of the
157 33 non-singleton OTUs (subset A: $n$ = 178 isolates; bipartite package; Dormann *et al*.,
158 2008; Blüthgen *et al*., 2006). The d′ index is useful for identifying discriminatory host
159 use because it accounts for interaction strength (i.e., strong, frequent vs. weak, occasional
160 interactions) and sampling intensity, while the number of interacting partners does neither
161 (Dormann, 2011; Blüthgen *et al*., 2006). The index estimates specialization by
162 calculating the degree to which observed host use differs from expected host use given
163 partner availability (Blüthgen *et al*., 2006; Dormann, 2011). We could not adjust for the
164 relative abundances of tree species when calculating d′ as the seven forest sites span a
165 marked gradient in plant diversity, and tree species are not uniformly distributed spatially
166 (Pyke *et al*., 2001). Thus, we relied on the interaction matrix's marginal sums (column-
167 and row-wise totals; total interactions per species) as a measure of partner availability,
168 meaning that, for our data, a generalized microbe infects tree species proportional to their
169 sampling intensities, while a specialized microbe infects tree species independent of their
170 sampling intensities (Blüthgen *et al*., 2006). We considered d' values ranging from 0–
171 0.33, 0.34–0.67, and 0.68–1.00 to represent low, moderate, and high specialization,
172 respectively (Blüthgen *et al*., 2006; Spear & Mordecai, 2018). We (3) calculated the
173 observed number of shared OTUs (rich package; Rossi, 2011) and, because that metric
174 generally results in an underestimation of similarity due to undetected species, we (4)
175 estimated similarities in the communities of putative pathogens shared between pairs of
176 tree species via the inverse of the Chao dissimilarity index (vegan package; Oksanen *et*
177 *al*., 2019) (full dataset: $n$ = 211 isolates; subset B: $n$ = 144 isolates). The Chao index
178 accounts for the relative abundances of observed OTUs and unseen shared OTUs, making
179 it relatively robust to unequal sampling and diverse guilds with many rare taxa (Chao *et*
180 *al*., 2005).
181 To determine if there is a phylogenetic signal to the host ranges of the OTUs, (i)
182 we used the mean pairwise phylogenetic distance (MPD) to determine if a given multi-
183 host OTU infects phylogenetically clustered or dispersed hosts, and (ii) we used two
184 binomial generalized linear models (GLMs) (logit link) to determine whether putative

185 pathogen community similarity is positively correlated with host relatedness.
186 Specifically, for the 31 OTUs isolated from ≥2 tree species (subset C: $n$ = 174 isolates),
187 we compared the observed MPD between host tree species infected by a given OTU to
188 the MPD generated in a null model with random host-OTU associations (999
189 permutations generated via randomization of the host phylogeny tips; picante and ape
190 packages; Kembel *et al*., 2010; Paradis & Schliep, 2018). For the GLMs, we used our full
191 dataset ($n$ = 211 isolates) and subset B ($n$ = 144 isolates), and we regressed and plotted
192 the pairwise estimated community similarities (1 - Chao index) against the pairwise
193 phylogenetic distances. The phylogeny of tree species was generated by Phylomatic
194 (phylodiversity.net/phylomatic/), which called the supertree published by Zanne *et al*.
195 (2014).
196 We tested for correlations between (i) the frequency with which a given fungal
197 OTU was isolated and the number of tree species from which it was isolated, and (ii)
198 sampling intensity (number of seedlings collected) and the number of OTUs isolated
199 from a given tree species using one-tailed, non-parametric Spearman's rank tests (full
200 dataset: $n$ = 211 isolates; stats package; R Core Team, 2020). One-tailed tests were used
201 because we had directional, literature-based *a priori* hypotheses (Quinn & Keough, 2002;
202 Ruxton & Neuhäuser, 2010) that: (i) the most abundant fungal taxa would be multi-host,
203 and (ii) observed fungal richness associated with a given plant species would increase
204 with increased sampling (Ferrer & Gilbert, 2003; Spear & Mordecai, 2018).
205 *Experimental assessments of pathogenicity and host range*
206 In 2011 and 2012, inoculation experiments were used to assess the pathogenicity
207 and host specificities of 27 fungal isolates collected in 2010 and 2011 (Fig. **S2a**). We
208 tested the 27 isolates, representing 18 OTUs, against seedlings of 35 tree species,
209 representing 20 families (Table **S1**). The tree species-by-fungal isolate combinations
210 were not fully reciprocal (285 of 945 possible combinations; 3,154 seedlings total) due to
211 space and time constraints and because seeds were unavailable or available in insufficient
212 quantities. A given fungal isolate was tested against three to 28 tree species and 48% of
213 isolates evaluated were tested against seedlings of ≥10 tree species. Because an objective
214 of the inoculation experiments was to describe pathogen host range, once isolates
215 displayed pathogenicity, we prioritized testing those isolates against additional tree

216 species (12-28 tree species, median 15) over expanding the testing of isolates failing to
217 generate significant disease. A given tree species was tested against one to 26 fungal
218 isolates and 29% of tree species were tested against ≥10 isolates.
219 Our inoculation technique simulated infested plant material in the soil to passively
220 inoculate the seedlings with the fungal isolates selected for screening (modified from
221 Augspurger & Wilkinson, 2007). To generate seedlings for the inoculation experiments,
222 seeds were collected from forests bordering the Panama Canal (May-Nov. of 2011, May-
223 June 2012), surface-sterilized (sequential washes of 95% EtOH for 10 s, 10% commercial
224 bleach for 2 min, 70% EtOH for 2 min), and germinated in flats of autoclave-sterilized (1
225 hour at 121º C, twice) commercial soil (Fig. **S2b**). Seedlings were transplanted to
226 individual pots containing autoclaved commercial soil and either rice visibly colonized
227 by one of the fungal isolates or inoculum-free, autoclave-sterilized rice (control/sham
228 treatment) (Fig. **S2c-f**). Depending on seed availability, five to 12 seedlings per species
229 were included in each treatment.
230 Inoculation experiments were conducted in Smithsonian Tropical Research
231 Institute shadehouses in Gamboa, Panama during the wet seasons of 2011 and 2012 (Fig.
232 **S2a**). The two screened-in shadehouses were covered on all sides with shadecloth and
233 their roofs were covered with plastic to minimize external contamination and to mimic
234 the forest understory. Shadehouse air temperatures (26.1 and 25.6ºC) did not significantly
235 differ from those recorded simultaneously in a nearby forest understory (25.4 and 25.3ºC;
236 Paton, 2019a). Relative humidity was significantly lower in the shadehouses (85.1 and
237 87.6%) than in the forest understory (95.6 and 96.7%; Paton, 2019b). Photosynthetically
238 active radiation (PAR) reaching the shadehouse seedlings was slightly higher (1.4 and
239 1.7% of full PAR) than observed in a nearby forest understory (1.3%; Brenes-Arguedas
240 *et al*., 2011). See Table **S3** for details. Light was somewhat variable within a given
241 shadehouse. To counteract this potentially confounding factor, seedlings were rotated
242 among the tables weekly.
243 Disease symptoms were documented every three days, for up to five weeks, and
244 were categorized as: (i) seedling mortality; (ii) stem damage (Fig. **S2g-o**); (iii) wilted
245 tissue (Fig. **S2p,q**); and (iv) stunted seedling growth (Fig. **S2r,s**). A seedling was
246 classified as morbid if it had any of the four disease symptoms. Wilted tissue was

247 considered a symptom of disease because the soil was not allowed to dry out (seedlings
248 were hand-watered every three days) and pathogens attacking roots can interfere with
249 water uptake (Yadeta & Thomma, 2013). Similarly, stunted growth can be indicative of
250 pathogen-caused damage to roots and/or the vascular system (Yadeta & Thomma, 2013).
251 Additionally, stunting may occur because energy and resources are allocated to defenses
252 and/or tissue replacement rather than growth (Huot *et al*., 2014). Root and foliar damage
253 could not be reliably observed and were not included in any analyses. Regarding the
254 latter, we observed a synchronous occurrence of morphologically similar foliar damage
255 across distinct treatments and the controls, which suggested that causative biotic agent
256 (*Xylaria* sp.; E. R. Spear, unpublished data) may have blown into the shadehouses.
257 To compare the proportion of symptomatic seedlings in the inoculated versus
258 inoculum-free sham (control) treatments, we used generalized linear models (GLMs)
259 with binomial error distributions, probit link functions, and mean bias-reducing score
260 adjustments (brglm package; Firth, 1993; Kosmidis & Firth, 2009; Kosmidis, 2019). We
261 used bias-reduced GLMs because we observed complete and quasi-complete separation
262 of data for some fungal isolate-by-tree species combinations (i.e., treatment perfectly, or
263 near perfectly, predicted the binary outcome; Albert & Anderson, 1984). The response
264 variables were presence/absence of (i) general morbidity and (ii) mortality. Each tree
265 species-by-fungal isolate combination was considered individually for each response
266 variable. We considered an isolate pathogenic if, for at least one tree species, a
267 significantly greater proportion of inoculated seedlings were symptomatic than paired
268 control seedlings (using $\alpha = 0.10$ because small sample sizes limited our power to detect
269 treatment effects; Quinn & Keough, 2002; Thiese *et al*., 2016). We tested for a
270 correlation between the frequency with which a fungal OTU was isolated and the number
271 of tree species in which it caused statistically significant disease using a non-parametric
272 Spearman's rank test. We used a one-tailed test because we had the *a priori* hypothesis
273 that the most abundant fungal taxa would be multi-host.
274 *Plant habitats, seed sizes, and disease susceptibility*
275 We used logit-link beta-binomial generalized linear models to explore disease
276 susceptibility as a function of habitat associations and seed size (aod package; Lesnoff &
277 Lancelot, 2012). We obtained data for seed size, shade tolerance, and spatial distribution

relative to annual rainfall from published sources (Table **S1**). In all models, the response variable was presence/absence of morbidity in inoculated seedlings, controlling for the morbidity observed in paired control seedlings and considering all 27 isolates tested (pathogens and non-pathogens alike). Because negative relative proportions are biologically equivalent to zero (no disease caused), and for statistical purposes, we converted all negative values to zero. We used two global models that contained (i) the additive main effects of seed size (continuous) and shade tolerance (categorical), and their interaction, or (ii) the additive main effects of seed size and distribution relative to rainfall (categorical), and their interaction. We excluded seeds with average dry masses greater than 3,000 mg to improve model stability. For both categorical predictors, we limited our comparisons to binary extremes (i.e., only shade-intolerant and -tolerant tree species, and only wet- and dry-site tree species). We analyzed disease as a function of shade tolerance (26 tree species, 179 observations: 42 for intolerant spp., 137 for tolerant spp.) and spatial distribution (15 tree species, 154 observations: 108 for dry-site spp., 46 for wet-site spp.) separately due to differences in the availability of trait data. For the two sets of models, automated model selection and averaging was done based on the global models, the Akaike information criterion with a correction for small sample sizes (AICc), and $\Delta$AICc $\leq 2$ (MuMIn package, Bartoń, 2020).

All data were processed in R v. 4.0.0 (R Core Team, 2020). In addition to the packages used for analyses, we used these for data formatting and figures: fossil (Vavrek, 2011), ggplot2 (Wickham, 2016), otuSummary (Yang, 2020), plot.matrix (Klinke, 2019), plyr (Wickham, 2011), reshape2 (Wickham, 2007).

## Results

*Relative abundances and taxonomic assignments of putative pathogens*

The 211 isolates represent 66 operational taxonomic units (OTUs); however, the taxon accumulation curve is non-asymptotic, indicating incomplete sampling (Fig. **S3b**). Most of the OTUs are relatively rare, 33 are singletons (Figures **1**, **S3a**; Table **S4**). Four OTUs are common (observed ≥10) and comprised 35% of all isolates: A–*Colletotrichum xanthorrhoeae*, B–Nectriaceae, C–*Mycoleptodiscus suttonii*, and F–*Diaporthe tulliensis* (Figures **1**, **S3a**; Table **S4**). The 209 fungal and two oomycete isolates represent five classes (Sordariomycetes, Dothideomycetes, Agaricomycetes, Eurotiomycetes, and

309 Oomycetes) and 11 orders. Most (81.5%) of the isolates could be assigned to 26 genera.
310 *Colletotrichum*, *Diaporthe,* and *Mycoleptodiscus* were the three genera most frequently
311 isolated (Fig. **1**; Table **S4**). We observed overlap in fungal OTUs among the three tissues
312 sampled: leaves, stems, and roots (Fig. **S4d**). While sampling occurred over five years,
313 there was overlap in the tree species and forest sites sampled and the isolation and
314 molecular techniques used (Table **S2**). Accordingly, we observed overlap in fungal OTUs
315 among the years, seedling collection methods, and isolation media (Fig. **S4a-c**).
316 *Fungi associated with symptomatic seedlings are host generalists*
317 The fungi isolated from symptomatic seedlings are predominately multi-host
318 generalists with wide host ranges. Of the 33 non-singleton OTUs, 31 were isolated from
319 2-9 tree species (median = 3) (Fig. **1**; Table **S4**). The number of tree species from which a
320 fungal OTU was isolated is positively correlated with isolation frequency (one-tailed
321 Spearman's rank correlation: rho = 0.963, $P < 0.001$; blue points Fig. **S5**). A–*C.*
322 *xanthorrhoeae* was isolated with the greatest frequency (35 times) and from the greatest
323 number of tree species (9 spp. in 7 families) (Fig. **1**; Table **S4**). Only two of the non-
324 singleton OTUs (T–*Macrophomina* and X–*Ceratobasidium*) were not multi-host, but
325 each was isolated only twice, and from the best-sampled tree species, suggesting their
326 observed host ranges may be a sampling artifact (Fig. **1**; Tables **S1, S4**). Based on the
327 specialization index d', 57.6% of 33 the non-singleton OTUs, including A–*C.*
328 *xanthorrhoeae* and B–Nectriaceae, exhibit low host-specialization (d' ≤ 0.33) and 42.4%,
329 including C–*M. suttonii* and F–*D. tulliensis*, exhibit moderate host-specialization (d'
330 0.33–0.67) at the species level (Table **S4**). Twelve of the 31 multi-host OTUs infect hosts
331 that are more closely related than expected by chance, but none significantly so (Table
332 **S4**). For two of those OTUs (Q–*Diaporthe cf. columnaris* and R–*Diaporthe fraxini-*
333 *angustifoliae*), phylogenetic clustering of host use was marginally significant ($P = 0.09$;
334 Fig. **1**; Table **S4**), which is tentative evidence of a phylogenetic signal to host range. Both
335 OTUs were solely isolated from *Anacardium excelsum* (Anacardiaceae, Sapindales) and
336 *Protium panamense* (Burseraceae, Sapindales) seedlings.
337 *Tree species are infected by various microbes, and their microbial communities overlap*
338 Multiple OTUs were isolated for all tree species with >2 seedlings collected (Fig.
339 **1**; Table **S1**). Predictably, the number of OTUs isolated from a given tree species is

340 strongly positively correlated with sampling intensity (one-tailed Spearman's rank
341 correlation: rho = 0.951, $P < 0.001$; Fig. **1**). The greatest richness of fungi (24 OTUs) was
342 recovered from the best-sampled tree species (41 isolates), *A. excelsum* (Table **S1**).
343 We observed overlap among the microbial communities of distantly related tree
344 species (Fig. **1**). Based on subset B, excluding singleton and doubleton observations of
345 tree species and OTUs, the estimated average similarity (1 - Chao index) between the
346 microbial communities of pairs of tree species is 0.21 (median = 0.15; Table **S5**). The
347 GLMs suggest that the similarity of microbial communities is unrelated to phylogenetic
348 distance between tree species (Fig. **2**; full dataset: Est. ± SE = 0.002 ± 0.003, *z*-value =
349 0.60, $P = 0.55$; subset B: Est. ± SE = 0.002 ± 0.004, *z*-value = 0.36, $P = 0.72$).
350 *Identities and impacts of fungi with confirmed pathogenicity*
351 We experimentally confirmed the pathogenicity of 10 of the 27 fungal isolates
352 tested (Fig. **3**). The isolates with confirmed pathogenicity are assigned to six OTUs, all in
353 the phylum Ascomycota, and were originally isolated from stems, roots, and leaves of
354 seedlings of seven tree species in seven families (Fig. **3**; Table **1**). Based on morbidity
355 (i.e., any of the four specific disease symptoms: wilt, stunting, stem damage, or
356 mortality), significant pathogenicity was observed for 20 of the 285 tree species-by-
357 fungal isolate combinations (Fig. **3**; Table **1**). For 13 of these combinations, infection led
358 to seedling mortality (Table **1**). Multiple disease symptoms were often observed for a
359 single tree species-by-pathogen combination (e.g., *Annona glabra* seedlings suffered
360 stem damage, wilt, and mortality when inoculated with isolate ERS029).
361 *Fungi with confirmed pathogenicity are multi-host*
362 We show that fungal pathogens can attack tree species belonging to
363 phylogenetically dispersed families. Five of the 10 pathogenic isolates successfully
364 attacked multiple tree species (Table **1**). The 10 isolates with confirmed pathogenicity
365 generated significant disease symptoms in 6-31% of the tree species against which they
366 were tested (median = 10%). At the high end, ERS029 (C–*Mycoleptodiscus suttonii*)
367 caused significant disease in 31% of the 13 tree species it was tested against and the four
368 susceptible tree species belong to three orders (Fig. **3**; Tables **1**, **S1**).
369 Three of the OTUs were represented by multiple isolates: B–Nectriaceae, G–
370 *Clonostachys rosea*, and C–*M. suttonii*. The two isolates of B–Nectriaceae were

371 recovered from *Trichilia tuberculata* and *Calophyllum longifolium*, and combined were
372 pathogenic on six of the 28 tree species tested (Fig. **3**; Table **1**). The three isolates of G–
373 *C. rosea* were isolated from *Virola surinamensis* and *Protium tenuifolium*, and combined
374 caused significant disease on four tree species (Fig. **3**; Table **1**). The two isolates of C–*M.*
375 *suttonii* were isolated from *V. surinamensis* and *Randia armata*, and combined caused
376 significant disease on five species (Fig. **3**; Table **1**). While the tree species-by-fungal
377 isolate combinations were not fully reciprocal, limiting conclusions, we observed
378 possible strain-specific effects (e.g., B–Nectriaceae: ERS034 caused significant disease
379 on seedlings of *Guapira standleyana* while ERS036 did not; Fig. **3**). Finally, there is a
380 marginally significant positive association between the frequency with which a fungal
381 OTU was isolated and the number of tree species in which it caused statistically
382 significant ($\alpha = 0.10$) disease (one-tailed Spearman's rank correlation rho = 0.721, $P$ =
383 0.053; green points Fig. **S5**).
384 *Tree species are differentially impacted by pathogens*
385 We documented interspecific differences in disease susceptibility and impact
386 among tree species. Most tree species tested were seemingly resistant to, or tolerant of,
387 all fungal isolates tested, suffering minimal to no fitness impacts despite soil inoculation
388 (Fig. **3**). For 13 of the 35 tree species tested, at least one of the 27 fungal isolates tested
389 caused significant disease (Table **1**). Five tree species experienced significant disease
390 from two to three of the isolates tested (Table **1**). Those five tree species experienced
391 significant disease from 10-50% (median = 15%) of the fungal isolates against which
392 they were tested (Fig. **3**).
393 The 13 susceptible tree species differed in their tolerance of attack. For most of
394 the susceptible tree species, pathogen attack did not consistently lead to death, the impact
395 from infection differed depending on the pathogenic isolate with which it was inoculated.
396 For example, while *Castilla elastica* seedlings suffered significant disease when
397 inoculated with two fungal isolates (ERS036 and ERS040), they only suffered significant
398 seedling mortality when inoculated with ERS040 (AAF–Nectriaceae) (Table **1**).
399 Furthermore, for those tree species that were susceptible to the same multi-host pathogen,
400 the impact from attack differed among species. For example, isolate ERS036 (B–
401 Nectriaceae) caused significant morbidity on seedlings of *C. elastica, Cochlospermum*

402 *vitifolium,* and *Dalbergia retusa*, but only caused significant mortality on *C. vitifolium*
403 seedlings (Table **1**).
404 *Plant habitats, seed sizes, and disease susceptibility*
405 When considering all 27 fungal isolates tested (pathogens and non-pathogens), the
406 proportion of seedlings with morbidity was influenced by a tree species' seed size, spatial
407 distribution relative to annual rainfall, and shade tolerance (Fig. **4a-d**; Tables **S6**, **S7**).
408 Shade-intolerant tree species were more likely to exhibit disease than shade-tolerant
409 species ($P = 0.003$; Fig. **4a,b**; Table **S6**). For both groups, the probability of disease
410 decreases with increasing seed size, but to a greater extent for shade-intolerant species ($P$
411 $= 0.014$; Fig. **4a,b**; Table **S6**). Tree species restricted to, or more abundant in, drier sites
412 (dry-site species) were more likely to exhibit disease than those restricted to, or more
413 abundant in, wetter sites (wet-site species) ($P = 0.042$; Fig. **4c,d**; Table **S7**). While we
414 found no correlation between seed size and disease risk for wet-site tree species, disease
415 risk decreases with increasing seed size for dry-site tree species (Fig. **4c,d**; Table **S7**).
416 **Discussion**
417 Pathogens are believed to shape the distributions and abundances of their hosts,
418 effects often ascribed to host-specialized pathogens (Comita *et al*., 2014). However, there
419 is increasing evidence that multi-host pathogens are prevalent, in natural plant
420 communities (Augspurger & Wilkinson, 2007; Gallery *et al*., 2007; Gilbert & Webb,
421 2007; Kluger *et al*., 2008; Hersh *et al*., 2012; Schweizer *et al*., 2013; Sarmiento *et al*.,
422 2017; Spear & Mordecai, 2018). These multi-host pathogens may contribute to the
423 maintenance of these communities via host-specific impacts (disease severity differs
424 among a pathogen's hosts), a form of effective specialization (Augspurger & Wilkinson
425 2007; Benítez *et al*., 2013; Sarmiento *et al*., 2017). Using a multifaceted approach, we
426 identified fungal pathogens attacking seedlings in the forests of Panama, and show that
427 they can attack phylogenetically dispersed hosts. Furthermore, we demonstrate that tree
428 species differ in their susceptibility to pathogens, with trees adapted to environments with
429 relatively low and/or inconsistent disease pressure being more susceptible to disease.
430 Collectively, these results suggest that generalist pathogens contribute to the maintenance
431 of local and regional plant diversity.

432 *Pathogens and their impacts*

433 We observed 66 OTUs, but only four were observed more than 10 times (Figures **1,**
434 **S3a**; Table **S4**). The relative rarity of the OTUs, coupled with a non-asymptomatic taxon
435 accumulation curve, indicate incomplete sampling and a diverse community of seedling-
436 associated fungi (Fig. **S3**). The most frequently isolated fungal genera (Fig. **1**) and those
437 with experimentally confirmed pathogenicity (Table **1**) include known plant pathogens
438 (Nguyen *et al*., 2016).

439 Seven of the 10 isolates with confirmed pathogenicity caused significant seedling
440 mortality for at least one tree species during the inoculation experiments (Table **1**),
441 reinforcing the importance of pathogens as a selective pressure and source of nonrandom
442 seedling mortality that can directly shape plant community composition (Green *et al*.,
443 2014). We also observed pathogen-caused stem damage, stunted growth, and wilting.
444 These nonlethal infections can also be a structuring force by compromising a seedling's
445 ability to survive future stressors (Ditommaso & Watson, 1995).

446 Seventeen of the 27 fungal isolates tested appear to be nonpathogenic. However,
447 some of the isolates categorized as nonpathogenic may be pathogenic under optimal
448 conditions. These isolates may not have caused disease in our experiments because we
449 did not replicate the environmental conditions necessary for disease development
450 (Álvarez-Loayza *et al*., 2011) or due to the absence of a susceptible host (each isolate
451 was only tested against three to 28 tree species, a fraction of the locally available hosts).
452 While a number of the fungi tested can infect multiple tissue types (Table **1**; Fig. **S4d**),
453 some of the isolates deemed nonpathogenic based on our soil inoculation technique may
454 only be able to colonize leaves or stems.

455 *Pathogens can infect phylogenetically dispersed hosts*

456 The host associations of the fungi isolated from symptomatic seedlings, in
457 combination with the results of the inoculation experiments, provide strong support for
458 our hypothesis that seedling pathogens in tropical forests tend to be multi-host (Figures **1,**
459 **3**; Table **1**). Multi-host pathogens of seedlings, leaves, and seeds have been observed in
460 previous Panama-based studies (Augspurger & Wilkinson, 2007; Gilbert & Webb, 2007;
461 Schweizer *et al*., 2013; Sarmiento *et al*., 2017). We speculate that our study

462 underestimates the true breadth of the fungi's host ranges, as we sampled and tested a
463 small fraction of the >824 tree species present in the local forests (Pyke *et al*., 2001).
464 In general, a given fungal OTU was isolated from heterofamilial tree species, host use
465 is phylogenetically dispersed, and pairwise similarities in community composition of
466 seedling-associated fungi are unrelated to pairwise phylogenetic distances between tree
467 species (Figures **1, 2**; Tables **S4**, **S5**). Together these results suggest that, contrary to our
468 second hypothesis, there is no phylogenetic signal to host range for the pathogens
469 attacking seedlings in these tropical forests. The absence of a significant phylogenetic
470 signal is consistent with one Panama-based study of foliar pathogens (Schweizer *et al*.,
471 2013), but conflicts with other studies in temperate and tropical plant communities
472 (Gilbert & Webb, 2007; Gilbert *et al*., 2012; Parker *et al*., 2015; Chen *et al*., 2019). Our
473 study did not include as many congeneric or confamilial host pairs as Gilbert and Webb
474 (2007) or Gilbert *et al*. (2012), which may have hindered our ability to detect a signal.
475 Alternatively, pathogen host use may track phylogenetically labile plant defenses (Kursar
476 *et al*., 2009) rather than phylogenetically conserved defenses (e.g., Fine *et al*., 2006).
477 Parallel evolution of similar defenses in unrelated lineages would explain why the
478 pathogens in this and other studies can infect many distantly related tree species.
479 The positive relationships between OTU isolation frequency and the number of hosts
480 observed during our survey and inoculation experiments (Fig. **S5**) suggest that host
481 generalists are abundant members of the pathogen community and that, if our sampling
482 effort were expanded, a given fungal OTU would be detected in additional tree species.
483 Many seedling pathogens, such as the soil-borne fungi and oomycetes responsible for
484 seedling damping-off, are passively dispersed (e.g., by splashing rain), with limited
485 dispersal distances (Madden, 1997). Thus, the ability to infect a wide range of hosts,
486 particularly spatially aggregated hosts, would increase a pathogen's likelihood of
487 persistence. For pathogens with limited dispersal, host use may be more tightly linked
488 with plant habitat associations than with phylogenetic relatedness, making them more
489 likely to infect tree species that commonly co-occur spatially and temporally (Roy, 2001).
490 Furthermore, just as selection should favor multi-host pathogens in species-rich
491 assemblages, selection should favor true generalists, whose host ranges are decoupled
492 from plant relatedness, in plant communities that are phylogenetically overdispersed on

493 the small spatial scale relevant to dispersal-limited pathogens (Swenson *et al*., 2007;
494 Swenson *et al*., 2012; Pearse *et al*., 2013).

495 *Tree species share pathogens, but are differentially impacted*

496 A given plant species is often infected by multiple microorganisms and these
497 microorganisms are frequently shared among members of a plant community (Hersh *et*
498 *al*., 2012; Sarmiento *et al*., 2017; Spear & Mordecai, 2018). We observed overlap among
499 the fungal communities of distantly related tree species (Fig. **1**; Table **S5**), a pattern likely
500 to intensify with increased sampling. The presence of a particular fungus in the seedlings
501 of multiple host tree species does not mean that those hosts are equally likely to be
502 infected by that fungus. Pathogens may disproportionally infect certain hosts based on
503 availability, defense traits, and/or habitat (Ferrer & Gilbert, 2003).

504 Disease progression among susceptible hosts is mediated by multiple factors,
505 including defense traits. Based on our inoculation experiments, tree species were
506 differentially impacted by shared pathogens (Fig. **3**; Table **1**); in support of our third
507 hypothesis and consistent with the host-specific impacts of multi-host pathogens
508 observed in previous studies (Davidson *et al*., 2000; Augspurger & Wilkinson, 2007;
509 Hersh *et al*., 2012; Sarmiento *et al*., 2017). Most of the tree species we tested were
510 seemingly resistant to the 10 pathogenic isolates (Fig. **3**), which does not preclude their
511 susceptibility to numerous untested fungi. Moreover, these tree species may actually be
512 susceptible to infection by the pathogenic isolates in our study, but their tolerance
513 mechanisms prevent extensive pathogen colonization and symptom development. In
514 which case, these tolerant tree species may act as reservoir hosts that facilitate the
515 persistence of pathogens within the community (Haas *et al*., 2011).

516 *Plant habitats, seed sizes, and disease susceptibility*

517 Disease pressure varies spatially, particularly across environmental gradients
518 (Augspurger, 1984a; Brenes-Arguedas *et al*., 2009; Defossez *et al*., 2011; Haak *et al*.,
519 2012; Hersh *et al*., 2012; Spear *et al*., 2015). For plants adapted to areas with relatively
520 low and/or inconsistent disease pressure, such as areas with higher light, lower humidity,
521 and inconsistent precipitation (Augspurger, 1984a; Spear *et al*., 2015), the allocation
522 costs of physical and chemical defenses may outweigh the benefits and vice versa for
523 plants adapted to areas with relatively high and/or consistent disease pressure (Coley &

524 Barone, 1996; Strauss & Agrawal, 1999; Endara & Coley, 2010). Consistent with
525 variable selective pressures, and previous field and greenhouse studies (Augspurger,
526 1984b; Augspurger & Kelly, 1984; McCarthy-Neumann & Kobe, 2008), seedlings of
527 shade-intolerant tree species and tree species restricted to, or more abundant in, drier
528 forests (dry-site species) were more likely to suffer disease than seedlings of shade-
529 tolerant and wet-site tree species in our inoculation experiments (Fig. **4**; Tables **S6**, **S7**).
530 Engelbrecht *et al*. (2007) found no correlation between a species' light requirements and
531 its occurrence in dry- versus wet-sites, but evergreen trees are more common in wet-sites,
532 while deciduous trees dominate dry-sites (Santiago *et al*., 2004). Shade-tolerant and
533 evergreen tree species, both with long-lived leaves, tend to invest more in pest defenses
534 than shade-intolerant and deciduous tree species (Coley & Barone, 1996; Santiago *et al*.,
535 2004).
536 For shade-tolerant, shade-intolerant, and dry-site tree species, the probability of
537 disease decreases with increasing seed size (Fig. **4**). This is perhaps unsurprising as large-
538 seeded species can invest more in defenses and better compensate for lost tissue
539 (Armstrong & Westoby, 1993; Green & Juniper, 2004). Our results are inconsistent with
540 two Panama-based studies, which found no correlation between seed size and pathogen
541 susceptibility, possibly because they only included wind-dispersed species while our
542 analyses included wind- and vertebrate-dispersed species (average seed dry masses: 1.9 -
543 686 versus 3 - 2334 mg) (Augspurger, 1984a; Augspurger & Kelly, 1984).
544 *Consequences for plant communities*
545 Neither disturbance nor microhabitat specialization are believed to be sufficient to
546 maintain the hyperdiversity of tropical forests (Leigh *et al*., 2004). While host-specialized
547 pests are commonly touted as the true drivers of diversity maintenance (Leigh *et al*.,
548 2004; Comita *et al*., 2014), we show that generalist pathogens are frequently responsible
549 for seedling death and disease. The host-specific impacts of multi-host pathogens and
550 differences among tree species in pathogen susceptibility observed by this and other
551 studies (Augspurger & Wilkinson, 2007; Hersh *et al*., 2012; Spear *et al*., 2015) suggest
552 that generalized pathogens have the potential to influence plant community diversity by
553 unevenly affecting seedling recruitment. First, variability in host competence may
554 maintain plant community diversity by creating a dilution effect (Ostfeld & Keesing,

555 2000). Overall disease risk should be lowered if the most competent host(s) is/are
556 relatively rare within the community because pathogen transmission would be slowed
557 between individuals of susceptible species. Accordingly, the presence of less competent
558 hosts should protect poorly defended host species from pathogen-caused local extinction.
559 Second, generalist pathogens with host-specific impacts may alter direct and indirect
560 interspecific competitive interactions among co-occurring species (Alexander & Holt,
561 1998; Barrett *et al*., 2009; Mordecai, 2011). Host-generalized pathogens could promote
562 coexistence if a competition-defense tradeoff exists among plant species (i.e., if superior
563 competitors are more susceptible; Mordecai, 2011). Finally, interspecific variation in host
564 susceptibility to generalist pathogens may maintain diversity because disease-sensitive
565 tree species are excluded from disease-prone habitats. By reinforcing resource
566 partitioning and limiting the geographic distributions of species, generalist pathogens
567 contribute to the spatial turnover of plant species and the maintenance of local and
568 regional forest diversity.

569 **Acknowledgments**

570 This research was funded by Simons Foundation Award 429440, Center for Tropical
571 Forest Science, Smithsonian Tropical Research Institute, Garden Club of America,
572 University of Utah's Global Change and Sustainability Center, and A. Herbert and
573 Marian W. Gold Scholarship. Additional logistical support and facilities were provided
574 by the Smithsonian Tropical Research Institute, S. Mangan, and the Arnold Lab at
575 University of Arizona. We thank J. Sertich, D. Martinez, B. Wolfe, J. Ibarra, H. Espinosa,
576 C. Swatek, M. Schmoker, D. Sandberg, J. Shaffer, and C. Gonzalez for field and lab
577 assistance; R. Perez for help with tree species identification; J. Wright, G. Gilbert, S.
578 Mangan, A. E. Arnold, S. Higginbotham, T. Kursar, and P. Coley for advice; T. Brenes-
579 Arguedas for sharing her culture collection, and N. Anaya and G. LaRosa, who
580 contributed to that collection; and Parque Natural Metropolitano, D. Roubik, Barro
581 Colorado Nature Monument, and the Ministerio de Ambiente for authorizing this
582 research.

583 **Author Contributions:** ERS designed and conducted the research, analyzed the data,
584 and wrote the first draft of the manuscript, ERS and KDB contributed to manuscript
585 revisions and approved the final version.

586 **References**

587 **Abarenkov K, Zirk A, Piirmann T, Pöhönen R, Ivanov F, Nilsson RH, Kõljalg U.**
588 **2020.** Full UNITE+INSD dataset for Fungi. UNITE Community.
589 10.15156/BIO/786372

590 **Agrawal AA. 2007.** Macroevolution of plant defense strategies. *Trends in Ecology &*
591 *Evolution* **22**: 103-109.

592 **Albert A, Anderson JA. 1984.** On the existence of maximum likelihood estimates in
593 logistic regression models. *Biometrika* **71**: 1-10.

594 **Álvarez-Loayza P, White JF Jr, Torres MS, Balslev H, Kristiansen T, Svenning JC,**
595 **Gil N. 2011.** Light converts endosymbiotic fungus to pathogen, influencing
596 seedling survival and niche-space filling of a common tropical tree, *Iriartea*
597 *deltoidea*. *PloS ONE* **6**: e16386.

598 **Armstrong DP, Westoby M. 1993.** Seedlings from large seeds tolerated defoliation
599 better: a test using phylogeneticaly independent contrasts. *Ecology* **74**: 1092-
600 1100.

601 **Augspurger CK. 1984a.** Seedling survival of tropical tree species: interactions of
602 dispersal distance, light-gaps, and pathogens. *Ecology* **65**:1705-1712.

603 **Augspurger CK. 1984b.** Light requirements of neotropical tree seedlings: a comparative
604 study of growth and survival. *Journal of Ecology* **72**: 777-795.

605 **Augspurger CK, Kelly CK. 1984.** Pathogen mortality of tropical tree seedlings:
606 experimental studies of the effects of dispersal distance, seedling density, and
607 light conditions. *Oecologia* **61**: 211-217.

608 **Augspurger CK, Wilkinson HT. 2007.** Host specificity of pathogenic *Pythium* species:
609 Implications for tree species diversity. *Biotropica* **39**: 702–708.

610 **Bagchi R, Gallery RE, Gripenberg S, Gurr SJ, Narayan L, Addis CE, Freckleton**
611 **RP, Lewis OT. 2014.** Pathogens and insect herbivores drive rainforest plant
612 diversity and composition. *Nature* **506**: 85-88.

613 **Barrett LG, Kniskern JM, Bodenhausen N, Zhang W, Bergelson J. 2009.** Continua
614 of specificity and virulence in plant host–pathogen interactions: causes and
615 consequences. *New Phytologist* **183**: 513-529.

616 **Bartoń K. 2020.** MuMIn: Multi-Model Inference. R package version 1.43.17. [WWW
617 document] URL https://CRAN.R-project.org/package=MuMIn [accessed 2 June
618 2020].
619 **Bell T, Freckleton RP, Lewis OT. 2006.** Plant pathogens drive density-dependent
620 seedling mortality in a tropical tree. *Ecology Letters* **9**: 569–574.
621 **Benítez MS, Hersh MH, Vilgalys R, Clark JS. 2013.** Pathogen regulation of plant
622 diversity via effective specialization. *Trends in Ecology and Evolution* **28**: 705–
623 711.
624 **Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J,**
625 **Sayers EW. 2012.** GenBank. *Nucleic Acids Research* **41**: D36-D42.
626 **Blüthgen N, Menzel F, Blüthgen N. 2006.** Measuring specialization in species
627 interaction networks. *BMC Ecology* **6**: 9.
628 **Bradley DJ, Gilbert GS, Parker IM. 2003.** Susceptibility of clover species to fungal
629 infection: the interaction of leaf surface traits and environment. American *Journal*
630 *of Botany* **90**: 857-864.
631 **Brenes-Arguedas T, Coley PD, Kursar TA. 2009.** Pests versus drought as determinants
632 of plant distribution along a tropical rainfall gradient. *Ecology* **90**: 1751-1761.
633 **Brenes-Arguedas T, Roddy AB, Coley PD, Kursar TA. 2011.** Do differences in
634 understory light contribute to species distributions along a tropical rainfall
635 gradient? *Oecologia* **166**: 443-456.
636 **Chao A, Chazdon RL, Colwell RK, Shen T. 2005.** A new statistical approach for
637 assessing similarity of species composition with incidence and abundance data.
638 *Ecology Letters* **8**: 148-159.
639 **Chen L, Swenson NG, Ji N, Mi X, Ren H, Guo L, Ma K. 2019.** Differential soil fungus
640 accumulation and density dependence of trees in a subtropical forest. *Science* **366**:
641 124-128.
642 **Coley PD, Barone JA. 1996.** Herbivory and plant defenses in tropical forests. *Annual*
643 *Review of Ecology and Systematics* **27**: 305-335.
644 **Connell JH. 1971.** On the role of natural enemies in preventing competitive exclusion in
645 some marine animals and in rain forest trees. In Boer PJD, Gradwell GR, eds.

646 *Dynamics of numbers in populations.* Wageningen, Netherlands: Centre for
647 Agricultural Publication and Documentation, 298–312.
648 **Comita LS, Muller-Landau HC, Aguilar S, Hubbell SP. 2010.** Asymmetric density
649 dependence shapes species abundances in a tropical tree community. *Science* **329**:
650 330-332.
651 **Comita LS, Queenborough SA, Murphy SJ, Eck JL, Xu K, Krishnadas M, Beckman**
652 **N, Zhu Y. 2014**. Testing predictions of the Janzen–Connell hypothesis: a meta-
653 analysis of experimental evidence for distance-and density-dependent seed and
654 seedling survival. *Journal of Ecology* **102**: 845-856.
655 **Davidson JM, Rehner SA, Santana M, Lasso E, Urena de Chapet O, Herre EA.**
656 **2000.** First report of *Phytophthora heveae* and *Pythium* spp. on tropical tree
657 seedlings in Panama. *Plant Disease* **84**: 706-706.
658 **Defossez E, Courbaud B, Marcais B, Thuiller W, Granda E, Kunstler G. 2011.** Do
659 interactions between plant and soil biota change with elevation? A study on *Fagus*
660 *sylvatica*. *Biology Letters* **7**: 699-701.
661 **Ditommaso A, Watson AK. 1995.** Impact of a fungal pathogen, *Colletotrichum*
662 *coccodes* on growth and competitive ability of *Abutilon theophrasti*. *New*
663 *Phytologist* **131**: 51-60.
664 **Dormann CF. 2011.** How to be a specialist? Quantifying specialisation in pollination
665 networks. *Network Biology* **1**: 1-20.
666 **Dormann CF, Gruber B, Fruend J. 2008.** Introducing the bipartite Package: analysing
667 ecological networks. *R news* **8**: 8-11.
668 **Endara MJ, Coley PD. 2011.** The resource availability hypothesis revisited: a meta-
669 analysis. *Functional Ecology* **25**: 389-398.
670 **Engelbrecht BM, Comita LS, Condit R, Kursar TA, Tyree MT, Turner BL, Hubbell**
671 **SP. 2007.** Drought sensitivity shapes species distribution patterns in tropical
672 forests. *Nature* **447**: 80–82.
673 **Fine PVA, Miller ZJ, Mesones I, Irazuzta S, Appel HM, Stevens MHH, Sääksjärvi I,**
674 **Schultz JC, Coley PD. 2006.** The growth-defense trade-off and habitat
675 specialization by plants in Amazonian forests. *Ecology* **87**: S150–S162.
676 **Firth D. 1993.** Bias reduction of maximum likelihood estimates. *Biometrika* **80**: 27–38.

677 **Ferrer A, Gilbert GS. 2003.** Effect of tree host species on fungal community
678 composition in a tropical rain forest in Panama. *Diversity and Distributions* **9**:
679 455-468.
680 **Gallery RE, Dalling JW, Arnold AE. 2007.** Diversity, host affinity, and distribution of
681 seed-infecting fungi: A case study with *Cecropia*. *Ecology* **88**: 582–588.
682 **Gilbert GS, Magarey R, Suiter K, Webb CO. 2012.** Evolutionary tools for
683 phytosanitary risk analysis: phylogenetic signal as a predictor of host range of
684 plant pests and pathogens. *Evolutionary Applications* **5**: 869-878.
685 **Gilbert GS, Webb CO. 2007.** Phylogenetic signal in plant pathogen-host range.
686 *Proceedings of the National Academy of Sciences, USA* **104**: 4979-4983.
687 **Gillett JB. 1962.** Pest pressure, and underestimated factor in evolution. *Systematics*
688 *Association Publication* **4**: 37–46.
689 **Green PT, Harms KE, Connell JH. 2014.** Nonrandom, diversifying processes are
690 disproportionately strong in the smallest size classes of a tropical forest.
691 *Proceedings of the National Academy of Sciences, USA* **111**: 18649-18654.
692 **Green PT, Juniper PA. 2004.** Seed mass, seedling herbivory and the reserve effect in
693 tropical rainforest seedlings. *Functional Ecology* **18**: 539-547.
694 **Grünwald NJ, Martin FN, Larsen MM, Sullivan CM, Coffey MD, Hansen EM,**
695 **Parke JL. 2011.** Phytophthora-ID.org: A sequence-based Phytophthora
696 identification tool. *Plant Disease* **95**: 337-342.
697 **Haak DC, McGinnis LA, Levey DJ, Tewksbury JJ. 2012.** Why are not all chilies hot?
698 A trade-off limits pungency. *Proceedings of the Royal Society B: Biological*
699 *Sciences* **279**: 2012-2017.
700 **Haas SE, Hooten MB, Rizzo DM, Meentemeyer RK. 2011.** Forest species diversity
701 reduces disease risk in a generalist plant pathogen invasion. *Ecology Letters* **14**:
702 1108-1116.
703 **Harms KE, Wright SJ, Calderon O, Hernandez A, Herre EA. 2000.** Pervasive
704 density-dependent recruitment enhances seedling diversity in a tropical forest.
705 *Nature* **404**: 493–495.
706 **Hersh MH, Vilgalys R, Clark JS. 2012**. Evaluating the impacts of multiple generalist
707 fungal pathogens on temperate tree seedling survival. *Ecology* **93**: 511-520.

708 **Higgins KL, Coley PD, Kursar TA, Arnold AE. 2011.** Culturing and direct PCR
709 suggest prevalent host generalism among diverse fungal endophytes of tropical
710 forest grasses. *Mycologia* **103**: 247-260.
711 **Hofstetter V, Buyck B, Eyssartier G, Schnee S, Gindro K. 2019.** The unbearable
712 lightness of sequenced-based identification. *Fungal Diversity* **96**: 243-284.
713 **Huot B, Yao J, BMontgomery BL, He SY. 2014.** Growth–defense tradeoffs in plants: a
714 balancing act to optimize fitness. *Molecular Plant* **7**: 1267-1287.
715 **Janzen DH. 1970.** Herbivores and the number of tree species in tropical forests.
716 *American Naturalist* **104**: 501–528.
717 **Kang S, Mansfield MA, Park B, Geiser DM, Ivors KL, Coffey MD, Grünwald NJ,**
718 **Martin FN, Lévesque CA, Blair JE. 2010.** The promise and pitfalls of sequence-
719 based identification of plant-pathogenic fungi and oomycetes. *Phytopathology*
720 **100**: 732-737.
721 **Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD,**
722 **Blomberg SP, Webb CO. 2010.** Picante: R tools for integrating phylogenies and
723 ecology. *Bioinformatics* **26**: 1463-1464.
724 **Kindt R, Coe R. 2005.** *Tree diversity analysis. A manual and software for common*
725 *statistical methods for ecological and biodiversity studies*. Nairobi, Kenya: World
726 Agroforestry Centre (ICRAF).
727 **Klinke S. 2019.** plot.matrix: Visualizes a Matrix as Heatmap. R package version 1.4.
728 [WWW document] URL https://CRAN.R-project.org/package=plot.matrix
729 [accessed 2 June 2020].
730 **Kluger CG, Dalling JW, Gallery RE, Sanchez E, Weeks-Galindo C, Arnold AE.**
731 **2008.** Host generalists dominate fungal communities associated with seeds of four
732 neotropical pioneer species. *Journal of Tropical Ecology* **24**: 351–354.
733 **Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates**
734 **ST, Bruns TD, Bengtsson-Palme J, Callaghan TM *et al.* 2013.** Towards a
735 unified paradigm for sequence-based identification of fungi. *Molecular Ecology*
736 **22**: 5271–5277.

**Kosmidis I. 2019.** brglm: Bias Reduction in Binary-Response Generalized Linear Models. R package version 0.6.2. [WWW document] URL https://cran.r-project.org/package=brglm [accessed 2 June 2020].

**Kosmidis I, Firth D. 2009.** Bias reduction in exponential family nonlinear models. *Biometrika* **96**: 793–804.

**Kursar TA, Dexter KG, Lokvam J, Pennington RT, Richardson JE, Weber MG, Murakami ET, Drake C, McGregor R, Coley PD. 2009.** The evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus *Inga*. *Proceedings of the National Academy of Sciences, USA* **106**: 18073-18078.

**Leigh EG, Davdar P, Dick CW, Puyravaud J-P, Terborgh J, ter Steege H, Wright SJ. 2004.** Why do some tropical forests have so many species of trees? *Biotropica* **36**: 447-473.

**Lesnoff M, Lancelot R. 2012.** aod: Analysis of Overdispersed Data. R package version 1.3.1. [WWW document] URL http://cran.r-project.org/package=aod [accessed 2 June 2020].

**Liu Y, Yu S, Xie Z, Staehelin C. 2012.** Analysis of a negative plant–soil feedback in a subtropical monsoon forest. *Journal of Ecology* **100**: 1019–1028.

**Lücking R, Aime MC, Robbertse B, Miller AN, Ariyawansa HA, Aoki T, Cardinali G, Crous PW, Druzhinina IS, Geiser DM. 2020.** Unambiguous identification of fungi: where do we stand and how accurate and precise is fungal DNA barcoding? *IMA Fungus* **11**: 1-32.

**Madden LV. 1997.** Effects of rain on splash dispersal of fungal pathogens. *Canadian Journal of Plant Pathology* **19**: 225-230.

**Mangan SA, Schnitzer SA, Herre EA, Mack KM, Valencia MC, Sanchez EI, Bever J. 2010.** Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* **466**: 752-755.

**May RM. 1991.** A fondness for fungi. *Nature* **352**: 475-476.

**McCarthy-Neumann S, Kobe RK. (2008).** Tolerance of soil pathogens co-varies with shade tolerance across species of tropical tree seedlings. *Ecology* **89**: 1883-1892.

767 **Mordecai EA. 2011.** Pathogen impacts on plant communities: Unifying theory, concepts,
768 and empirical work. *Ecological Monograph* **81**: 429-441.
769 **Mordecai EA. 2012.** Soil moisture and fungi affect seed survival in California grassland
770 annual plants. *PloS ONE* **7:** e39083.
771 **Nguyen NH, Song Z, Bates ST, Branco, S, Tedersoo L, Menke J, Schilling JS,**
772 **Kennedy PG. 2016.** FUNGuild: an open annotation tool for parsing fungal
773 community datasets by ecological guild. *Fungal Ecology* **20**: 241-248.
774 **Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin**
775 **PR, O'Hara RB, Simpson GL, Solymos P *et al*. 2019.** vegan: Community
776 Ecology Package. R package version 2.5-6. [WWW document] URL
777 https://CRAN.R-project.org/package=vegan [accessed 2 June 2020].
778 **Ostfeld RS, Keesing F. 2000.** Biodiversity and disease risk: the case of Lyme disease.
779 *Conservation Biology* **14**: 722–728.
780 **Packer A, Clay K. 2000.** Soil pathogens and spatial patterns of seedling mortality in a
781 temperate tree. *Nature* **404**: 278-281.
782 **Paradis E, Schliep K. 2018.** ape 5.0: an environment for modern phylogenetics and
783 evolutionary analyses in R. *Bioinformatics* **35**: 526-528.
784 **Parker IM, Saunders M, Bontrager M, Weitz AP, Hendricks R, Magarey R, Suiter**
785 **K, Gilbert GS. 2015.** Phylogenetic structure and host abundance drive disease
786 pressure in communities. *Nature* **520**: 542-544.
787 **Paton S. 2019a.** Barro Colorado Island, Lutz tower 1m_Air Temperature. The
788 Smithsonian Institution. Dataset. https://doi.org/10.25573/data.10042394.v7
789 **Paton S. 2019b.** Barro Colorado Island, Lutz tower 1m_Relative Humidity. The
790 Smithsonian Institution. Dataset. https://doi.org/10.25573/data.10042400.v7
791 **Pearse WD, Jones FA, Purvis A. 2013.** Barro Colorado Island's phylogenetic
792 assemblage structure across fine spatial scales and among clades of different ages.
793 *Ecology* **94**: 2861-2872.
794 **Pyke CR, Condit R, Aguilar S, Lao S. 2001.** Floristic composition across a climatic
795 gradient in a neotropical lowland forest. *Journal of Vegetation Science* **12**: 553–
796 566.

797 **Quinn GP, Keough MJ. 2002.** *Experimental design and data analysis for biologists*.
798 Cambridge University Press.
799 **R Core Team. 2020.** R: a language and environment for statistical computing. R
800 Foundation for Statistical Computing, Vienna, Austria.
801 **Robbertse B, Strope PK, Chaverri P, Gazis R, Ciufo S, Domrachev M, Schoch CL.**
802 **2017.** Improving taxonomic accuracy for fungi in public sequence databases:
803 applying 'one name one species' in well-defined genera with
804 *Trichoderma*/*Hypocrea* as a test case. *Database* **2017**
805 **Roy BA. 2001.** Patterns of association between crucifers and their flower-mimic
806 pathogens: host jumps are more common than coevolution or cospeciation.
807 *Evolution* **55**: 41-53.
808 **Rossi J-P. 2011.** rich: an R package to analyse species richness. *Diversity* **3**: 112-120.
809 **Ruxton GD, Neuhäuser M. 2010.** When should we use one-tailed hypothesis testing?
810 *Methods in Ecology and Evolution* **1**: 114-117.
811 **Sarmiento C, Zalamea PC, Dalling JW, Davis AS, Stump SM, U'Ren JM, Arnold**
812 **AE. 2017.** Soilborne fungi have host affinity and host-specific effects on seed
813 germination and survival in a lowland tropical forest. *Proceedings of the National*
814 *Academy of Sciences, USA* **114**: 11458-11463.
815 **Santiago LS, Kitajima K, Wright SJ, Mulkey SS. 2004.** Coordinated changes in
816 photosynthesis, water relations and leaf nutritional traits of canopy trees along a
817 precipitation gradient in lowland tropical forest. *Oecologia* **139**: 495-502.
818 **Schweizer D, Gilbert GS, Holl KD. 2013.** Phylogenetic ecology applied to enrichment
819 planting of tropical native tree species. *Forest Ecology and Management* **297**: 57-
820 66.
821 **Spear ER. 2017.** Phylogenetic relationships and spatial distributions of putative fungal
822 pathogens of seedlings across a rainfall gradient in Panama. *Fungal Ecology* **26**:
823 65-73.
824 **Spear ER, Coley PD, Kursar TA. 2015.** Do pathogens limit the distributions of tropical
825 trees across a rainfall gradient? *Journal of Ecology* **103**: 165-174.

826 **Spear ER, Mordecai EA. 2018.** Foliar pathogens of California grasses are multi-host
827 and spatially widespread: implications for grassland diversity. *Ecology* **99**: 2250-
828 2259.
829 **Strauss SY, Agrawal AA. 1999.** The ecology and evolution of plant tolerance to
830 herbivory. *Trends in Ecology and Evolution* **14**: 179–185.
831 **Swenson NG, Enquist BJ, Thompson J, Zimmerman JK. 2007.** The influence of
832 spatial and size scale on phylogenetic relatedness in tropical forest communities.
833 *Ecology* **88**: 1770-1780.
834 **Swenson NG, Erickson DL, Mi X, Bourg NA, Forero-Montaña J, Ge X, Howe R,**
835 **Lake JK, Liu X, Ma K *et al*. 2012.** Phylogenetic and functional alpha and beta
836 diversity in temperate and tropical tree communities. *Ecology* **93**: S112-S125.
837 **Talley SM, Coley PD, Kursar TA. 2002.** Anti-fungal leaf surface metabolites correlate
838 with fungal abundance in sagebrush populations. *Journal of Chemical Ecology*
839 **28**: 2141–2168.
840 **Vavrek MJ. 2011.** fossil: palaeoecological and palaeogeographical analysis tools.
841 *Palaeontologia Electronica* **14**: 1T.
842 **Wickham H. 2007.** Reshaping data with the reshape package. *Journal of Statistical*
843 Software **21**: 1-20.
844 **Wickham H. 2011.** The split-apply-combine strategy for data analysis. *Journal of*
845 *Statistical Software* **40**: 1-29.
846 **Wickham H. 2016.** *ggplot2: elegant graphics for data analysis*. New York, NY, USA:
847 Springer-Verlag.
848 **Yadeta KA, Thomma, BPHJ. 2013.** The xylem as battleground for plant hosts and
849 vascular wilt pathogens. *Frontiers in Plant Science* **4**: 97.
850 **Yang S. 2020.** otuSummary: summarizing OTU table regarding the composition,
851 abundance and beta diversity of abundant and rare biospheres. R package version
852 0.1.1. [WWW document] URL https://CRAN.R-
853 project.org/package=otuSummary [accessed 2 June 2020].
854 **Zanne AE, Tank DC, Cornwell WY, Eastman JM, Smith SA, FitzJohn RG,**
855 **McGlinn DJ, O'Meara BC, Moles AT, Reich PB *et al*. 2014.** Three keys to the
856 radiation of angiosperms into freezing environments. *Nature* **506**: 89-92.

857 **Supporting Information**

858

859 **Figure S1** Examples of disease in the forests of Panama.

860

861 **Figure S2** Details of shadehouse-based inoculation experiments.

862

863 **Figure S3** Rank abundance plot and OTU accumulation curve.

864

865 **Figure S4** Overlap in fungal OTUs among sampling years, methods used to obtain
866 symptomatic seedlings, isolation media, and tissue sampled.

867

868 **Figure S5** Correlation between OTU host range and isolation frequency.

869

870 **Table S1** Taxonomic assignments, traits, sampling effort, and observed OTUs for tree
871 species evaluated in our survey and experiments.

872

873 **Table S2** Methodological details pertaining to the multi-year collection of symptomatic
874 seedlings, and microbial isolation and sequencing.

875

876 **Table S3** Average light levels, air temperatures, and relative humidities of the
877 shadehouses used for the inoculation experiments versus ambient conditions.

878

879 **Table S4** Estimated taxonomic placement, isolation frequency, number of observed
880 hosts, estimated host specialization, and phylogenetic pattern of host use of the OTUs.

881

882 **Table S5** Overlap in seedling-associated OTUs among tree species.

883

884 **Table S6** Results of the beta-binomial generalized linear regression with the proportion
885 of diseased seedlings as a function of seed size and shade tolerance.

886

887 **Table S7** Average estimates based on the best-ranked beta-binomial generalized linear
888 regressions with the proportion of diseased seedlings as a function of seed size and spatial
889 distribution relative to annual rainfall.
890
891 **Methods S1** Methods used to estimate the taxonomic placements of the 66 OTUs and
892 assign nomenclature.

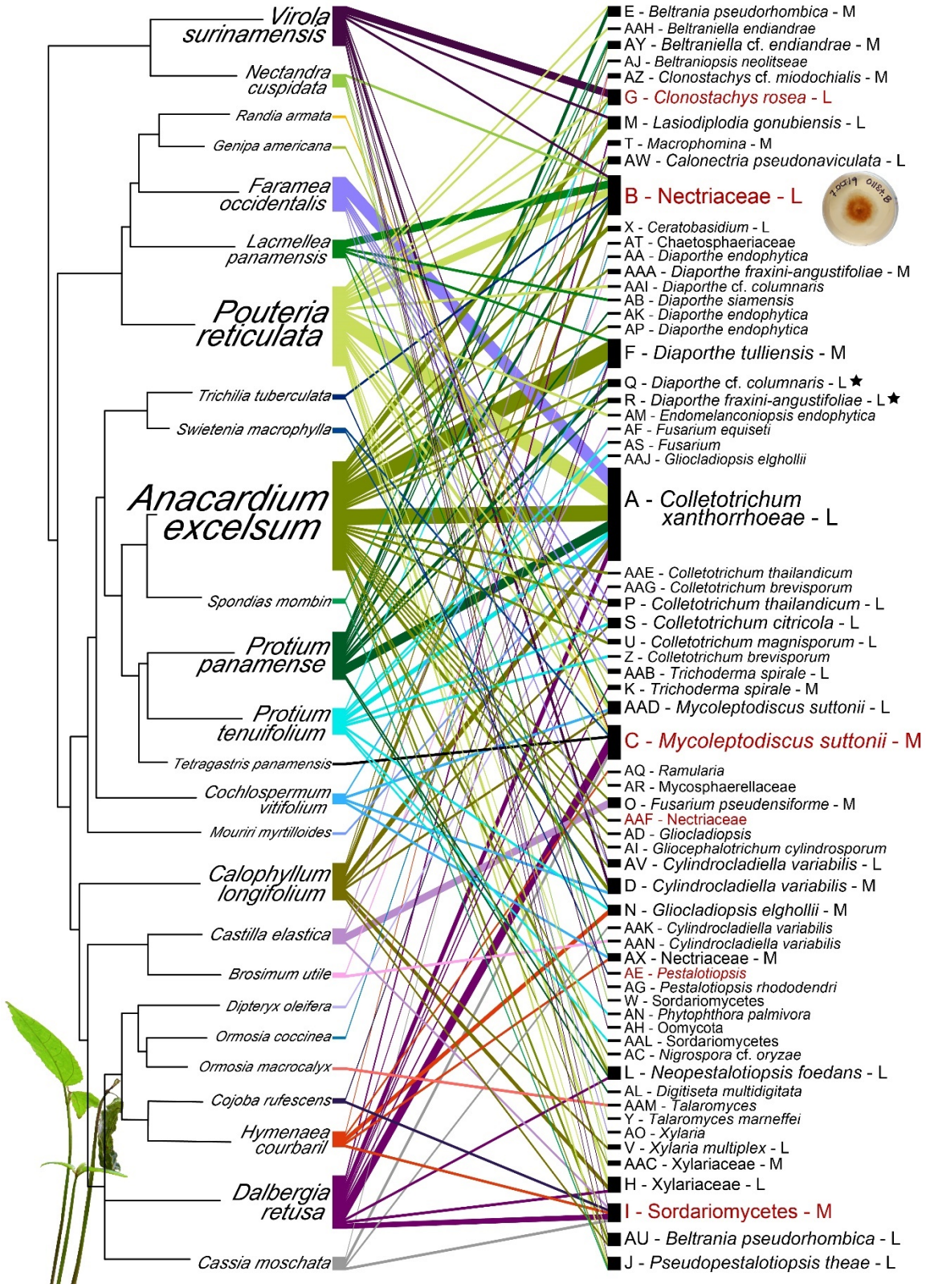


**Figure 1.** Host associations of the fungi and oomycetes isolated from symptomatic seedlings (full dataset: $n = 211$ isolates). The 26 host tree species (left) are arranged

896 according to their phylogenetic relationships. The estimated taxonomic placement of each
897 of the 66 microbial OTUs (right) is preceded by a unique letter identifier. Because our
898 OTU grouping strategy (99% sequence similarity) often differed from that of the UNITE
899 database, distinct OTUs in our study sometimes share the same nomenclature based on
900 the species hypothesis of the best-matching reference sequence in UNITE (e.g., Z and
901 AAG). For the non-singleton OTUs, nomenclature is followed by an L or M, indicating
902 low or moderate estimated specialization (d'). The width of each line represents the
903 number of times a given OTU was isolated from seedlings of a given tree species. For
904 both tree species and OTUs, font size is scaled to the number of interactions. For two
905 OTUs (Q and R, starred), phylogenetic clustering of host use was marginally significant
906 ($P = 0.09$). The six fungal OTUs with members that exhibited statistically significant ($\alpha =$
907 $0.10$) pathogenicity during the inoculation experiments are listed in red.
908

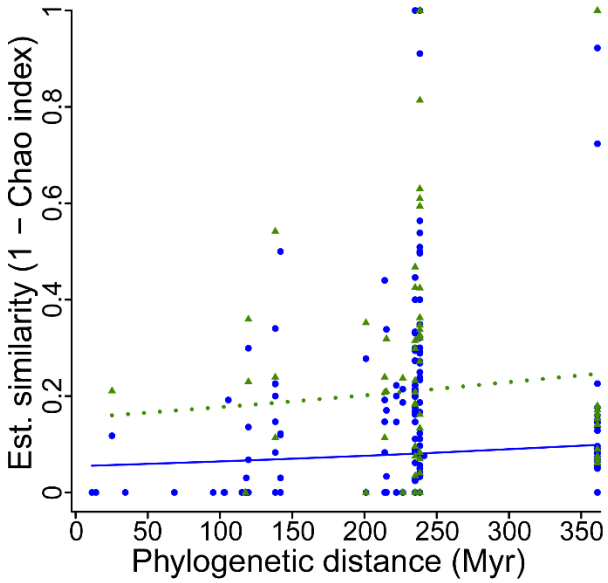


**Figure 2.** Estimated pairwise similarities of OTU communities are unrelated to pairwise phylogenetic distances (time of independent evolution in Myr). The blue circles and solid line (GLM: Est. ± SE = 0.002 ± 0.003, *z*-value = 0.60, *P* = 0.55) are based on the full dataset (*n* = 211 isolates, representing 66 OTUs, from 26 tree species), while the green triangles and dotted line (GLM: Est. ± SE = 0.002 ± 0.004, *z*-value = 0.36, *P* = 0.72) are based on subset B, excluding singleton and doubleton observations of tree species and OTUs (*n* = 144 isolates, representing 22 OTUs, from 13 tree species).

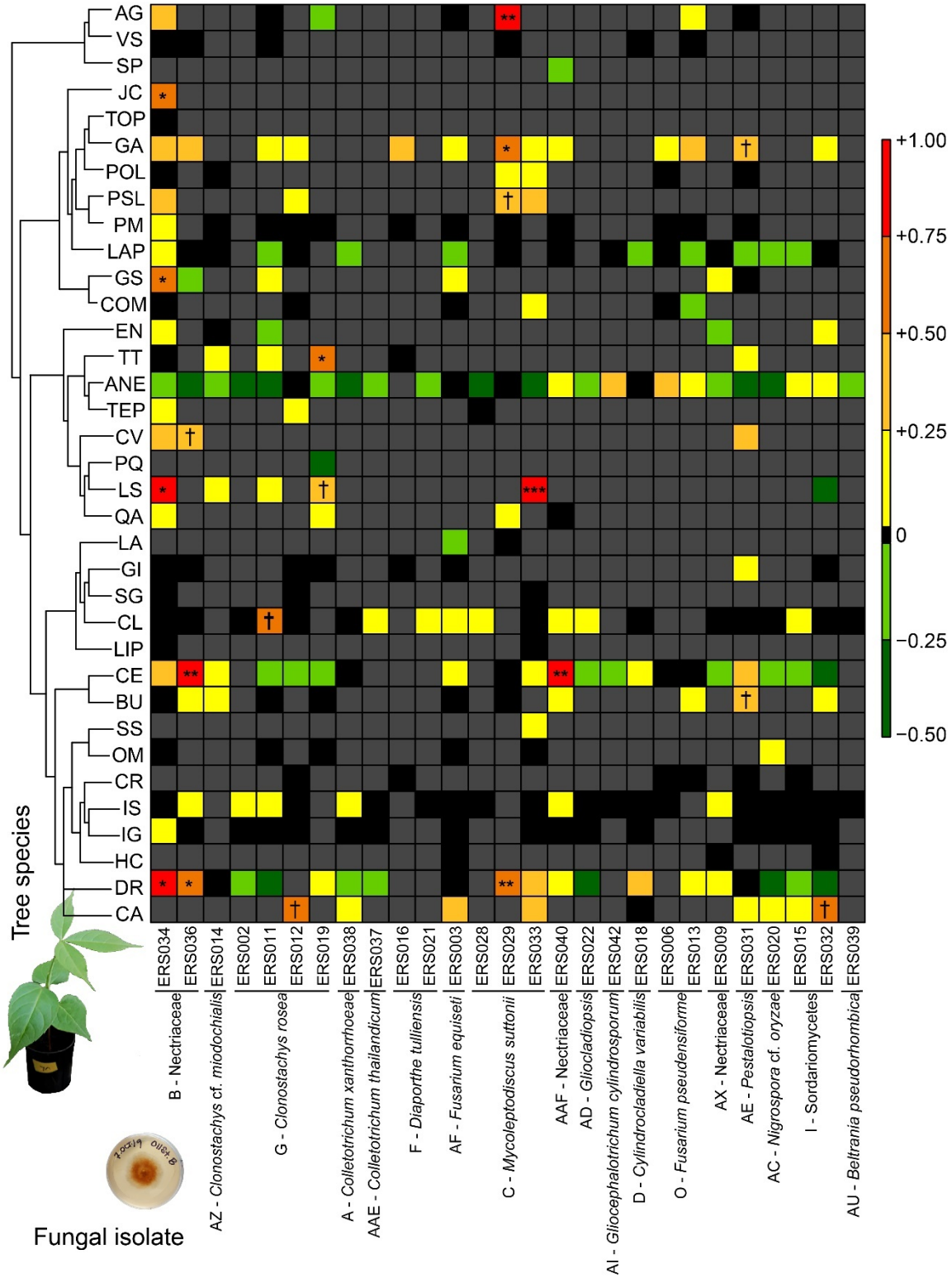


**Figure 3.** Pathogenicity and host ranges of the 27 fungal isolates tested, and susceptibility of the 35 tree species tested, based on seedling morbidity. Fungal isolates are grouped according to their OTU (horizontal axis). Tree species (Table **S1**) are

922 arranged based on their phylogenetic relationships (vertical axis). Tree species-by-fungal
923 isolate combinations for which evidence of pathogenicity was observed (proportion of
924 inoculated seedlings with symptoms > proportion in paired control group) are depicted in
925 shades of yellow (pale for minimal) to red (dark for substantial). Combinations for which
926 there was no evidence of pathogenicity (proportion of inoculated seedlings with
927 symptoms ≤ proportion in paired control group) are depicted in black and shades of
928 green. Gray cells represent an untested combination. For 20 of the 285 combinations
929 tested, we observed significant pathogenicity (***$P \leq 0.001$, **$P \leq 0.01$, *$P \leq 0.05$, †$P \leq$
930 0.10; Table **1**).

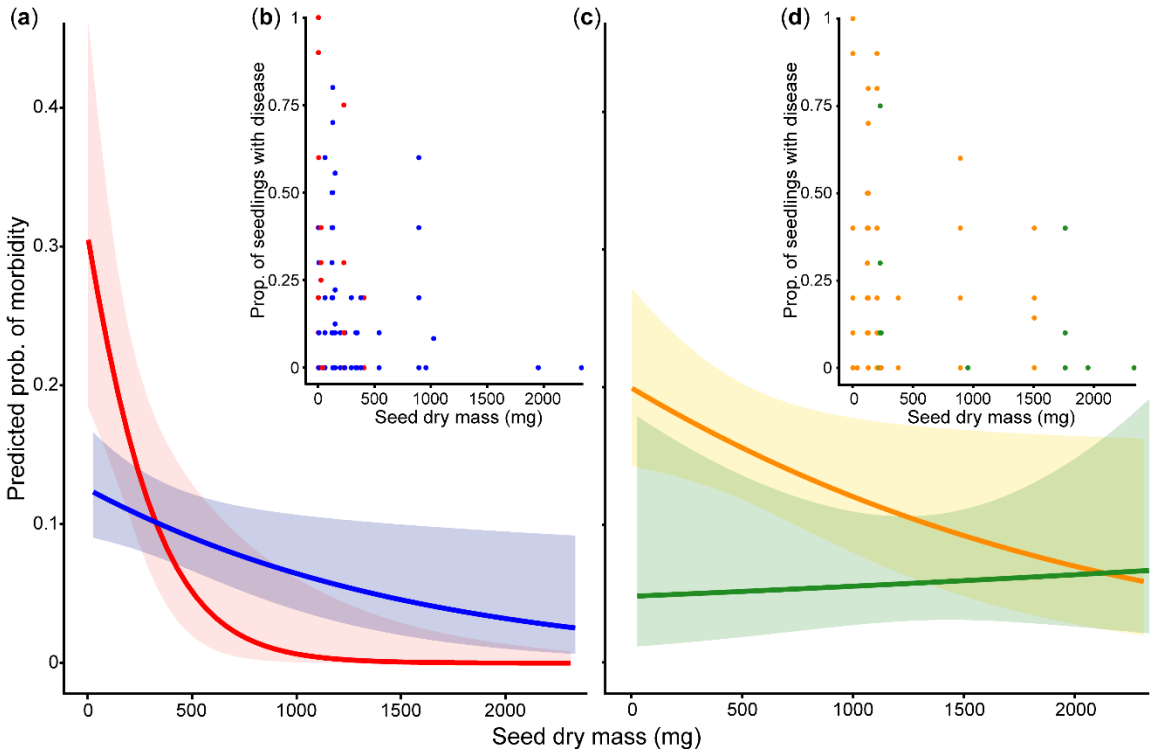


**Figure 4.** Relationships between disease susceptibility and seed size and shade tolerance (a,b), and seed size and spatial distribution relative to annual rainfall (c,d). Shade-intolerant tree species (red) are more likely to experience disease than shade-tolerant species (blue) ($P = 0.003$), and for both groups the probability of disease decreases with increasing seed size (a,b) (Table **S6**). Dry-site tree species (orange) are more likely to experience disease than wet-site species (green) ($P = 0.042$; Table **S7**). For dry-site species (orange) the probability of disease decreases with increasing seed size (c,d) (Table **S7**).

940 **Table 1.** The 20 fungal isolate-by-tree species combinations for which significant pathogenicity was observed based on the
941 presence/absence of (i) general morbidity and (ii) mortality in the inoculated versus control seedlings, analyzed with bias-reduced
942 generalized linear models assuming binomial error distributions and probit link functions and using α = 0.10 because small sample
943 sizes limited our power to detect treatment effects.
944

| **OTU – Estimated taxonomic placement (isolate, origin tree sp. & tissue, no. of tree spp. tested)** | **Susceptible tree species** | ***n*** **Cntrl** | ***n*** **Inoc** | **Disease metric** | **Prop. rel. to control** | **Est (SE)** | **z** | ***P*** |
|---|---|---|---|---|---|---|---|---|
| B - Nectriaceae (ERS034, TT root, 28) | *D. retusa*[1] | 5 | 5 | morbidity | 0.8 | 2.169 (1.044) | 2.076 | 0.038 |
| | | | | mortality | 1 | 2.932 (1.195) | 2.454 | 0.014 |
| | *G. standleyana* | 10 | 10 | morbidity | 0.6 | 1.609 (0.651) | 2.471 | 0.014 |
| | *J. copaia*[1] | 10 | 10 | morbidity | 0.6 | 1.996 (0.828) | 2.411 | 0.016 |
| | | | | mortality | 0.6 | 1.996 (0.828) | 2.411 | 0.016 |
| | *L. seemannii*[1] | 5 | 5 | morbidity | 1 | 2.932 (1.195) | 2.454 | 0.014 |
| | | | | mortality | 1 | 2.932 (1.195) | 2.454 | 0.014 |
| B - Nectriaceae (ERS036, CL stem[2], 13) | *C. elastica* | 10 | 10 | morbidity[8] | 0.8 | 2.252 (0.711) | 3.168 | 0.002 |
| | *C. vitifolium* | 10 | 10 | morbidity[9] | 0.4 | 1.126 (0.640) | 1.759 | 0.079 |
| | | | | mortality | 0.4 | 1.126 (0.640) | 1.759 | 0.079 |
| | *D. retusa* | 10 | 10 | morbidity[10] | 0.5 | 1.36 (0.643) | 2.117 | 0.034 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| G – *Clonostachys rosea*, Bionectriaceae (ERS011, VS stem[3], 17) | *C. longifolium* | 5 | 5 | morbidity | 0.6 | 1.684 (1.017) | 1.657 | 0.098 |
| G – *C. rosea* (ERS012, PT leaf[4], 15) | *C. aromatica* | 5 | 5 | morbidity[11,12] | 0.6 | 1.684 (1.017) | 1.657 | 0.098 |
| G – *C. rosea* (ERS019, VS root, 12) | *L. seemannii* | 10 | 10 | morbidity | 0.4 | 1.126 (0.640) | 1.759 | 0.079 |
| | | | | mortality | 0.4 | 1.126 (0.640) | 1.759 | 0.079 |
| | *T. tuberculata* | 8 | 9 | morbidity | 0.56 | 1.796 (0.867) | 2.072 | 0.038 |
| | | | | mortality | 0.44 | 1.540 (0.867) | 1.776 | 0.076 |
| C - *Mycoleptodiscus suttoni*, Muyocopronaceae (ERS029, VS stem[5], 13) | *A. glabra* | 12 | 12 | morbidity | 0.75 | 2.462 (0.800) | 3.078 | 0.002 |
| | | | | mortality | 0.58 | 2.033 (0.788) | 2.579 | 0.010 |
| | *D. retusa* | 10 | 10 | morbidity | 0.7 | 1.891 (0.669) | 2.826 | 0.005 |
| | | | | mortality | 0.8 | 2.526 (0.848) | 2.977 | 0.003 |
| | *G. americana* | 10 | 10 | morbidity | 0.5 | 1.360 (0.643) | 2.117 | 0.034 |
| | *P. limonensis* | 10 | 10 | morbidity | 0.4 | 1.527 (0.828) | 1.844 | 0.065 |
| | | | | mortality | 0.4 | 1.527 (0.828) | 1.844 | 0.065 |
| C - *M. suttoni* (ERS033, RA stem[6], 16) | *L. seemannii* | 10 | 10 | morbidity[13] | 0.9 | 2.887 (0.882) | 3.274 | 0.001 |
| | | | | mortality | 0.9 | 2.887 (0.882) | 3.274 | 0.001 |
| AAF - Nectriaceae (ERS040, ANE stem[7], 12) | *C. elastica* | 10 | 9 | morbidity[14] | 0.9 | 2.843 (0.895) | 3.178 | 0.002 |
| | | | | mortality | 0.89 | 2.827 (0.890) | 3.177 | 0.002 |
| AE – *Pestalotiopsis*, Sporocadaceae (ERS031, TT root, 18) | *B. utile* | 10 | 10 | morbidity | 0.4 | 1.527 (0.828) | 1.844 | 0.065 |
| | | | | mortality | 0.4 | 1.527 (0.828) | 1.844 | 0.065 |
| | *G. americana* | 10 | 10 | morbidity | 0.4 | 1.126 (0.640) | 1.759 | 0.079 |
| | | | | mortality | 0.4 | 1.126 (0.640) | 1.759 | 0.079 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| I - Sordariomycetes (ERS032, HC stem, 14) | *C. aromatica* | 5 | 5 | morbidity | 0.6 | 1.684 (1.017) | 1.657 | 0.098 |

See Table **S1** for the full names of the tree species. Prop. rel. to control is the proportion of inoculated seedlings with the disease metric minus the proportion in the paired control group. We considered an isolate pathogenic if, for at least one tree species, a significantly greater proportion of inoculated seedlings were symptomatic than paired control seedlings.

[1] Experiment end date = date all inoculated seedlings had died (i.e., used truncated observations of paired control seedlings).

[2,3,4,5,6,7]See **Fig. S1j,t,f,q,r,v**, respectively, for photos.

[8,9,10,11,12,13,14] See **Fig. S2r,n,k,g,h,m,s**, respectively, for photos.