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

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Summary

- Host-specialized pathogens are credited with the maintenance of tropical forest diversity under the Janzen-Connell hypothesis. Yet, in diverse forests, selection may 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 we observed phylogenetically overdispersed host use for 19 OTUs. 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.
- Our results suggest that generalist pathogens contribute to the maintenance of local and regional forest diversity via host-specific impacts and the exclusion of disease-sensitive trees from disease-prone habitats.

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; Augspurger & Wilkinson, 2007; 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 and phylogenetic relatedness of host species (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). Assuming that closely related plants are more likely to possess similar defense traits, the likelihood of tree species sharing a pathogen should decrease with decreasing

63 phylogenetic relatedness (Barrett *et al.*, 2009). Pathogens specialized at the clade-level
64 could still maintain plant community diversity, but at higher taxonomic levels (Parker *et*
65 *al*., 2015).

66 While specialist pathogens are invoked under JCH, generalist pathogens may
67 contribute to the maintenance of host community diversity via effective specialization
68 (Benítez *et al*., 2013). One form is host-specific impacts (Sarmiento *et al*., 2017),
69 meaning disease severity differs among hosts of a given pathogen. Host-specific impacts
70 could be an equalizing mechanism that maintains plant community diversity if the growth
71 and survival of infected seedlings varies among host species (Barrett *et al*., 2009);
72 particularly, if differential impacts reflect ecological trade-offs between allocation of
73 resources to defense, competitive ability, and/or tolerance of stressors. For example, for
74 tree species adapted to environments with high and/or consistent disease pressure, the
75 fitness benefits of physical and chemical resistance and/or tolerance traits may outweigh
76 the associated allocation costs of those traits, while the costs may outweigh the benefits
77 for tree species adapted to environments with low and/or inconsistent disease pressure
78 (Haak *et al*., 2012). The risk of pathogen attack, referred to here as pathogen pressure,
79 varies with environmental conditions that impact pathogen and plant fitness (Barrett *et*
80 *al*., 2009). High pathogen pressure has been observed in areas with low light, high soil
81 moisture, and persistent fog and dew (Augspurger & Kelly, 1984; Bradley *et al*., 2003;
82 Mordecai, 2012; Spear *et al*., 2015). Plants adapted to environments with high and/or
83 consistent pathogen pressure may be under selection for increased defenses, whereas
84 plants adapted to low and/or inconsistent pathogen pressure environments may be poorly
85 defended (Coley & Barone, 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 are often multi-host. Second, there is a phylogenetic signal to the host
91 ranges of these multi-host pathogens (i.e., pathogen sharing is positively correlated with
92 host relatedness). Third, tree species differ in their susceptibility to pathogens because the
93 cost-to-benefit ratio of defense traits differs among species (Strauss & Agrawal, 1999;

94 Endara & Coley, 2010). Fourth, plants adapted to environments with low and/or
95 inconsistent disease pressure are more susceptible to disease than those adapted to high
96 and/or consistent disease pressure.
97
98 **Materials and Methods**
99 *Identification of putative pathogens*
100 Potentially pathogenic fungi and oomycetes were isolated from 124 seedlings,
101 representing 26 tree species in 13 families, with disease on the leaf, stem, and/or root
102 (Supporting Information Fig. **S1;** Table **S1**). Symptomatic seedlings were collected from
103 seven forest sites in 2007, 2010-2012, and 2019 by: (1) opportunistic collection of
104 naturally occurring seedlings (n = 59); (2) collection of seedlings germinated in a
105 shadehouse and then transplanted to forest sites (n = 22), and (3) collection of seedlings
106 that germinated from surface-sterilized seeds planted directly in the forests (n = 43)
107 (Table **S2**). Most putative pathogens were isolated from leaves (52.13%), followed by
108 stems (37.44%) and roots (10.43%). Molecular identification was completed by
109 bidirectional Sanger sequencing of either the nuclear ribosomal Internal Transcribed
110 Spacer (ITS) region (149 isolates) or the ITS plus an adjacent portion of the large subunit
111 (ITS+LSU) (62 isolates). See Table **S2** and Spear (2017) for details.
112 All 211 unique isolates were identified and assigned to operational taxonomic
113 units (OTUs) based on 99% sequence similarity (minimum overlap of 40 bp) in
114 Sequencher v. 5.4.6. We used a stringent similarity threshold that splits rather than lumps,
115 while accounting for ≤1% sequencing error, because we make statements about host
116 specificity and to avoid masking strain-specific effects (Higgins *et al*., 2011). We
117 estimated the taxonomic placement of the fungal isolates by querying all 209 edited
118 sequences against the well-curated Full UNITE+INSD dataset for Fungi (v. 8.2, released
119 2020-02-04; Abarenkov *et al*., 2020) (detailed in Methods **S1**). For the two oomycetes,
120 we queried their sequences against GenBank (accessed 8-Oct-2020; Benson *et al*., 2012)
121 and the curated database *Phytophthora*-ID (v. 2.0, accessed 8-Oct-2020; Grünwald *et al.*,
122 2011). All assigned nomenclatures represent estimates as: (i) the ITS region is an
123 imprecise barcode for species discrimination for certain taxa; (ii) multi-locus phylogenies
124 are required for accuracy; and (iii) precision is limited by incomplete taxonomic and

125 geographic coverage of databases (Kang *et al*., 2010; Hofstetter *et al*., 2019; Lücking *et*
126 *al*., 2020). Additionally, sequences in the UNITE database are clustered into
127 hypothesized species-level groups (Species Hypotheses, SHs) based on sequence
128 similarity thresholds ranging from 97–100% (Kõljalg *et al*., 2013; Robbertse *et al*.,
129 2017), while we used 99% sequence similarity to designate OTUs because 97-98.5% is
130 too relaxed for species delimitation for some taxa (Lücking *et al*., 2020). Because our
131 grouping strategy often differed from that of the UNITE database, multiple, distinct
132 OTUs in our study sometimes share the same nomenclature based on the SH of the best-
133 matching UNITE reference sequence (e.g., OTUs Z and AAG are both annotated as
134 *Colletotrichum brevisporum*). Finally, for certain groups of fungi, UNITE SHs
135 erroneously include distinct species (Robbertse *et al*., 2017).
136 Sequences for the 90 previously published fungal isolates (Spear, 2017) were
137 submitted to the NCBI GenBank database (KY413686–KY413775). Sequences for the
138 121 isolates collected in 2019 will be submitted to GenBank (XXXXXXX–
139 XXXXXXX).
140 To characterize sampling efficacy and the relative abundance of OTUs, we
141 calculated the richness of observed OTUs and plotted the taxon accumulation curve
142 (vegan package; Oksanen *et al*., 2019) and rank-abundance distribution (BiodiversityR
143 package; Kindt & Coe, 2005) (n = 211 isolates from 124 seedlings). We defined OTUs
144 observed ≥10 times as abundant.
145 *Survey-based assessments of host associations and ranges of putative pathogens*
146 Our survey-based assessments of the host associations and ranges of putative
147 pathogens utilize our full dataset (n = 211 isolates, representing 66 OTUs, from 26 tree
148 species) and two subsets to ameliorate the influence of undersampled taxa. Subset A
149 excludes singleton OTUs (n = 178 isolates, representing 33 OTUs, from 24 tree species)
150 and B excludes singleton and doubleton OTUs and tree species (n = 144 isolates,
151 representing 22 OTUs, from 13 tree species). All three datasets include isolates collected
152 in all 5 yr.
153 We used four approaches to characterize host associations. We (1) summed and
154 plotted the number of observed host tree species (unweighted links) (full dataset), and (2)
155 calculated Blüthgen's weighted specialization index d' for each of the 33 non-singleton

156 OTUs (subset A; bipartite package; Blüthgen *et al*., 2006; Dormann *et al*., 2008). The d′
157 index is useful for identifying discriminatory host use because it accounts for interaction
158 strength (i.e., strong, frequent vs. weak, occasional interactions) and sampling intensity,
159 while the number of interacting partners does neither (Blüthgen *et al*., 2006; Dormann,
160 2011). The index estimates specialization by calculating the degree to which observed
161 differs from expected host use given partner availability (Blüthgen *et al*., 2006; Dormann,
162 2011). We could not adjust for the relative abundances of tree species when calculating d′
163 as the tree species are not uniformly distributed across the seven forest sites (Pyke *et al*.,
164 2001). Thus, we relied on the interaction matrix's marginal sums (column- and row-wise
165 totals) as a measure of partner availability, meaning that, for our data, a generalized
166 microbe infects tree species proportional to their sampling intensities, while a specialized
167 microbe infects tree species independent of their sampling intensities (Blüthgen *et al*.,
168 2006). We considered d' values ranging from 0–0.33, 0.34–0.67, and 0.68–1.00 to
169 represent low, moderate, and high specialization, respectively (Blüthgen *et al*., 2006;
170 Spear & Mordecai, 2018). We (3) calculated the observed number of shared OTUs (rich
171 package; Rossi, 2011) and, because that metric generally results in an underestimation of
172 similarity due to undetected species, we (4) estimated similarities in the communities of
173 putative pathogens shared between pairs of tree species via the inverse of the Chao
174 dissimilarity index (vegan package; Oksanen *et al*., 2019) (full dataset and subset B). The
175 Chao index accounts for the relative abundances of observed OTUs and unseen shared
176 OTUs, making it relatively robust to unequal sampling and diverse guilds with many rare
177 taxa (Chao *et al*., 2005).
178 To determine if there is a phylogenetic signal to the host ranges of the OTUs, (i)
179 we used mean pairwise phylogenetic distance (MPD) to determine if a given multi-host
180 OTU infects phylogenetically clustered or dispersed hosts, and (ii) we used two binomial
181 generalized linear models (GLMs) (logit link) to determine whether putative pathogen
182 community similarity is positively correlated with host relatedness. Specifically, for the
183 31 OTUs isolated from ≥2 tree species, we compared the observed MPD between tree
184 species infected by a given OTU to the MPD expected under a null model with random
185 host-OTU associations (full dataset; 999 randomizations of the host phylogeny tip labels;
186 picante and ape packages; Kembel *et al*., 2010; Paradis & Schliep, 2018). For the GLMs,

187 we used our full dataset and subset B, and we regressed and plotted the pairwise
188 estimated community similarities (1 - Chao index) against the pairwise phylogenetic
189 distances. The phylogeny of tree species was generated by Phylomatic
190 (phylodiversity.net/phylomatic/), which called the Zanne *et al*. (2014) supertree.
191 We tested for correlations between (i) the frequency with which a given fungal
192 OTU was isolated and the number of tree species from which it was isolated, and (ii)
193 sampling intensity (number of seedlings collected) and the number of OTUs isolated
194 from a given tree species using non-parametric Spearman's rank tests (full dataset; stats
195 package; R Core Team, 2020). One-tailed tests were used because we had directional,
196 literature-based *a priori* hypotheses (Quinn & Keough, 2002; Ruxton & Neuhäuser,
197 2010) that: (i) the most abundant fungal taxa would be multi-host, and (ii) observed
198 fungal richness associated with a given plant species would increase with increased
199 sampling (Ferrer & Gilbert, 2003; Spear & Mordecai, 2018).
200 *Experimental assessments of pathogenicity and host range*
201 In 2011 and 2012, inoculation experiments were used to assess the pathogenicity
202 and host specificities of 27 fungal isolates collected in 2010 and 2011 (Fig. **S2a**). We
203 tested the isolates, representing 18 OTUs, against seedlings of 35 tree species,
204 representing 20 families (Table **S1**). We could not test four isolates against the tree
205 species from which they were originally isolated because seeds were unavailable or
206 available in insufficient quantities (e.g., ERS033; Fig. **3**; Table **S1**). Related, recreating
207 the exact conditions under which disease developed for a specific fungus-plant
208 combination is challenging. Thus, some tree species were included as phytometers of
209 pathogenicity based on previously observed disease susceptibility (e.g., *Luehea*
210 *seemannii*, Augspurger & Wilkinson 2007; *Dalbergia retusa*, Spear, personal
211 observation). Tree species-by-isolate combinations were not fully reciprocal (285 of 945
212 possible combinations; 3,154 seedlings total) due to space, time, and seed availability
213 constraints. A given fungal isolate was tested against 3–28 tree species and 48% of
214 isolates evaluated were tested against seedlings of ≥10 tree species. Because an objective
215 of the experiments was to describe pathogen host range, once isolates displayed
216 pathogenicity, we prioritized testing those isolates against additional tree species (12-28
217 tree species, median 15) over expanding the testing of isolates failing to generate

218 significant disease. A given tree species was tested against 1–26 isolates, and 29% of tree
219 species were tested against ≥10 isolates.
220 Our inoculation technique simulated infested plant material in the soil to passively
221 inoculate the seedlings with the fungal isolates selected for screening (modified from
222 Augspurger & Wilkinson, 2007). To generate seedlings for the experiments, seeds were
223 collected from forests bordering the Panama Canal (May-Nov. 2011, May-June 2012),
224 surface-sterilized (sequential washes of 95% EtOH [10 s], 10% commercial bleach [2
225 min], 70% EtOH [2 min]), and germinated in flats of autoclave-sterilized (1 hour at 121º
226 C, twice) commercial soil (Fig. **S2b**). Seedlings were transplanted to individual pots
227 containing autoclaved commercial soil and either rice visibly colonized by one of the
228 isolates or inoculum-free, autoclave-sterilized rice (control/sham treatment) (Fig. **S2c-f**).
229 Depending on seed availability, 5–12 seedlings per species were included in each
230 treatment.
231 Inoculation experiments were conducted in Smithsonian Tropical Research
232 Institute shadehouses in Gamboa, Panama during the wet seasons of 2011 and 2012 (Fig.
233 **S2a**). The two screened-in shadehouses were covered on all sides with shadecloth and
234 their roofs were covered with plastic to minimize external contamination and to mimic
235 the forest understory. Shadehouse air temperatures (26.1 and 25.6ºC) did not significantly
236 differ from those recorded simultaneously in a nearby forest understory (25.4 and 25.3ºC;
237 Paton, 2019a). Relative humidity was significantly lower in the shadehouses (85.1 and
238 87.6%) than in the forest understory (95.6 and 96.7%; Paton, 2019b). Photosynthetically
239 active radiation (PAR) reaching the shadehouse seedlings was slightly higher (1.4 and
240 1.7% of full PAR) than observed in a nearby forest understory (1.3%; Brenes-Arguedas
241 *et al*., 2011). See Table **S3** for details. Light was somewhat variable within a given
242 shadehouse. To counteract this potentially confounding factor, seedlings were rotated
243 among the tables weekly.
244 Disease was documented every 3 d (for ≤5 wk) and categorized as: (i) mortality;
245 (ii) stem damage (Fig. **S2g-o**); (iii) wilting (Fig. **S2p,q**); and (iv) stunting (Fig. **S2r,s**). A
246 seedling was classified as morbid if it had any of the four symptoms. Wilting was
247 considered a disease symptom because the soil did not dry out (seedlings were hand-
248 watered every 3 d) and pathogens attacking roots can impede water uptake (Yadeta &

249 Thomma, 2013). Similarly, stunting can indicate pathogen-caused damage to roots and/or
250 the vascular system (Yadeta & Thomma, 2013). Additionally, stunting may occur
251 because energy and resources are allocated to defenses and/or tissue replacement rather
252 than growth (Huot *et al*., 2014). We did not document root damage and could not ascribe
253 foliar damage to a specific treatment because we observed a synchronous occurrence of
254 morphologically similar foliar necrosis across distinct treatments and the controls,
255 suggesting that the causative pathogen (*Xylaria* sp., unpublished data) blew into the
256 shadehouses. Thus, neither damage type was included in our analyses.
257 To compare the proportion of symptomatic seedlings in the inoculated versus
258 inoculum-free control treatments, we used generalized linear models (GLMs) with
259 binomial error distributions (probit link) and mean bias-reducing score adjustments
260 (brglm package; Firth, 1993; Kosmidis & Firth, 2009; Kosmidis, 2019). We used bias-
261 reduced GLMs because we observed complete and quasi-complete separation of data for
262 some isolate-by-tree species combinations (i.e., treatment perfectly, or near perfectly,
263 predicted the binary outcome; Albert & Anderson, 1984). The response variables were
264 presence/absence of (i) general morbidity and (ii) mortality. Each isolate-by-tree species
265 combination was considered individually for each response variable. We considered an
266 isolate pathogenic if, for at least one tree species, a significantly greater proportion of
267 inoculated seedlings were symptomatic than paired control seedlings (using $\alpha = 0.10$
268 because small sample sizes limited our power to detect treatment effects; Quinn &
269 Keough, 2002; Thiese *et al*., 2016). We tested for a correlation between the frequency
270 with which a fungal OTU was isolated and the number of tree species in which it caused
271 statistically significant disease using a non-parametric Spearman's rank test. We used a
272 one-tailed test because we had the *a priori* hypothesis that the most abundant fungal taxa
273 would be multi-host.
274 *Plant traits 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
278 relative to annual rainfall from published sources (Table **S1**). In all models, the response
279 variable was presence/absence of morbidity in inoculated seedlings, controlling for the

280 morbidity observed in paired control seedlings and considering all 27 isolates tested
281 (pathogens and non-pathogens alike). Because negative relative proportions are
282 biologically equivalent to zero (no disease caused), and for statistical purposes, we
283 converted all negative values to zero. We used two global models that contained (i) the
284 additive main effects of seed size (continuous) and shade tolerance (categorical), and
285 their interaction, or (ii) the additive main effects of seed size and distribution relative to
286 rainfall (categorical), and their interaction. We excluded seeds with average dry masses
287 >3,000 mg to improve model stability. For both categorical predictors, we limited our
288 comparisons to binary extremes (i.e., only shade-intolerant and -tolerant, and wet- and
289 dry-site tree species). We analyzed disease as a function of shade tolerance (26 tree
290 species, 179 observations: 42 for intolerant, 137 for tolerant spp.) and spatial distribution
291 (15 tree species, 154 observations: 108 for dry-site, 46 for wet-site spp.) separately due to
292 differences in the availability of trait data. For the two sets of models, automated model
293 selection and averaging was done based on the global models, the Akaike information
294 criterion with a correction for small sample sizes (AICc), and ΔAICc ≤ 2 (MuMIn
295 package; Bartoń, 2020).
296 All data were processed in R v. 4.0.0 (R Core Team, 2020). In addition to the
297 aforementioned packages, we used fossil (Vavrek, 2011), ggplot2 (Wickham, 2016),
298 otuSummary (Yang, 2020), plot.matrix (Klinke, 2019), plyr (Wickham, 2011), reshape2
299 (Wickham, 2007), and VennDiagram (Chen, 2018) for data formatting and figures.
300
301 **Results**
302 *Abundances and taxonomic assignments of putative pathogens*
303 The 211 isolates represent 66 operational taxonomic units (OTUs); however, the
304 taxon accumulation curve is non-asymptotic (Fig. **S3b**). Most of the OTUs are relatively
305 rare, 33 are singletons (Figs **1**, **S3a**; Table **S4**). Four OTUs are common (observed ≥10)
306 and comprised 35% of all isolates: A–*Colletotrichum xanthorrhoeae*, B–Nectriaceae, C–
307 *Mycoleptodiscus suttonii*, and F–*Diaporthe tulliensis* (Figs **1**, **S3a**; Table **S4**). The 209
308 fungal and two oomycete isolates represent five classes (Sordariomycetes,
309 Dothideomycetes, Agaricomycetes, Eurotiomycetes, Oomycetes) and 11 orders. Most
310 (81.5%) of the isolates could be assigned to 26 genera; *Colletotrichum*, *Diaporthe,* and

311 *Mycoleptodiscus* were most frequently isolated (Fig. **1**; Table **S4**). We observed overlap
312 in fungal OTUs among the tissues sampled: leaves, stems, and roots (Fig. **S4d**). While
313 sampling occurred over 5 yr, there was overlap in the tree species and forest sites
314 sampled, and the isolation and molecular techniques used (Table **S2**). Accordingly, we
315 observed overlap in fungal OTUs among the years, seedling collection methods, and
316 isolation media (Fig. **S4a-c**).
317 *Fungi associated with symptomatic seedlings are often host generalists*
318 The non-singleton fungi isolated from symptomatic seedlings are predominately
319 multi-host generalists with wide host ranges. Of the 33 non-singleton OTUs, 31 were
320 isolated from 2–9 tree species (median = 3) (Fig. **1**; Table **S4**). The number of tree
321 species from which a fungal OTU was isolated is positively correlated with isolation
322 frequency (Spearman's rank correlation: rho = 0.96, $P < 0.001$; blue points Fig. **S5**). A–*C.*
323 *xanthorrhoeae* was isolated with the greatest frequency (35 times) and from the greatest
324 number of tree species (nine in seven families) (Fig. **1**; Table **S4**). Only two of the non-
325 singleton OTUs (T–*Macrophomina*, X–*Ceratobasidium*) were not multi-host, but each
326 was isolated only twice, and from the best-sampled tree species, suggesting their
327 observed host ranges are a sampling artifact (Fig. **1**; Tables **S1, S4**). Based on the
328 specialization index d', 57.6% of 33 the non-singleton OTUs, including A–*C.*
329 *xanthorrhoeae* and B–Nectriaceae, exhibit low host-specialization and 42.4%, including
330 C–*M. suttonii* and F–*D. tulliensis*, exhibit moderate host-specialization at the species
331 level (Table **S4**). We observed phylogenetically overdispersed host use for 19 of the 31
332 multi-host OTUs, significant for B–Nectriaceae and AV–*Cylindrocladiella variabilis*,
333 and marginally significant for M–*Lasiodiplodia gonubiensis* and AU–*Beltrania*
334 *pseudorhombica* (Fig. **1**; Table **S4**). Conversely, 12 OTUs exhibited phylogenetically
335 clustered host use, marginally significant for R–*Diaporthe fraxini-angustifoliae* and Q–
336 *Diaporthe* cf. *columnaris* (Fig. **1**; Table S**4**). Both OTUs were solely isolated from
337 *Anacardium excelsum* (Anacardiaceae, Sapindales) and *Protium panamense*
338 (Burseraceae, Sapindales), providing tentative evidence of a phylogenetic signal to host
339 range.

340 *Tree species are infected by various microbes, and their microbial communities overlap*

341 Multiple OTUs were isolated for all tree species with >2 seedlings collected (Fig.
342 **1**; Table **S1**). Predictably, the number of OTUs isolated from a given tree species is
343 strongly positively correlated with sampling intensity (Spearman's rank correlation: rho =
344 0.95, $P < 0.001$; Fig. **1**). The greatest richness of fungi (24 OTUs) was recovered from
345 the best-sampled tree species (41 isolates), *A. excelsum* (Table **S1**).

346 We observed overlap among the microbial communities of distantly related tree
347 species (Fig. **1**). Based on subset B, only tree species and OTUs observed ≥3 times, the
348 estimated average similarity (1 - Chao index) between the microbial communities of pairs
349 of tree species is 0.21 (median = 0.15; Table **S5**). The GLMs suggest microbial
350 community similarity is unrelated to phylogenetic distance between tree species (Fig. **2**;
351 full dataset: Est. ± SE = 0.002 ± 0.003, *z*-value = 0.60, $P = 0.55$; subset B: Est. ± SE =
352 0.002 ± 0.004, *z*-value = 0.36, $P = 0.72$).

353 *Identities and impacts of confirmed pathogens*

354 We experimentally confirmed the pathogenicity of 10 of the 27 fungal isolates
355 tested (Fig. **3**). The pathogenic isolates belong to six OTUs, all in the phylum
356 Ascomycota, and were isolated from stems, roots, and leaves of seedlings of seven tree
357 species in seven families (Fig. **3**; Table **1**). Based on morbidity (i.e., any of the four
358 symptoms: wilting, stunting, stem damage, or mortality), significant pathogenicity was
359 observed for 20 of the 285 tree species-by-isolate combinations (Fig. **3**; Table **1**). For 13
360 of these combinations, infection led to mortality (Table **1**). Multiple symptoms were often
361 observed for a single tree species-by-pathogen combination (e.g., *Annona glabra*
362 seedlings suffered stem damage, wilt, and mortality when inoculated with isolate
363 ERS029). Seven of the pathogenic isolates were tested against the tree species from
364 which they were originally isolated, but for those tree species-by-isolate combinations
365 neither morbidity nor mortality significantly differed between the inoculated and control
366 seedlings (Fig. **3**).

367 *Fungi with confirmed pathogenicity are multi-host*

368 We show that fungal pathogens can attack tree species belonging to
369 phylogenetically dispersed families. Five of the 10 pathogenic isolates successfully
370 attacked multiple tree species (Table **1**). The 10 pathogenic isolates generated significant

371 disease in 6–31% of the tree species against which they were tested (median = 10%). At
372 the high end, ERS029 (C–*Mycoleptodiscus suttonii*) caused significant disease in 31% of
373 the 13 tree species it was tested against, and the four susceptible tree species belong to
374 three orders (Fig. **3**; Tables **1**, **S1**).
375 Three of the OTUs were represented by multiple isolates: B–Nectriaceae, G–
376 *Clonostachys rosea*, and C–*M. suttonii*. The two isolates of B–Nectriaceae were
377 recovered from *Trichilia tuberculata* and *Calophyllum longifolium*, and combined were
378 pathogenic on six of the 28 tree species tested (Fig. **3**; Table **1**). The three isolates of G–
379 *C. rosea* were isolated from *Virola surinamensis* and *Protium tenuifolium*, and combined
380 caused significant disease on four tree species (Fig. **3**; Table **1**). The two isolates of C–*M.*
381 *suttonii* were isolated from *V. surinamensis* and *Randia armata*, and combined caused
382 significant disease on five species (Fig. **3**; Table **1**). While the tree species-by-isolate
383 combinations were not fully reciprocal, limiting conclusions, we observed possible strain-
384 specific effects (e.g., B–Nectriaceae: ERS034 caused significant disease on *Guapira*
385 *standleyana* while ERS036 did not; Fig. **3**). Finally, there is a marginally significant
386 positive association between the frequency with which a fungal OTU was isolated and
387 the number of tree species in which it caused statistically significant ($\alpha = 0.10$) disease
388 (Spearman's rank correlation rho = 0.72, $P = 0.053$; green points Fig. **S5**).
389 *Tree species are differentially impacted by pathogens*
390 We documented interspecific differences in disease susceptibility and impact
391 among tree species. Most tree species tested were seemingly resistant to, or tolerant of,
392 all isolates tested, suffering minimal to no fitness impacts despite soil inoculation (Fig.
393 **3**). For 13 of the 35 tree species tested, at least one of the 27 fungal isolates tested caused
394 significant disease (Table **1**). Five tree species experienced significant disease from 2–3
395 of the isolates tested (Table **1**). Those five species experienced significant disease from
396 10–50% (median = 15%) of the isolates against which they were tested (Fig. **3**).
397 The 13 susceptible tree species differed in their tolerance of attack. For most of
398 the susceptible tree species, pathogen attack did not consistently lead to death, the impact
399 from infection depended on the pathogenic isolate with which it was inoculated. For
400 example, while *Castilla elastica* seedlings suffered significant disease when inoculated
401 with two isolates (ERS036, ERS040), they only suffered significant mortality when

402 inoculated with ERS040 (AAF–Nectriaceae) (Table **1**). Furthermore, for those tree
403 species that were susceptible to the same multi-host pathogen, the impact from attack
404 differed among species. For example, isolate ERS036 (B–Nectriaceae) caused significant
405 morbidity on *C. elastica, Cochlospermum vitifolium,* and *Dalbergia retusa*, but only
406 caused significant mortality on *C. vitifolium* (Table **1**).
407 *Plant traits and disease susceptibility*
408 When considering all 27 fungal isolates tested (pathogens and non-pathogens), the
409 proportion of morbid seedlings was influenced by a tree species' seed size, spatial
410 distribution relative to annual rainfall, and shade tolerance (Fig. **4a-d**; Tables **S6**, **S7**).
411 Shade-intolerant tree species were more likely to exhibit disease than shade-tolerant
412 species ($P = 0.003$; Fig. **4a,b**; Table **S6**). For both groups, the probability of disease
413 decreases with increasing seed size, but to a greater extent for shade-intolerant species (P
414 $= 0.014$; Fig. **4a,b**; Table **S6**). Tree species restricted to, or more abundant in, drier sites
415 (dry-site) were more likely to exhibit disease than those restricted to, or more abundant
416 in, wetter sites (wet-site) ($P = 0.042$; Fig. **4c,d**; Table **S7**). While we found no correlation
417 between seed size and disease risk for wet-site species, disease risk decreases with
418 increasing seed size for dry-site species (Fig. **4c,d**; Table **S7**).
419
420 **Discussion**
421 Pathogens are believed to shape the distributions and abundances of their hosts,
422 effects often ascribed to host-specialized pathogens (Comita *et al*., 2014). However, there
423 is increasing evidence that multi-host pathogens are prevalent in natural plant
424 communities (Augspurger & Wilkinson, 2007; Gallery *et al*., 2007; Gilbert & Webb,
425 2007; Kluger *et al*., 2008; Hersh *et al*., 2012; Schweizer *et al*., 2013; Sarmiento *et al*.,
426 2017; Spear & Mordecai, 2018). These multi-host pathogens may contribute to the
427 maintenance of these communities via host-specific impacts (disease severity differs
428 among a pathogen's hosts), a form of effective specialization (Augspurger & Wilkinson
429 2007; Benítez *et al*., 2013; Sarmiento *et al*., 2017). Using a multifaceted approach, we
430 identified fungal pathogens attacking seedlings in the forests of Panama, and show that
431 they can attack phylogenetically dispersed hosts. Furthermore, we demonstrate that tree
432 species differ in their susceptibility to pathogens, with trees adapted to environments with

433 low and/or inconsistent disease pressure being more susceptible to disease. Collectively,
434 these results suggest that generalist pathogens contribute to the maintenance of local and
435 regional plant diversity.

436 *Pathogens and their impacts*

437 We observed 66 OTUs, but only four were observed >10 times (Figs **1, S3a**; Table
438 **S4**). The relative rarity of the OTUs, coupled with a non-asymptomatic taxon
439 accumulation curve, indicate incomplete sampling and a diverse community of seedling-
440 associated fungi (Fig. **S3**). The most frequently isolated fungal genera (e.g.,
441 *Colletotrichum*; Fig. **1**) and those with experimentally confirmed pathogenicity (e.g.,
442 *Clonostachys*; Table **1**) include previously identified phytopathogens (Nguyen *et al*.,
443 2016).

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

451 Seventeen of the 27 fungal isolates tested appear to be nonpathogenic; however, some
452 categorized as nonpathogenic may be pathogenic under optimal conditions. These
453 isolates may not have caused disease in our experiments because we failed to: (1) test
454 them against a susceptible host (each isolate was only tested against 3–28 tree species, a
455 fraction of the species locally present); (2) test them against susceptible individuals of a
456 host species, attributable to intraspecific genotypic variation in resistance; or (3) replicate
457 the environmental conditions necessary for disease development (Barrett *et al.*, 2009;
458 Álvarez-Loayza *et al*., 2011). Additionally, while many of the fungi tested infect multiple
459 tissue types (Table **1**; Fig. **S4d**), (4) some isolates deemed nonpathogenic based on root
460 inoculation may only colonize leaves or stems. Experimental challenges two through four
461 may also explain why the 10 pathogenic isolates did not generate significant disease on
462 the host species from which they were originally isolated (Fig. **3**).

463 *Pathogens can infect phylogenetically dispersed hosts*

464 The host associations of the fungi isolated from symptomatic seedlings, in
465 combination with the results of our inoculation experiments, provide support for our
466 hypothesis that seedling pathogens in these tropical forests are often multi-host (Figs **1, 3**;
467 Table **1**). We speculate that our study underestimates the true breadth of the host ranges
468 of the observed multi-host fungi, as we sampled and tested a small fraction of the >824
469 tree species present locally (Pyke *et al*., 2001). We cannot assess host specialization for
470 the 33 singleton OTUs and some may be strict specialists. Moreover, host-specialized
471 fungi may have gone unobserved due to our culture-based approach, which limited
472 sampling and excluded biotrophic fungi, generally exhibiting greater specificity than
473 necrotrophs (Agrios, 2005). That said, multi-host pathogens of seedlings, leaves, and
474 seeds were also observed in previous Panama-based studies (Augspurger & Wilkinson,
475 2007; Gilbert & Webb, 2007; Schweizer *et al*., 2013; Sarmiento *et al*., 2017).

476 In general, a given fungal OTU was isolated from heterofamilial tree species, host use
477 was phylogenetically overdispersed, and pairwise similarities in community composition
478 of seedling-associated fungi were unrelated to pairwise phylogenetic distances between
479 tree species (Figs **1, 2**; Tables **S4**, **S5**). Together these results suggest that, contrary to our
480 second hypothesis, there is no phylogenetic signal to host range for these multi-host
481 seedling pathogens. The absence of a significant phylogenetic signal is consistent with
482 one Panama-based study of foliar pathogens (Schweizer *et al*., 2013), but conflicts with
483 other studies in temperate and tropical plant communities (Gilbert & Webb, 2007; Gilbert
484 *et al*., 2012; Parker *et al*., 2015; Chen *et al*., 2019). Our study did not include as many
485 congeneric or confamilial host pairs as Gilbert and Webb (2007) or Gilbert *et al*. (2012),
486 potentially hindering our ability to detect a signal. Alternatively, pathogen host use may
487 track phylogenetically labile plant defenses (Kursar *et al*., 2009) rather than
488 phylogenetically conserved defenses (Fine *et al*., 2006). Parallel evolution of similar
489 defenses in unrelated lineages would explain why the pathogens in this and other studies
490 can infect many distantly related tree species.

491 The positive relationships between OTU isolation frequency and the number of hosts
492 observed during our survey and inoculation experiments (Fig. **S5**) suggest that host
493 generalists are abundant members of the pathogen community and that, if sampling was

494 expanded, a given fungal OTU would be detected in additional tree species. Many
495 seedling pathogens, including the soil-borne fungi and oomycetes causing seedling
496 damping-off, are passively dispersed (e.g., by splashing rain), with limited dispersal
497 distances (Madden, 1997). Thus, the ability to infect a wide range of hosts, particularly
498 spatially aggregated hosts, would increase a pathogen's likelihood of persistence. For
499 pathogens with limited dispersal, host use may be more tightly linked with plant habitat
500 associations than phylogenetic relatedness, making them more likely to infect tree species
501 that commonly co-occur spatially and temporally (Roy, 2001). Furthermore, just as
502 selection should favor multi-host pathogens in species-rich assemblages, selection should
503 favor true generalists, whose host ranges are decoupled from plant relatedness, in plant
504 communities that are phylogenetically overdispersed on the small spatial scale relevant to
505 dispersal-limited pathogens (Swenson *et al*., 2007; Swenson *et al*., 2012; Pearse *et al*.,
506 2013).

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

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

516 Disease progression among susceptible hosts is mediated by multiple factors,
517 including defense traits. Based on our inoculation experiments, tree species were
518 differentially impacted by shared pathogens (Fig. **3**; Table **1**), in support of our third
519 hypothesis and consistent with the host-specific impacts of multi-host pathogens
520 previously observed (Davidson *et al*., 2000; Augspurger & Wilkinson, 2007; Hersh *et al*.,
521 2012; Sarmiento *et al*., 2017). Most of the tree species we tested were seemingly resistant
522 to the 10 pathogenic isolates (Fig. **3**), but that does not preclude their susceptibility to
523 numerous untested fungi. Moreover, these tree species may actually be susceptible to
524 infection by the pathogenic isolates in our study, but their resistance mechanisms prevent

525 extensive pathogen colonization and symptom development. In which case, these
526 resistant tree species may act as reservoir hosts that facilitate the persistence of pathogens
527 within the community (Haas *et al*., 2011).

528 *Plant traits and disease susceptibility*

529 Disease pressure varies spatially, particularly across environmental gradients
530 (Augspurger, 1984a; Brenes-Arguedas *et al*., 2009; Defossez *et al*., 2011; Haak *et al*.,
531 2012; Hersh *et al*., 2012; Spear *et al*., 2015). For plants adapted to areas with low and/or
532 inconsistent disease pressure, such as areas with higher light, lower humidity, and
533 inconsistent precipitation (Augspurger, 1984a; Spear *et al*., 2015), the allocation costs of
534 physical and chemical defenses may outweigh the benefits and vice versa for plants
535 adapted to areas with high and/or consistent disease pressure (Coley & Barone, 1996;
536 Strauss & Agrawal, 1999; Endara & Coley, 2010). Consistent with variable selective
537 pressures, and previous field and greenhouse studies (Augspurger, 1984b; Augspurger &
538 Kelly, 1984; McCarthy-Neumann & Kobe, 2008), seedlings of shade-intolerant tree
539 species and tree species restricted to, or more abundant in, drier forests (dry-site species)
540 were more likely to suffer disease than seedlings of shade-tolerant and wet-site tree
541 species in our inoculation experiments (Fig. **4**; Tables **S6**, **S7**). Engelbrecht *et al*. (2007)
542 found no correlation between a species' light requirements and its occurrence in drier
543 versus wetter sites, but evergreen trees are more common in wetter sites, while deciduous
544 trees dominate drier sites (Santiago *et al*., 2004). Shade-tolerant and evergreen tree
545 species, both with long-lived leaves, tend to invest more in pest defenses than shade-
546 intolerant and deciduous species (Coley & Barone, 1996; Santiago *et al*., 2004).

547 For shade-tolerant, shade-intolerant, and dry-site tree species, the probability of
548 disease decreases with increasing seed size (Fig. **4**). This is perhaps unsurprising as large-
549 seeded species can invest more in defenses and better compensate for lost tissue
550 (Armstrong & Westoby, 1993; Green & Juniper, 2004). Our results are inconsistent with
551 two Panama-based studies (Augspurger, 1984a; Augspurger & Kelly, 1984), which found
552 no correlation between seed size and pathogen susceptibility, possibly because they only
553 included wind-dispersed species while our analyses included wind- and vertebrate-
554 dispersed species (average seed dry masses: 1.9 - 686 versus 3 - 2334 mg).

555 *Consequences for plant communities*

556 Neither disturbance nor microhabitat specialization are considered sufficient to
557 maintain the hyperdiversity of tropical forests (Leigh *et al*., 2004). While host-specialized
558 pests are commonly touted as the drivers of diversity maintenance (Leigh *et al*., 2004;
559 Comita *et al*., 2014), we show that generalist pathogens are frequently responsible for
560 seedling death and disease. The host-specific impacts of multi-host pathogens and
561 differences among tree species in pathogen susceptibility observed by this and other
562 studies (Augspurger & Wilkinson, 2007; Hersh *et al*., 2012; Spear *et al*., 2015) suggest
563 that generalized pathogens may also influence plant community composition by unevenly
564 affecting seedling recruitment. First, variability in host competence may maintain plant
565 community diversity by creating a dilution effect (Ostfeld & Keesing, 2000). Overall
566 disease risk should be lowered if the most competent host(s) is/are rare within the
567 community because pathogen transmission would be slowed between individuals of
568 susceptible species. Accordingly, the presence of less competent hosts should protect
569 poorly defended host species from pathogen-caused local extinction. Second, generalist
570 pathogens with host-specific impacts may alter direct and indirect competition among co-
571 occurring species (Alexander & Holt, 1998; Barrett *et al*., 2009; Mordecai, 2011). Host-
572 generalized pathogens could promote coexistence if a competition-defense tradeoff exists
573 among plant species and superior competitors are more susceptible (Mordecai, 2011).
574 Finally, interspecific variation in host susceptibility to generalist pathogens may maintain
575 diversity because disease-sensitive trees (e.g., shade-intolerant and dry-site spp.) are
576 excluded from disease-prone habitats (e.g., wetter, aseasonal forests and low-light
577 understories). By reinforcing resource partitioning and limiting the geographic
578 distributions of species, generalist pathogens contribute to the spatial turnover of plant
579 species and the maintenance of local and regional forest diversity.

580

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597 ERS designed and conducted the research, analyzed the data, and wrote the first draft of
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600

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Supporting Information

Fig. S1 Examples of disease in the forests of Panama.

Fig. S2 Details of shadehouse-based inoculation experiments.

Fig. S3 Rank abundance plot and OTU accumulation curve.

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

890 **Fig. S5** Correlation between OTU host range and isolation frequency.
891
892 **Table S1** Taxonomic assignments, traits, sampling effort, and observed OTUs for tree
893 species evaluated in our survey and experiments.
894
895 **Table S2** Methodological details pertaining to the multi-year collection of symptomatic
896 seedlings, and microbial isolation and sequencing.
897
898 **Table S3** Average light levels, air temperatures, and relative humidities of the
899 shadehouses used for the inoculation experiments versus ambient conditions.
900
901 **Table S4** Estimated taxonomic placement, isolation frequency, number of observed
902 hosts, estimated host specialization, and phylogenetic pattern of host use of the OTUs.
903
904 **Table S5** Overlap in seedling-associated OTUs among tree species.
905
906 **Table S6** Results of the beta-binomial generalized linear regression with the proportion
907 of diseased seedlings as a function of seed size and shade tolerance.
908
909 **Table S7** Average estimates based on the best-ranked beta-binomial generalized linear
910 regressions with the proportion of diseased seedlings as a function of seed size and spatial
911 distribution relative to annual rainfall.
912
913 **Methods S1** Methods used to estimate the taxonomic placements of the 66 OTUs and
914 assign nomenclature.

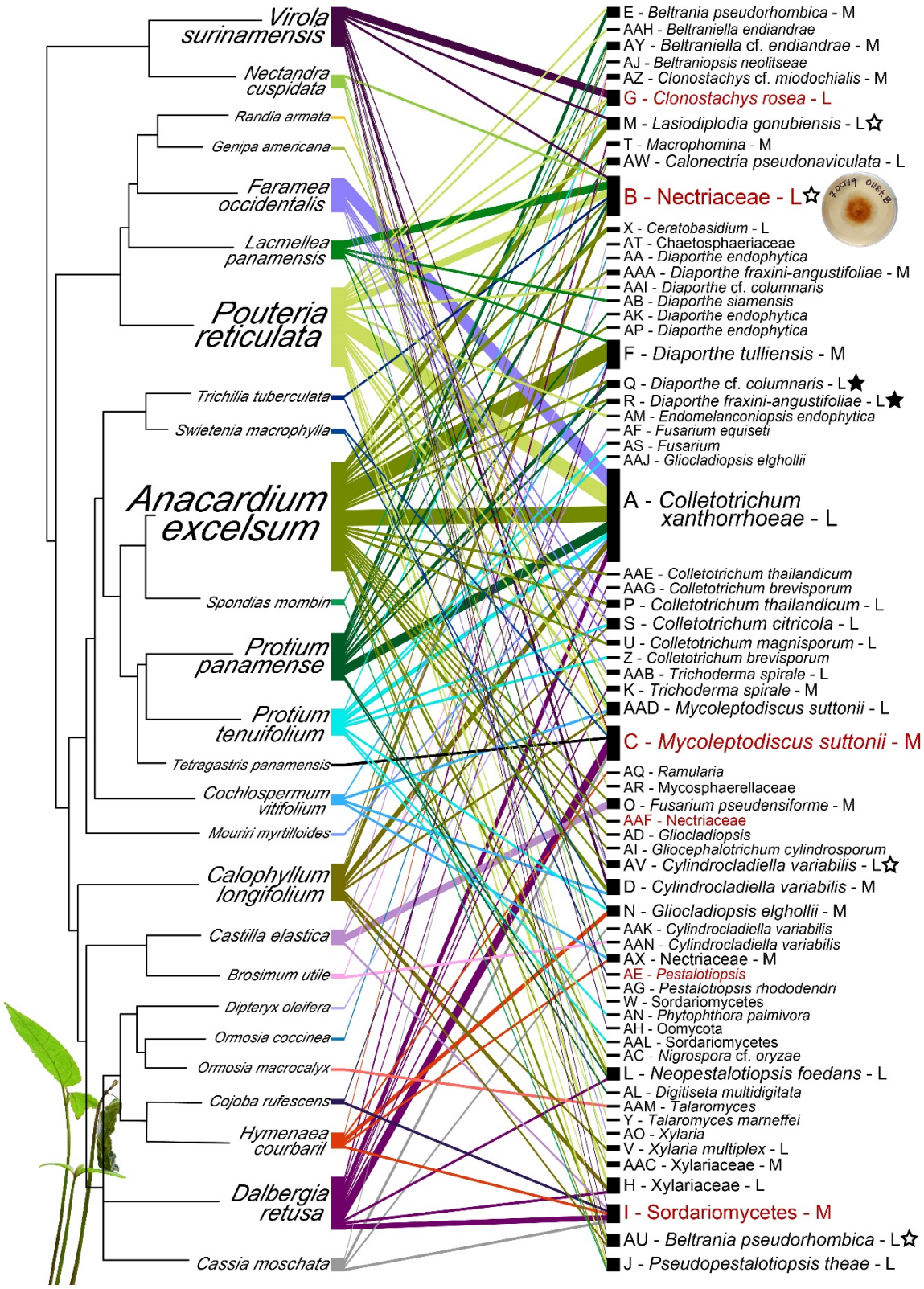
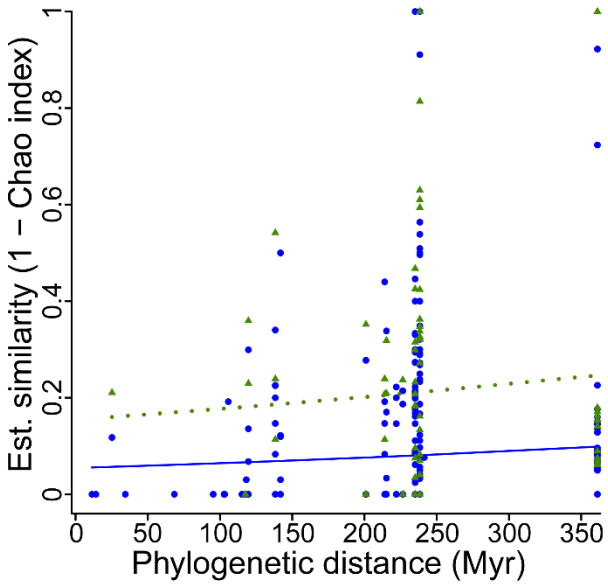


Figure 1. Host associations of the fungi and oomycetes isolated from symptomatic seedlings (full dataset: n = 211 isolates). The 26 host tree species (left) are arranged

918 according to their phylogenetic relationships. The estimated taxonomic placement of each
919 of the 66 microbial OTUs (right) is preceded by a unique letter identifier. Because our
920 OTU grouping strategy (99% sequence similarity) often differed from that of the UNITE
921 database, distinct OTUs in our study sometimes share the same nomenclature based on
922 the species hypothesis of the best-matching reference sequence in UNITE (e.g., Z and
923 AAG). For the non-singleton OTUs, nomenclature is followed by an L or M, indicating
924 low or moderate estimated specialization (d'). The width of each line represents the
925 number of times a given OTU was isolated from seedlings of a given tree species. For
926 both tree species and OTUs, font size is scaled to the number of interactions. OTUs with
927 significant and marginally significant phylogenetic patterns of host use (Table S4) are
928 marked with stars, solid indicating clustered and open indicating overdispersed. The six
929 fungal OTUs with members that exhibited statistically significant ($\alpha = 0.10$)
930 pathogenicity during the inoculation experiments are listed in red.



931

932 **Figure 2.** Estimated pairwise similarities of OTU communities are unrelated to pairwise
933 phylogenetic distances (time of independent evolution in Myr). The blue circles and solid
934 line (GLM: Est. ± SE = 0.002 ± 0.003, *z*-value = 0.60, *P* = 0.55) are based on the full
935 dataset, while the green triangles and dotted line (GLM: Est. ± SE = 0.002 ± 0.004, *z*-
936 value = 0.36, *P* = 0.72) are based on subset B, only tree species and OTUs observed ≥3
937 times.

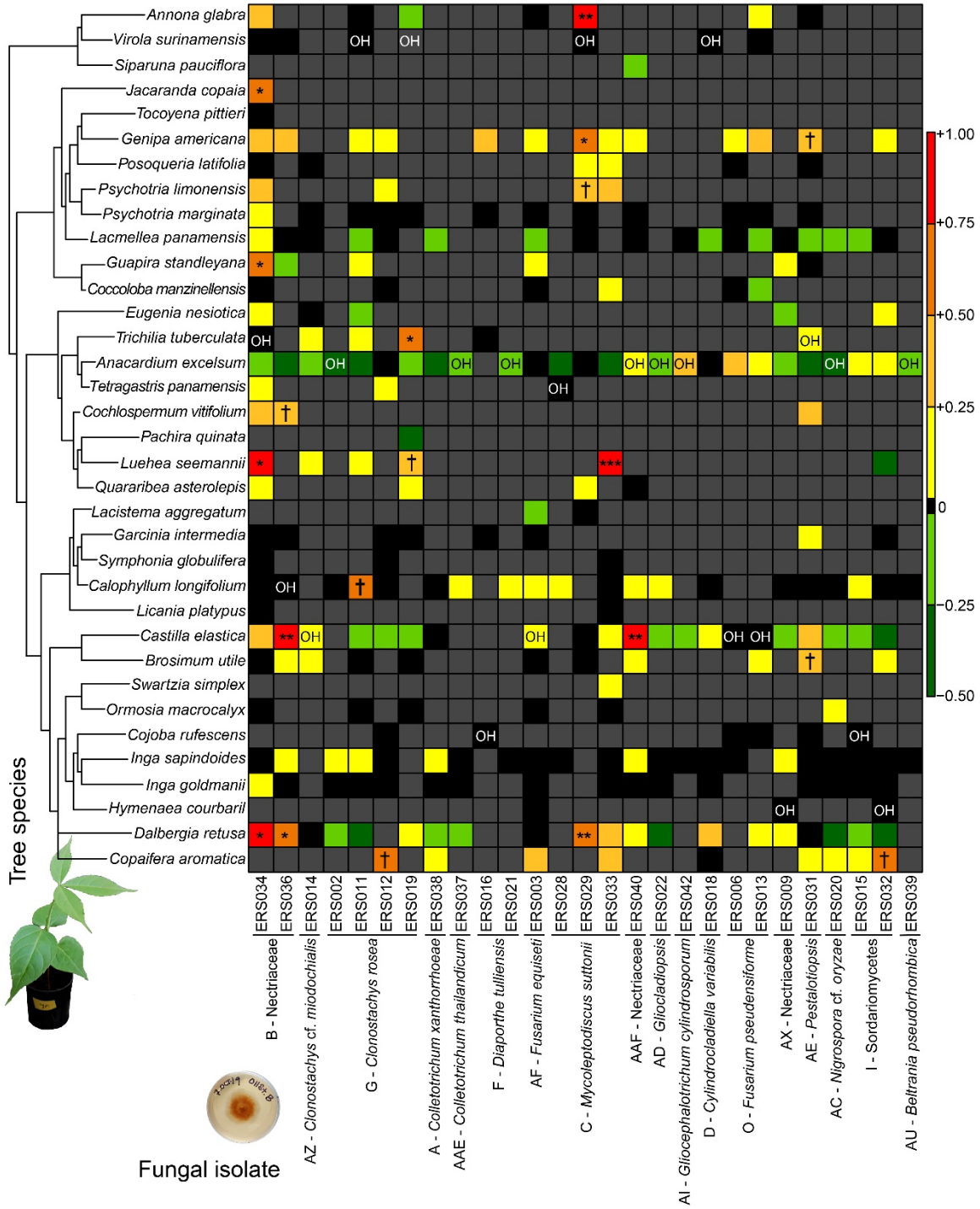


Figure 3. Pathogenicity and host ranges of the 27 fungal isolates tested, and susceptibility of the 35 tree species tested, based on seedling morbidity. Fungal isolates are grouped according to their OTU (horizontal axis). Tree species are arranged based on their phylogenetic relationships (vertical axis). OH (original host) denotes the tree species from which an isolate originated. Tree species-by-isolate combinations for which

944 evidence of pathogenicity was observed (proportion of inoculated seedlings with
945 symptoms > proportion in paired control group) are depicted in shades of yellow (pale for
946 minimal) to red (dark for substantial). Combinations for which there was no evidence of
947 pathogenicity (proportion of inoculated seedlings with symptoms ≤ proportion in paired
948 control group) are depicted in black and shades of green. Gray cells represent an untested
949 combination. For 20 of the 285 combinations tested, we observed significant
950 pathogenicity (***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; †, $P \leq 0.10$; Table **1**).

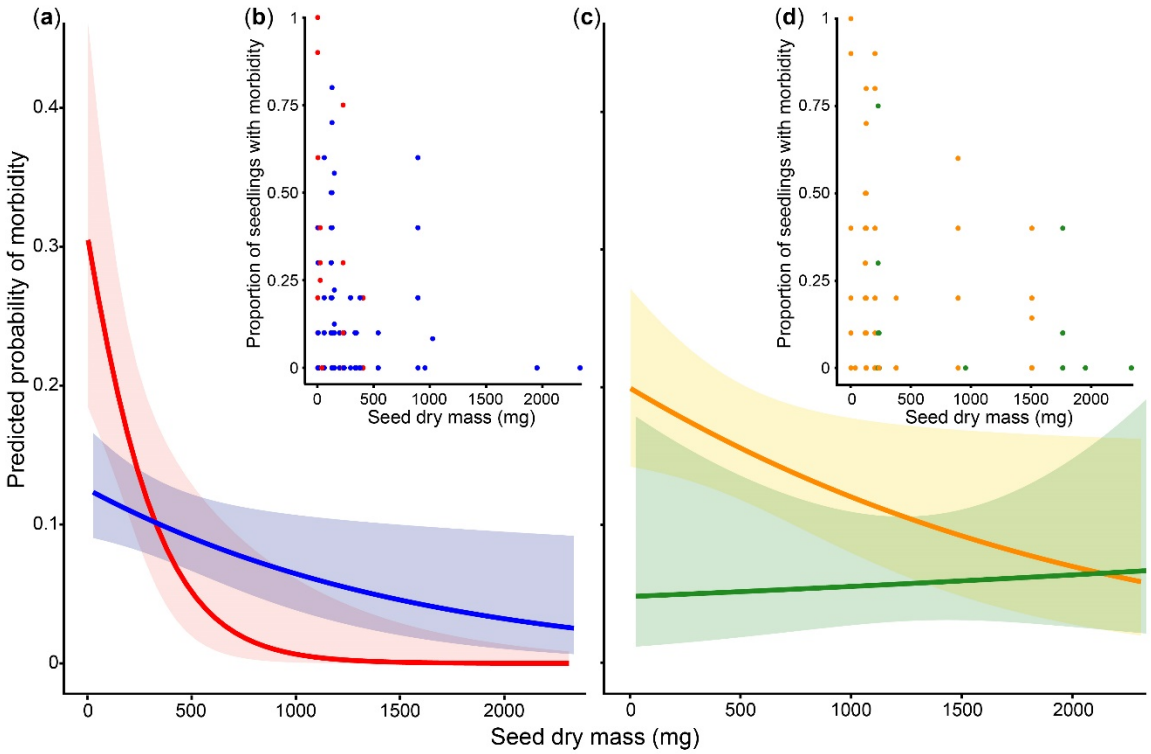


Figure 4. Relationships between disease susceptibility and seed size and shade tolerance (a,b), and seed size and spatial distribution relative to annual rainfall (c,d). Shade-intolerant tree species (red) are more likely to experience disease than shade-tolerant species (blue) ($P = 0.003$), and for 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**). The shaded areas represent 95% confidence intervals.

960 **Table 1.** The 20 fungal isolate-by-tree species combinations for which significant pathogenicity was observed based on the
961 presence/absence of (i) general morbidity and (ii) mortality in the inoculated versus control seedlings, analyzed with bias-reduced
962 generalized linear models assuming binomial error distributions (probit link) and using α = 0.10 because small sample sizes limited
963 our power to detect treatment effects.
964

OTU–Estimated taxonomic placement (isolate, origin tree sp. & tissue, no. of tree spp. tested)	**Susceptible tree species**	***n***		**Disease metric**	**Prop. rel. to control**	**Est (SE)**	**z**	***P***
		Cntrl	**Inoc**					
B–Nectriaceae sp. (ERS034, TT root, 28)	*D. retusa*[1]	5	5	morbidity	0.8	2.17 (1.04)	2.08	0.038
				mortality	1	2.93 (1.2)	2.45	0.014
	G. standleyana	10	10	morbidity	0.6	1.61 (0.65)	2.47	0.014
	J. copaia[1]	10	10	morbidity	0.6	2 (0.83)	2.41	0.016
				mortality	0.6	2 (0.83)	2.41	0.016
	L. seemannii[1]	5	5	morbidity	1	2.93 (1.2)	2.45	0.014
				mortality	1	2.93 (1.2)	2.45	0.014
B–Nectriaceae sp. (ERS036, CL stem[2], 13)	*C. elastica*	10	10	morbidity[8]	0.8	2.25 (0.71)	3.17	0.002
	C. vitifolium	10	10	morbidity[9]	0.4	1.13 (0.64)	1.76	0.079
				mortality	0.4	1.13 (0.64)	1.76	0.079
	D. retusa	10	10	morbidity[10]	0.5	1.36 (0.64)	2.12	0.034

G–*Clonostachys rosea*, Bionectriaceae (ERS011, VS stem[3], 17)	*C. longifolium*	5	5	morbidity	0.6	1.68 (1.02)	1.66	0.098
G–*C. rosea* (ERS012, PT leaf[4], 15)	*C. aromatica*	5	5	morbidity[11,12]	0.6	1.68 (1.02)	1.66	0.098
G–*C. rosea* (ERS019, VS root, 12)	*L. seemannii*	10	10	morbidity	0.4	1.13 (0.64)	1.76	0.079
				mortality	0.4	1.13 (0.64)	1.76	0.079
	T. tuberculata	8	9	morbidity	0.56	1.8 (0.87)	2.07	0.038
				mortality	0.44	1.54 (0.87)	1.78	0.076
C–*Mycoleptodiscus suttoni*, Muyocopronaceae (ERS029, VS stem[5], 13)	*A. glabra*	12	12	morbidity	0.75	2.46 (0.8)	3.08	0.002
				mortality	0.58	2.03 (0.79)	2.58	0.010
	D. retusa	10	10	morbidity	0.7	1.89 (0.67)	2.83	0.005
				mortality	0.8	2.53 (0.85)	2.98	0.003
	G. americana	10	10	morbidity	0.5	1.36 (0.64)	2.12	0.034
	P. limonensis	10	10	morbidity	0.4	1.53 (0.83)	1.84	0.065
				mortality	0.4	1.53 (0.83)	1.84	0.065
C–*M. suttoni* (ERS033, RA stem[6], 16)	*L. seemannii*	10	10	morbidity[13]	0.9	2.89 (0.88)	3.27	0.001
				mortality	0.9	2.89 (0.88)	3.27	0.001
AAF–Nectriaceae sp. (ERS040, ANE stem[7], 12)	*C. elastica*	10	9	morbidity[14]	0.9	2.84 (0.9)	3.18	0.002
				mortality	0.89	2.83 (0.89)	3.18	0.002
AE–*Pestalotiopsis* sp., Sporocadaceae (ERS031, TT root, 18)	*B. utile*	10	10	morbidity	0.4	1.53 (0.83)	1.84	0.065
				mortality	0.4	1.53 (0.83)	1.84	0.065
	G. americana	10	10	morbidity	0.4	1.13 (0.64)	1.76	0.079
				mortality	0.4	1.13 (0.64)	1.76	0.079

I–Sordariomycetes sp. (ERS032, HC stem, 14)	*C. aromatica*	5	5	morbidity	0.6	1.68 (1.02)	1.66	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.