



**Manchester
Metropolitan
University**

Kennedy, John Paul, Antwis, Rachael E, Preziosi, Richard ORCID logoORCID: <https://orcid.org/0000-0003-0468-6655> and Rowntree, Jennifer ORCID logoORCID: <https://orcid.org/0000-0001-8249-8057> (2021) Evidence for the genetic similarity rule at an expanding mangrove range limit. *American Journal of Botany*, 108. pp. 1331-1342. ISSN 0002-9122

Downloaded from: <https://e-space.mmu.ac.uk/628339/>

Version: Accepted Version

Publisher: Botanical Society of America

DOI: <https://doi.org/10.1002/ajb2.1715>

Please cite the published version

<https://e-space.mmu.ac.uk>

1 **Evidence for the genetic similarity rule at an expanding mangrove range limit**

2 John Paul Kennedy^{1*}, Rachael E. Antwis², Richard F. Preziosi¹, and Jennifer K. Rowntree¹

3 ¹Ecology and Environment Research Centre, Department of Natural Sciences, Faculty of Science
4 and Engineering, Manchester Metropolitan University, Manchester, UK

5 ²School of Science, Engineering and Environment, University of Salford, Salford, UK

6 *Corresponding author.

7 E-mail address: john.p.kennedy@stu.mmu.ac.uk; kennedy3jp@gmail.com

8 Running head: Community genetics at a mangrove range limit

9

10 **Citation:** Kennedy, J. P., R. E. Antwis, R. F. Preziosi, and J. K. Rowntree. 2021. Evidence for
11 the genetic similarity rule at an expanding mangrove range limit. *American Journal of Botany*

12 108(8): 1331–1342.

13 DOI: <https://doi.org/10.1002/ajb2.1715>

14 **PREMISE:** Host-plant genetic variation can shape associated communities of organisms. These
15 community-genetic effects include (1) genetically-similar hosts harbouring similar associated
16 communities (i.e., the genetic similarity rule) and (2) host-plant heterozygosity increasing
17 associated community diversity. Community-genetic effects are predicted to be less prominent in
18 plant systems with limited genetic variation, such as those at distributional range limits. Yet,
19 empirical evidence from such systems is limited.

20 **METHODS:** We sampled a natural population of a mangrove foundation species (*Avicennia*
21 *germinans*) at an expanding range limit in Florida, USA. We measured genetic variation within
22 and among 40 host trees with 24 nuclear microsatellite loci and characterised their foliar
23 endophytic fungal communities with ITS1 gene amplicon sequencing. We evaluated
24 relationships among host-tree genetic variation, host-tree spatial location, and the associated
25 fungal communities.

26 **RESULTS:** Genetic diversity was low across all host trees (mean: 2.6 alleles per locus) and
27 associated fungal communities were relatively homogeneous (five sequence variants represented
28 78% of all reads). We found: (1) genetically-similar host trees harboured similar fungal
29 communities, with no detectable effect of inter-host geographic distance. (2) Host-tree
30 heterozygosity had no detectable effect, while host-tree absolute spatial location affected
31 community alpha diversity.

32 **CONCLUSIONS:** This research supports the genetic similarity rule within a range limit
33 population and helps broaden the current scope of community genetics theory by demonstrating
34 that community-genetic effects can occur even at expanding distributional limits where host-
35 plant genetic variation may be limited. Our findings also provide the first documentation of
36 community-genetic effects in a natural mangrove system.

37

38 **KEY WORDS**

39 associated communities; *Avicennia germinans*; black mangrove; community genetics;
40 endophytic fungi; foundation species; intra-individual heterozygosity; plant genetic variation

41

42

43 **INTRODUCTION**

44 Intraspecific diversity can shape the ecological dynamics of communities and entire ecosystems
45 (Raffard et al., 2019). For instance, a central principle of community genetics is that genetic
46 variation within a host plant can influence the structure and diversity of associated communities
47 of organisms (Whitham et al., 2003). Empirical evidence of community-genetic effects is found
48 across diverse systems, including terrestrial forests with low (Whitham et al., 2006) and high
49 (Zytynska et al., 2011) species diversity, agricultural landscapes (Stevenson et al., 2017), and
50 aquatic systems (Jormalainen et al., 2017). This pattern may be most prominent in systems
51 dominated by a limited number of plant foundation species (Whitham et al., 2006), which define
52 ecosystems with their physical structure and provide resources that directly influence diverse
53 community assemblages (Ellison et al., 2005).

54 Community-genetic effects are measured both in terms of host-plant genetic similarity and
55 diversity, plus spatial effects need to also be considered. First, genetically-similar host plants
56 may harbour similar associated communities, a pattern known as the genetic similarity rule
57 (Bangert, Allan, et al., 2006; Bangert, Turek, et al., 2006; Barbour et al., 2009; Kagiya et al.,
58 2018). Second, increased genetic diversity at the population level may lead to concomitant
59 increases in associated species diversity (Wimp et al., 2004; Crutsinger et al., 2006; Johnson et

60 al., 2006). Similar patterns are also found when considering the genetic diversity of individual
61 host plants (i.e., heterozygosity) (Tovar-Sánchez et al., 2013; Valencia-Cuevas et al., 2018). This
62 extension of community genetics theory is in line with extensive research on the link between
63 intra-individual heterozygosity and fitness (reviews by Hansson and Westerberg, 2002; Szulkin
64 et al., 2010). Lastly, in addition to host genetic variation, the spatial context of host plants,
65 including their relative position in relation to neighbouring conspecifics and variation in
66 environmental conditions, needs to also be considered as spatial effects can prove more
67 influential (Tack et al., 2010; Gossner et al., 2015; Barbour et al., 2019; but see Bangert, Allan,
68 et al., 2006; Lamit et al., 2015).

69 Community-genetic effects may also vary with the extent of genetic variation present in the
70 host population. Plant systems with limited genetic variation are predicted to exhibit less
71 prominent effects and, instead, environmental variation will exhibit a stronger effect on
72 associated community structure (Bangert, Turek, et al., 2006). However, only one study has
73 provided empirical evidence from such systems. Pohjanmies et al. (2015) documented that
74 genetic variation within a tree foundation species correlates with the structure and diversity of
75 associated herbivore communities at a distributional range limit. Range limits may exhibit
76 limited genetic variation (Pironon et al., 2017) and are shifting for many species with
77 anthropogenic climate change (Pecl et al., 2017). Further assessments of relationships between
78 host-plant genetic variation and associated communities at range limits, especially those where
79 foundation species are undergoing climate-driven range shifts, could help broaden the current
80 scope of community genetics theory and provide insights into the ecological and evolutionary
81 processes shaping these dynamic systems.

82 In this study, we evaluated relationships between genetic variation within a mangrove
83 foundation species at its expanding distributional range limit and the structure and diversity of
84 associated foliar endophytic fungal communities. Mangroves are (sub)tropical, intertidal woody
85 plants that provide vital ecosystem services to coastal habitats worldwide (Lee et al., 2014).
86 Mangrove forests consist of relatively few tree species (Alongi, 2009) and, as such, intraspecific
87 differences may be particularly influential in shaping ecological dynamics in these systems
88 (Farnsworth, 1998). Numbers of mangrove species are further reduced towards climate-sensitive,
89 poleward range limits where generally only one predominant species exists (Osland et al., 2017)
90 and often genetic variation is limited (e.g., Pil et al., 2011; De Ryck et al., 2016; Kennedy et al.,
91 2017; Binks et al., 2019; Ochoa-Zavala et al., 2019).

92 Mangrove systems harbour numerous associated communities of both terrestrial and marine
93 origin (Nagelkerken et al., 2008), including diverse fungal communities found on or within
94 multiple mangrove tissues (e.g., Gilbert et al., 2002; Arfi et al., 2012; de Souza Sebastianes et al.,
95 2013; Lee et al., 2019). Fungal endophytes are ubiquitous inhabitants within plant tissues, obtain
96 shelter and nutrition from their host plant, and may influence plant health and function (Arnold,
97 2007; Porrás-Alfaro and Bayman, 2011). Endophytic fungi in leaves and twigs vary among host
98 genotypes of diverse plant species (Elamo et al., 1999; Pan et al., 2008; Lamit et al., 2014;
99 Griffiths et al., 2020); however, whether intraspecific genetic differences among mangrove host
100 trees correlates with the structure and diversity of their associated fungal communities remains
101 unanswered.

102 We sampled a natural population of neotropical black mangrove (*Avicennia germinans*) at a
103 northern range limit on the Atlantic coast of Florida, USA. At this range limit, *A. germinans* is
104 the predominant mangrove species (Lonard et al., 2017), exists as discrete patches within a

105 landscape dominated by salt-marsh vegetation (Kangas and Lugo, 1990), and exhibits reduced
106 genetic variation (Kennedy, Preziosi, et al., 2020) and elevated levels of self-fertilisation
107 (Kennedy et al., 2021). A lack of extreme freeze events for several decades has been linked to *A.*
108 *germinans* proliferation (Cavanaugh et al., 2014; Osland et al., 2018) and further expansion is
109 forecast with climate change (Cavanaugh et al., 2015, 2019), which may have wide-reaching
110 effects on these coastal ecosystems (Kelleway et al., 2017). We genotyped *A. germinans* host
111 trees with 24 nuclear microsatellite loci, characterised communities of endophytic fungi in their
112 leaves with ITS1 gene amplicon sequencing, and accounted for potential spatial effects with
113 host-tree GPS coordinates and inter-host geographic distances. We asked: (1) Do inter-host
114 genetic similarity and inter-host geographic distance correlate with similarity among associated
115 endophytic fungal communities? (2) Do host-tree heterozygosity and host-tree absolute spatial
116 location correlate with alpha diversity of the associated endophytic fungal community?

117

118 **MATERIALS AND METHODS**

119 **Study design**

120 On 09 October 2017, we sampled from and collected GPS coordinates for 40 mature *A.*
121 *germinans* trees, all approximately the same height (~2 m), at a single collection site (29.7284, -
122 81.2425) near the Atlantic Florida range limit. Mangrove area has progressively increased for
123 several decades at this site (Rodriguez et al., 2016) which is flanked by a brackish lagoon to the
124 west and a fringe of terrestrial hammock forest to the east. Salinity during this time of the year
125 (Sept–Nov) increases from west to east along the site (38 to 67 ‰), then decreases adjacent to
126 the terrestrial fringe (40 ‰) (Guana Tolomato Matanzas National Estuarine Research Reserve,
127 *unpublished data*; Fig. 1). Our sampling area covered ~0.1 km², which included most of the total

128 spatial extent of this *A. germinans* population, with a minimum inter-tree distance of 11 m and a
129 maximum distance of 528 m (Fig. 1). For each tree, we sampled a total of three undamaged
130 leaves, each from the first fully mature leaf pair on branches located in direct sunlight. We
131 collected these leaves (generally the third leaf pair) to standardise leaf age and exposure to
132 sunlight, both of which can influence fungal community structure (Koide et al., 2017;
133 Younginger and Ballhorn, 2017). We placed leaves from each tree into separate, labelled plastic
134 bags and stored them in a portable cooler with an ice pack during fieldwork and subsequent
135 transport to the laboratory.

136

137 **Sample processing and DNA isolation**

138 Leaves were kept on ice and processed within 24 hours of sampling. We rinsed individual leaves
139 under running tap water for 30 sec, then surface sterilised with sequential immersion in 95%
140 ethanol for 10 seconds, 0.5% bleach for 2 minutes, and 70% ethanol for 2 minutes under a sterile
141 hood (U'Ren et al., 2014). We allowed leaves to air dry and then used sterilised surgical blades
142 to cut ~5 mm x 5 mm sections from the middle of each leaf at both sides of the midvein. We
143 combined the cut sections from each of the three leaves per tree into a single microcentrifuge
144 tube and isolated genomic DNA with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany)
145 following the standard protocol, with an extended incubation of 45 minutes. We also included
146 two extraction blanks (negative controls) during this process. We quantified DNA extracts on a
147 Qubit 2.0 Fluorometer (ThermoFisher Scientific, Waltham, Massachusetts, USA) and created
148 standardised aliquots of 35 ng/uL to be used for both host-tree genotyping and fungal community
149 sequencing. We stored DNA aliquots at -20°C until further processing.

150

151 **Host-tree genotyping**

152 We genotyped host trees at 32 nuclear microsatellite loci. Of this total, 12 loci were previously
153 developed (Nettel et al., 2005; Cerón-Souza et al., 2006, 2012; Mori et al., 2010) and genotyped
154 following the protocol outlined in Kennedy, Preziosi, et al. (2020). The remaining 20 loci were
155 more recently developed (Craig, Feller, et al., 2020) and genotyped following the author's
156 protocol. We performed PCR on a Prime thermal cycler (Techne, Staffordshire, UK), analysed
157 fragments on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Foster City,
158 California, USA) with LIZ 500 size standard, and scored alleles in the R-package Fragman
159 (Covarrubias-Pazaran et al., 2016). We evaluated the presence of null alleles in MICRO-
160 CHECKER 2.2.3 (van Oosterhout et al., 2004) and randomly amplified and genotyped 10% of
161 our DNA samples ($n = 4$) a second time to estimate a study error rate (Bonin et al., 2004). We
162 tested for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium, and
163 calculated the number of alleles and observed and expected heterozygosity per locus in FSTAT
164 2.9.3.2 (Goudet, 2002).

165 We calculated five measures of host-tree heterozygosity (i.e., proportion of heterozygous
166 loci, observed heterozygosity, expected heterozygosity, internal relatedness, homozygosity by
167 loci) for each of the 40 host trees with the R-function GENHET (Coulon, 2010). We also
168 manually calculated the number of alleles within the multi-locus genotype of each host tree. All
169 six measures were highly correlated (Pearson's correlation, $r = 0.96-1.0$, $p < 0.001$). Hence, we
170 present results only for homozygosity by loci (HL), an index that considers allelic variability at
171 each locus to estimate heterozygosity and, based on simulations, correlates better than other
172 measures with genome-wide heterozygosity (Aparicio et al., 2006). As this index varies from 0
173 (all loci are heterozygous) to 1 (all loci are homozygous), we used $1 - HL$ for statistical analyses

174 to provide more intuitive results (i.e., higher values represent higher heterozygosity). To evaluate
175 genetic similarity, we calculated pairwise inter-individual genetic distances (as outlined in
176 Smouse and Peakall, 1999) and geographic distances among the 40 host trees in GenAlEx 6.5
177 (Peakall and Smouse, 2012).

178

179 **Associated fungal community sequencing**

180 We performed ITS gene amplicon library preparation and sequencing at the University of
181 Salford, UK. Fungal DNA was amplified at the ITS 1F-2 gene (White et al., 1990) with modified
182 versions of the ITS1F (5'–CTT GGT CAT TTA GAG GAA GTA A–3') and ITS2 (5'–GCT
183 GCG TTC TTC ATC GAT GC –3') primer set that included Illumina adapters, a linker, and
184 unique barcodes (see Smith and Peay, 2014) as outlined in Griffiths et al. (2020). PCR products
185 for our samples and those of 80 additional fungal samples, which consisted of ITS1 gene
186 amplicons used for an unrelated study, were then pooled to equimolar concentrations. ITS1 gene
187 amplicon sequencing was performed using paired-end reads with an Illumina v3 (2 x 300 bp)
188 cartridge on an Illumina MiSeq (Illumina, San Diego, California, USA). Negative (extraction
189 blanks) and positive (synthetic mock community with 12 mock isolates; Palmer et al., 2018)
190 controls were also included in the sequence run.

191 We removed adaptor and primer sites from the ITS1 gene sequence data with cutadapt v2.4
192 (Martin, 2011), and performed all subsequent data processing and calculations in R v3.6.0 (R
193 Core Team, 2020). A total of 275,829 raw sequences across our 40 samples were generated. We
194 used the R-package DADA2 1.12.1 (Callahan et al., 2016) with default pipelines to perform
195 quality filtering and taxonomic assignment with the UNITE v8.0 database (UNITE Community,
196 2019). Here, we analysed only forward sequence reads because lower quality and quantity of

197 reverse reads resulted in a nearly 50% reduction in total sequence reads after quality filtering of
198 the assembled paired-end reads (Appendix S1a, see the Supplementary Data with this article).
199 Discarding low-quality reverse reads is a common strategy that often provides better results than
200 assembled paired-end reads (Nguyen et al., 2015; Pauvert et al., 2019). One chimera was
201 removed. We then removed amplicon sequence variants (ASVs) with <100 reads across all
202 samples as a conservative approach to deal with potential artifacts of high-throughput sequencing
203 (Pauvert et al., 2019). Modal contig length was 225 bp (range: 153 – 251 bp). No contaminants
204 were identified in the first negative control, and one ASV was identified in the second negative
205 control, but was not found in other samples. All 12 expected ASVs were identified in the
206 synthetic mock community. We did not further trim forward reads, we manually checked
207 whether ASVs with identical taxonomic assignments were indeed unique sequences (i.e., did not
208 simply vary at the start or end of the sequence), and all ASVs assigned as unidentified fungi were
209 further checked with default blastn analyses on the UNITE website (Nilsson et al., 2019). We
210 removed all ASVs that corresponded to the host-tree species (*A. germinans*), which included
211 64% of all sequence reads, and all additional unidentified fungi had significant alignments with
212 public fungal ITS sequences (e-values = $1e^{-13}$ – $4e^{-88}$). The resulting data set consisted of 64,308
213 reads across 40 samples, with a median of 748 reads per sample (range: 104 – 9,314).

214 We exported the ASV table, taxonomy table, and sample identifications to the R-package
215 phyloseq 1.28.0 (McMurdie and Holmes, 2013) for the following calculations. We calculated
216 alpha diversity of fungal communities with Hill numbers (Hill, 1973) at the scales of $q=0$
217 (species richness), $q=1$ (exponential of Shannon index), and $q=2$ (inverse of Simpson index),
218 which represent the effective number of species and put more weight on abundant species as the
219 value of q increases (Chao et al., 2014). We performed these calculations with the raw count data

220 rarefied to a standardised number of reads equal to the sample with the lowest read count (104
221 reads; see Appendix S2a). Although read counts were limited for certain samples, asymptotes
222 were reached in all rarefaction curves with few rank-order changes among samples past this
223 lowest read count (Appendix S2a). As such, our sampling effort seems to have captured most
224 diversity within these samples. Random sampling to generate rarefied counts can add noise to a
225 data set and undermine the performance of downstream methods (McMurdie and Holmes, 2014);
226 therefore, we also performed alpha diversity calculations and the subsequent statistical analyses
227 with the raw count data and results were equivalent to those presented here (Appendix S1b). To
228 evaluate community dissimilarity (beta diversity), we calculated Bray-Curtis dissimilarity with
229 the raw count data converted to relative abundances. We also calculated Aitchison distance by
230 centred log-ratio (clr) transforming the raw count data with the R-package microbiome (Lahti et
231 al., 2017) and then calculating pairwise Euclidean distances in phyloseq 1.28.0 (McMurdie and
232 Holmes, 2013). Aitchison distance accounts for the compositional nature of high-throughput
233 sequence data, which makes this measure more appropriate than many standard measures (Gloor
234 et al., 2017; Quinn et al., 2018).

235

236 **Statistical analyses**

237 We performed all statistical analyses in R v3.6.0 (R Core Team, 2020). To address our first
238 question, we tested for an effect of inter-host genetic distance and a relative spatial effect of
239 inter-host geographic distance on dissimilarity among associated endophytic fungal communities
240 across all samples with ranked Mantel tests of correlation. As spatial effects may not be linear
241 (Diniz-Filho et al., 2013; Legendre et al., 2015), we also performed multivariate Mantel
242 correlograms to assess these patterns at five discrete distance classes. All analyses were

243 performed in the R-package *ecodist* (Goslee and Urban, 2007). Significance for each analysis
244 was determined with 10^4 permutations, and p-values for correlograms were adjusted for multiple
245 comparisons with a false discovery rate correction method using the R-function *p.adjust*. For
246 both Mantel tests and Mantel correlograms, we first tested for a relationship between the two
247 predictor variables (i.e., inter-host genetic distance and inter-host geographic distance), then
248 performed separate tests between fungal community dissimilarity and each of the two predictor
249 variables, and finally performed partial analyses between fungal community dissimilarity and
250 inter-host genetic distance, while controlling for inter-host geographic distance.

251 To address our second question, we tested for an effect of host-tree heterozygosity and an
252 absolute spatial effect of host-tree spatial location on the alpha diversity of associated endophytic
253 fungal communities with multiple linear regressions. We fitted three additive models, with alpha
254 diversity of fungal communities at each Hill number ($q = 0, 1, 2$) as the response variable and
255 heterozygosity, longitude, and latitude of each host tree as predictor variables. We also tested full
256 models and subsets with interactions among two of the three predictor variables, but none of
257 these interactions proved statistically significant and none of these models provided better fits
258 based on the Bayesian Information Criterion (BIC; Schwarz, 1978). Hill numbers at $q=1$ and $q=2$
259 were natural log-transformed to meet the statistical assumption of normality, and we centred and
260 scaled the predictor variables to standardise regression coefficients.

261

262 **RESULTS**

263 **Host-tree genotyping**

264 We discarded seven of the 32 nuclear microsatellite loci that were monomorphic across all
265 samples, and discarded another locus that proved difficult to score. Our final host-tree genotypes

266 included 24 loci (Appendix S1c) with no missing data, and all 40 host-tree genotypes were
267 unique. We found no evidence for null alleles and each of the four samples that were amplified
268 and genotyped a second time produced consistent multi-locus genotypes. We found no evidence
269 for linkage disequilibrium or deviations from Hardy-Weinberg equilibrium. Genetic variation
270 was low across the 40 host trees, with 2.6 ± 1.4 (SD) alleles per locus and expected
271 heterozygosity of 0.37 ± 0.20 (Appendix S1c). Host-tree heterozygosity ($1 - HL$) ranged from
272 0.06 to 0.81 (mean: 0.45 ± 0.15).

273

274 **Associated fungal community sequencing**

275 A total of 49 amplicon sequence variants (ASVs) were identified across the 40 host trees. Most
276 ASVs were assigned to the phylum Ascomycota (35 of 49 ASVs, 87% of all reads) and 11% of
277 all reads were assigned only to the level of kingdom Fungi (Appendix S2b). Less than half (47%)
278 of all reads were assigned class level taxonomy, with the class Dothideomycetes as the most
279 common (28% of all reads; Appendix S2c). The endophytic fungal community was relatively
280 homogeneous, with one ASV (assigned taxonomy only to the level of phylum Ascomycota)
281 representing 41% of all reads (Appendix S1d). The five most abundant ASVs represented 78%
282 of all reads, and subsequent ASVs each represented $\leq 2\%$ of all reads (Appendix S1d). Alpha
283 diversity of fungal communities across the 40 host trees at $q=0$ (species richness) was 4.0 ± 1.7
284 (SD), at $q=1$ (exponential of Shannon index) was 2.8 ± 1.2 , and at $q=2$ (inverse of Simpson
285 index) was 2.5 ± 1.1 .

286

287 **Associated fungal community structure correlates with host-tree genetics**

288 Genetically-similar host trees harboured similar associated fungal communities, with no
289 detectable relative spatial effect of geographic distance among host trees both across all samples
290 (Mantel tests) and at five distance classes (Mantel correlograms) (Fig. 2). For Mantel tests, the
291 predictor variables (i.e., inter-host genetic distance and inter-host geographic distance) exhibited
292 no relationship (Mantel correlation, $r_M = 0.05$, $p = 0.181$; Appendix S2d). Fungal community
293 (Bray-Curtis) dissimilarity exhibited a weak, but statistically significant positive relationship
294 with inter-host genetic distance ($r_M = 0.26$, $p = 0.002$), and no relationship with inter-host
295 geographic distance ($r_M = 0.06$, $p = 0.164$) (Fig. 2a, b). Accounting for inter-host geographic
296 distance did not impact the relationship with inter-host genetic distance (partial $r_M = 0.26$, $p =$
297 0.002). Community dissimilarity measured with Aitchison distance provided equivalent results
298 (inter-host genetic distance: $r_M = 0.16$, $p = 0.041$; inter-host geographic distance: $r_M = 0.05$, $p =$
299 0.188) (Fig. 2e, f), with a weaker relationship with inter-host genetic distance (partial $r_M = 0.16$,
300 $p = 0.043$).

301 Mantel correlogram results were equivalent to those of the Mantel tests, with no
302 relationships between predictor variables ($r_M = -0.07 - 0.07$, $p \geq 0.568$; Appendix S2d), and
303 community (Bray-Curtis) dissimilarity exhibited statistically significant positive relationships
304 with the first two genetic distance classes ($r_M = 0.16$, $p = 0.002$; $r_M = 0.14$, $p = 0.050$;
305 respectively), a statistically significant negative relationship with the fourth genetic distance
306 class ($r_M = -0.16$, $p = 0.008$), and no relationships with inter-host geographic distance classes (r_M
307 $= -0.06 - 0.03$, $p \geq 0.810$) (Fig. 2c, d). Accounting for inter-host geographic distances did not
308 impact these relationships with inter-host genetic distance classes, except for the second genetic
309 distance class that was now statistically non-significant ($p = 0.090$) (Appendix S2e). Community
310 dissimilarity measured with Aitchison distance provided equivalent results (Fig. 2g, h), with

311 weaker relationships with inter-host genetic distance classes that were statistically significant at
312 only the first genetic distance class ($r_M = 0.13$, $p = 0.027$). Accounting for inter-host geographic
313 distances did not impact these relationships (Appendix S2e).

314

315 **Associated fungal community diversity correlates with host-tree spatial location**

316 Host-tree heterozygosity had no detectable effect on the alpha diversity of associated endophytic
317 fungal communities. Instead, the absolute spatial location of host trees affected these associated
318 fungal communities. Additive models explained limited variation in the alpha diversity of fungal
319 communities at each of the three Hill numbers. The model for $q=0$ was not statistically
320 significant ($F_{3,36} = 1.7$, $p = 0.195$, adjusted $r^2 = 0.05$) and models for $q=1$ ($F_{3,36} = 3.1$, $p = 0.038$,
321 adjusted $r^2 = 0.14$) and $q=2$ ($F_{3,36} = 3.4$, $p = 0.027$, adjusted $r^2 = 0.16$) were marginally
322 significant. Longitude was the only predictor variable to exhibit a significant partial regression
323 slope (for full model breakdown see Table 1). This increase in fungal community alpha diversity
324 with increased longitude (i.e., from the brackish lagoon to the landward margin) was statistically
325 significant at each of the three Hill numbers ($p = 0.043$, 0.009 , 0.009 , respectively; Table 1). Yet,
326 instead of a systematic increase, these effects seemed to be shaped primarily by the fact that
327 highest fungal alpha diversity was observed within trees closest to the landward margin (Fig. 3).

328

329 **DISCUSSION**

330 Community-genetic effects are predicted to be less prominent in plant systems with limited
331 genetic variation, such as those at distributional range limits. Yet, empirical evidence from such
332 systems is limited. Here, at the scale of an expanding range limit population of a mangrove
333 foundation species (*Avicennia germinans*), we found evidence for the genetic similarity rule

334 whereby genetically-similar host trees harboured similar associated endophytic fungal
335 communities. In contrast, we found no detectable effect of host-tree heterozygosity on fungal
336 community alpha diversity. This research demonstrates that community-genetic effects can occur
337 even at expanding distributional limits where host-plant genetic variation may be limited, and
338 provides the first documentation of these effects in a natural mangrove system.

339 Genetically-similar mangrove hosts harbouring similar endophytic fungal communities, with
340 no detectable relative spatial effect, may be explained by the mode of fungal transmission and/or
341 biotic filtering dictated by the physiology and anatomy of the host plant (Ricks and Koide,
342 2019). Horizontal transmission via airborne fungal spores is commonly observed in woody
343 plants (Arnold and Herre, 2003 and citations within), although vertical transmission from parent
344 tree to seed is also possible (e.g., Vega et al., 2010). Our studied species (*A. germinans*) produces
345 cryptoviviparous propagules (i.e., embryos emerge from the seed coat, but remain within the
346 fruit until abscission from maternal trees), with varying degrees of vivipary across many
347 mangrove species (Tomlinson, 1986). This form of reproduction, where developing propagules
348 remain attached to maternal trees for extended periods may lead to a greater contribution of
349 fungal transfer from parent to offspring. Consistent with this hypothesis, endophytic fungi (Lee
350 et al., 2019) and bacteria (Soldan et al., 2019) are found within surface-sterilised
351 cryptoviviparous mangrove propagules collected directly from maternal trees. Host physiology
352 may also dampen horizontal transfer in *A. germinans* as salt excretion through leaf glands (a
353 mechanism to tolerate salt stress) can reduce foliar fungal colonisation (Gilbert et al., 2002).
354 Fungal communities in trees also vary with differences in phenotypic leaf traits, such as internal
355 chemistry and surface characteristics (Valkama et al., 2005; Kembel and Mueller, 2014).
356 Additional research that compares fungal endophytes in both *A. germinans* maternal trees and

357 their offspring, with parallel leaf trait assessments, could evaluate the relative influence of fungal
358 transmission mode and biotic filtering in shaping these associated communities.

359 We did not detect an effect of host-tree heterozygosity on fungal community alpha diversity.
360 Instead, we found that alpha diversity varied with the absolute spatial location of host trees.
361 Increased host-tree heterozygosity can lead to greater growth rates (Charlesworth and Willis,
362 2009) and greater foliar phytochemical diversity (Campbell et al., 2013), factors that may
363 underlie increases in associated herbivore community alpha diversity observed elsewhere
364 (Tovar-Sánchez et al., 2013; Valencia-Cuevas et al., 2018). We suggest that, within this
365 mangrove population, the limited genetic variation present across host trees may not translate
366 into large enough variation in host-tree phenotypic traits that would augment the alpha diversity
367 of these associated communities. Rather, community alpha diversity increased with longitude
368 across our collection site (i.e., from the brackish lagoon to the landward margin), an absolute
369 spatial effect seemingly shaped by the fact that highest alpha diversity was observed within trees
370 closest to the landward margin. Soil salinity increases with longitude across the site, but then
371 declines at this landward margin adjacent to a fringe of terrestrial forest (Fig. 1). Salinity
372 differences can impact fungal communities associated with the *A. germinans* rhizosphere
373 (Vanegas et al., 2019), but their effect on foliar fungal communities remains to be formally
374 tested. Higher soil salinity closer to the centre of the collection site will demand greater salt
375 excretion through *A. germinans* leaf glands (Sobrado and Greaves, 2000; Suárez and Medina,
376 2008) that may further diminish foliar fungal colonisation in this species (Gilbert et al., 2002). In
377 addition, as mangrove leaves may contain fungi predominately from terrestrial sources (Lee et
378 al., 2019, 2020), the fringe of terrestrial forest is presumably a reservoir of unique fungal
379 diversity. Therefore, within the mangrove population studied here, trees located nearest to this

380 landward margin may harbour slightly more diverse fungal communities than conspecifics
381 elsewhere due to both reduced soil salinity and proximity to additional fungal sources. Whether
382 this pattern extends to additional mangrove populations remains to be tested.

383 Pohjanmies et al. (2015), with their research at a distributional range limit, provided the first
384 empirical evidence of community-genetic effects within a plant system with limited genetic
385 variation. Our documentation of the genetic similarity rule at a mangrove range limit, where host
386 trees possessed very limited genetic variation (on average, 2.6 alleles per locus), adds further
387 support to these previous findings and strengthens the argument that correlations between genetic
388 variation within foundation species and the dynamics of associated communities can occur even
389 at distributional limits that may be genetically depauperate. These correlations, however, will
390 ultimately depend on the strength of the community-genetic effect relative to the degree of
391 environmental variation and how this relationship varies with spatial scale (Bangert et al., 2008).
392 Both Pohjanmies et al. (2015) and our study assessed correlations between plant foundation
393 species and their associated communities within single range limit populations. Environmental
394 variation will inherently be small at this local scale compared to that across broader spatial scales
395 where community-genetic effects may be less influential (Hughes and Stachowicz, 2009; Tack et
396 al., 2010; Gossner et al., 2015; but see Bangert, Allan, et al., 2006; Davies et al., 2014; Lamit et
397 al., 2015). Spatial effects on foliar endophytic fungal communities in mangroves are evident
398 across greater geographic distances (Lee et al., 2019, 2020). As such, the relationship between
399 mangrove host-tree genetic variation and associated fungal communities documented here may
400 vary depending on the spatial extent under consideration and warrants additional research.

401 Although we sampled a relatively small spatial area, this is the scale at which species
402 expansion occurs as small isolated populations become colonised and begin to proliferate. This

403 process is particularly evident at the Atlantic Florida *A. germinans* range limit where initial
404 colonisation may consist of a single individual (Kennedy, Dangremond, et al., 2020), and for the
405 population studied here which has increased from only about 10% to 45% mangrove cover over
406 the past several decades (Rodriguez et al., 2016). In this context, our research demonstrates that
407 community-genetic effects can occur across the spatial extent of an expanding range limit
408 population, with potential implications for host fitness and population resilience as endophytic
409 fungi can vary greatly in function within plant hosts from latent pathogens to mutualistic
410 symbionts (Porrás-Alfaro and Bayman, 2011). Symbioses with endophytic fungi can contribute
411 to plant adaptation to high-stress environments (Rodriguez et al., 2004), with evidence that
412 variation in soil fungal communities can influence the fitness and susceptibility of *A. germinans*
413 to cold stress (Chen et al., 2020), although fungal infections can reduce recruitment (Devaney et
414 al., 2017). We documented a correlation between mangrove host-tree genetics and fungal
415 community differences, but does this relationship generate variation in stress tolerance among
416 mangrove hosts? If so, this insight could broaden the current discussion of how a shift from salt
417 marsh to mangrove dominance may shape these coastal communities (e.g., Kelleway et al., 2017;
418 Johnston and Gruner, 2018; Smith et al., 2019; Armitage et al., 2020) by including mangrove
419 intraspecific variation as a factor that could influence population resilience at these high-stress
420 range limits.

421 This research also provides the first documentation of community-genetic effects in a natural
422 mangrove system. Does the genetic similarity rule apply elsewhere across the broad
423 distributional range of mangroves and to further mangrove-associated communities?
424 Experimental plantings demonstrate that mangrove maternal genotypic identity can impact the
425 composition of associated soil microbial communities (Craig, Kennedy, et al., 2020), which

426 indicates that community-genetic effects can have a broader reach in mangrove systems than the
427 more intimately associated endophytic fungal communities assessed here. Moreover,
428 intraspecific differences in quantitative traits of mangroves, including trichome density (Piovia-
429 Scott, 2011), plant architecture (Silva et al., 2017), and leaf chemistry (Erickson et al., 2004), can
430 affect mangrove-associated communities. Heritable variation in these traits has been identified as
431 a potential factor linking associated communities to host-plant genetics (Whitham et al., 2012).
432 Assessments in additional mangrove-associated communities (of both terrestrial and marine
433 origin) would further our understanding of how host-tree genetic variation may relate to the
434 broader community of organisms associated with these plants, with direct implications for
435 conservation and restoration practices.

436

437 **CONCLUSIONS**

438 We found evidence for the genetic similarity rule at an expanding mangrove range limit. This
439 research helps broaden the current scope of community genetics theory by demonstrating that
440 community-genetic effects can occur even at expanding distributional limits where host-plant
441 genetic variation may be limited. Our findings also add to the growing number of diverse
442 systems where associated communities vary with host-plant genetics. As community-level
443 effects of host-plant genetic variation are found to be most prominent in systems dominated by
444 few plant foundation species (Whitham et al., 2006), mangrove forests and their low tree species
445 diversity may prove to be a system ripe for discovery.

446

447 **ACKNOWLEDGEMENTS**

448 This research was funded by a Manchester Metropolitan University studentship to J.P.K. Many
449 thanks to IC Feller and R Feller (and their cast iron skillet) and the Smithsonian Marine Station
450 at Fort Pierce, Florida for logistical support during fieldwork, to N Dix and P Marcum at the
451 Guana Tolomato Matanzas National Estuarine Research Reserve for permission to work at the
452 collection site and provision of monitoring data, to the US Forest Service and the University of
453 Wisconsin-Madison for provision of fungal mock communities for amplicon sequencing, to H
454 Craig for providing information and reagents for microsatellite genotyping, to G Fox for
455 computational assistance, and to the University of Manchester Genomic Technologies Core
456 Facility and F Combe for fragment analysis. Additional thanks to three anonymous reviewers
457 and the Associate Editor whose insightful comments much improved an earlier version of this
458 manuscript. As always, thank you to A Jara Cavieres, C Kennedy, and M Kennedy for
459 unconditional support and big smiles.

460

461 **AUTHOR CONTRIBUTIONS**

462 J.P.K., R.F.P. and J.K.R. conceived and designed the research. J.P.K. performed field
463 collections, DNA extractions, and host-tree genotyping. R.E.A. performed library preparation
464 and sequencing, and provided analysis tools. R.F.P. and J.K.R. supervised the research. J.P.K.
465 conducted bioinformatics analysis and statistical analyses. J.P.K. wrote the manuscript with input
466 from all co-authors.

467

468 **DATA AVAILABILITY**

469 Microsatellite genotype data are publicly available on figshare:
470 <https://doi.org/10.6084/m9.figshare.14252660.v1>. Sequence data are deposited on the NCBI
471 SRA database: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA643237/>.

472

473 **SUPPORTING INFORMATION**

474 Additional Supporting Information may be found online in the supporting information tab for
475 this article.

476 **APPENDIX S1 Supplemental Tables:**

477 **Appendix S1a.** Summary of ITS1 gene sequence data sets using only forward sequence reads
478 and using assembled paired-end reads.

479 **Appendix S1b.** Multiple linear regressions of associated endophytic fungal community diversity
480 (calculated with the raw count data) as a function of the heterozygosity and absolute spatial
481 location of host trees.

482 **Appendix S1c.** Genetic diversity of 24 nuclear microsatellite loci used for genotyping of
483 *Avicennia germinans* host trees.

484 **Appendix S1d.** Endophytic fungal diversity identified with ITS1 gene sequencing.

485

486 **APPENDIX S2 Supplemental Figures:**

487 **Appendix S2a.** Rarefaction curves of observed amplicon sequence variants (ASVs) in sampled
488 *Avicennia germinans* trees.

489 **Appendix S2b.** Relative abundance across all sequence data of fungal phyla for the forward-
490 reads data set.

491 **Appendix S2c.** Relative abundance across all sequence data of fungal class for the forward-reads
492 data set.

493 **Appendix S2d.** Graphical representation of Mantel test and Mantel correlogram between inter-
494 host genetic distance and inter-host geographic distance.

495 **Appendix S2e.** Graphical representation of partial Mantel correlograms between fungal
496 community dissimilarity, measured with Bray-Curtis dissimilarity and Aitchison distance, and
497 inter-host genetic distance.

498

499 LITERATURE CITED

- 500 Alongi, D. M. 2009. Paradigm shifts in mangrove biology. *In* G. M. E. Perillo, E. Wolanski, D.
501 R. Cahoon, and M. M. Brinson [eds.], *Coastal Wetlands An Integrated Ecosystem*
502 *Approach*, 615–640. Elsevier, Amsterdam.
- 503 Aparicio, J. M., J. Ortego, and P. J. Cordero. 2006. What should we weigh to estimate
504 heterozygosity, alleles or loci? *Molecular Ecology* 15: 4659–4665.
- 505 Arfi, Y., M. Buée, C. Marchand, A. Levasseur, and E. Record. 2012. Multiple markers
506 pyrosequencing reveals highly diverse and host-specific fungal communities on the
507 mangrove trees *Avicennia marina* and *Rhizophora stylosa*. *FEMS Microbiology Ecology* 79:
508 433–444.
- 509 Armitage, A. R., C. A. Weaver, A. A. Whitt, and S. C. Pennings. 2020. Effects of mangrove
510 encroachment on tidal wetland plant, nekton, and bird communities in the Western Gulf of
511 Mexico. *Estuarine, Coastal and Shelf Science*: 106767.
- 512 Arnold, A. E. 2007. Understanding the diversity of foliar endophytic fungi: progress, challenges,
513 and frontiers. *Fungal Biology Reviews* 21: 51–66.
- 514 Arnold, A. E., and E. A. Herre. 2003. Canopy cover and leaf age affect colonization by tropical
515 fungal endophytes: Ecological pattern and process in *Theobroma cacao* (Malvaceae).
516 *Mycologia* 95: 388–398.
- 517 Bangert, R. K., G. J. Allan, R. J. Turek, G. M. Wimp, N. Meneses, G. D. Martinsen, P. Keim,
518 and T. G. Whitham. 2006. From genes to geography: a genetic similarity rule for arthropod
519 community structure at multiple geographic scales. *Molecular Ecology* 15: 4215–4228.
- 520 Bangert, R. K., E. V. Lonsdorf, G. M. Wimp, S. M. Shuster, D. Fischer, J. A. Schweitzer, G. J.
521 Allan, et al. 2008. Genetic structure of a foundation species: Scaling community phenotypes
522 from the individual to the region. *Heredity* 100: 121–131.
- 523 Bangert, R. K., R. J. Turek, B. Rehill, G. M. Wimp, J. A. Schweitzer, G. J. Allan, J. K. Bailey, et
524 al. 2006. A genetic similarity rule determines arthropod community structure. *Molecular*
525 *Ecology* 15: 1379–1391.
- 526 Barbour, M. A., S. Erlandson, K. Peay, B. Locke, E. S. Jules, and G. M. Crutsinger. 2019. Trait

527 plasticity is more important than genetic variation in determining species richness of
528 associated communities. *Journal of Ecology* 107: 350–360.

529 Barbour, R. C., J. M. O'Reilly-Wapstra, D. W. De Little, G. J. Jordan, D. A. Steane, J. R.
530 Humphreys, J. K. Bailey, et al. 2009. A geographic mosaic of genetic variation within a
531 foundation tree species and its community-level consequences. *Ecology* 90: 1762–1772.

532 Binks, R. M., M. Byrne, K. McMahon, G. Pitt, K. Murray, and R. D. Evans. 2019. Habitat
533 discontinuities form strong barriers to gene flow among mangrove populations, despite the
534 capacity for long-distance dispersal. *Diversity and Distributions* 25: 298–309.

535 Bonin, A., E. Bellemain, P. B. Eidesen, F. Pompanon, C. Brochmann, and P. Taberlet. 2004.
536 How to track and assess genotyping errors in population genetics studies. *Molecular*
537 *Ecology* 13: 3261–3273.

538 Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes.
539 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature*
540 *Methods* 13: 581–583.

541 Campbell, S. A., J. S. Thaler, and A. Kessler. 2013. Plant chemistry underlies herbivore-
542 mediated inbreeding depression in nature. *Ecology Letters* 16: 252–260.

543 Cavanaugh, K. C., E. M. Dangremond, C. L. Doughty, A. P. Williams, J. D. Parker, M. A.
544 Hayes, W. Rodriguez, and I. C. Feller. 2019. Climate-driven regime shifts in a mangrove-
545 salt marsh ecotone over the past 250 years. *Proceedings of the National Academy of*
546 *Sciences* 116: 21602–21608.

547 Cavanaugh, K. C., J. R. Kellner, A. J. Forde, D. S. Gruner, J. D. Parker, W. Rodriguez, and I. C.
548 Feller. 2014. Poleward expansion of mangroves is a threshold response to decreased
549 frequency of extreme cold events. *Proceedings of the National Academy of Sciences* 111:
550 723–727.

551 Cavanaugh, K. C., J. D. Parker, S. C. Cook-Patton, I. C. Feller, A. P. Williams, and J. R. Kellner.
552 2015. Integrating physiological threshold experiments with climate modeling to project
553 mangrove species' range expansion. *Global Change Biology* 21: 1928–1938.

554 Cerón-Souza, I., E. Bermingham, W. O. McMillan, and F. A. Jones. 2012. Comparative genetic
555 structure of two mangrove species in Caribbean and Pacific estuaries of Panama. *BMC*
556 *Evolutionary Biology* 12: 205.

557 Cerón-Souza, I., E. Rivera-Ocasio, S. M. Funk, and W. O. McMillan. 2006. Development of six
558 microsatellite loci for black mangrove (*Avicennia germinans*). *Molecular Ecology Notes* 6:
559 692–694.

560 Chao, A., N. J. Gotelli, T. C. Hsieh, E. L. Sander, K. H. Ma, R. K. Colwell, and A. M. Ellison.
561 2014. Rarefaction and extrapolation with Hill numbers: A framework for sampling and
562 estimation in species diversity studies. *Ecological Monographs* 84: 45–67.

563 Charlesworth, D., and J. H. Willis. 2009. The genetics of inbreeding depression. *Nature Reviews*
564 *Genetics* 10: 783–796.

565 Chen, E., J. A. Blaze, R. S. Smith, S. Peng, and J. E. Byers. 2020. Freeze-tolerance of poleward-
566 spreading mangrove species weakened by soil properties of resident salt marsh competitor.
567 *Journal of Ecology* 108: 1725–1737.

568 Coulon, A. 2010. GENHET: An easy-to-use R function to estimate individual heterozygosity.
569 *Molecular Ecology Resources* 10: 167–169.

570 Covarrubias-Pazarán, G., L. Diaz-García, B. Schlautman, W. Salazar, and J. Zalapa. 2016.
571 Fragman: an R package for fragment analysis. *BMC Genetics* 17: 1–8.

572 Craig, H., I. C. Feller, and J. K. Rowntree. 2020. Development of additional microsatellite

573 primers for the mangrove tree species *Avicennia germinans*. *bioRxiv*: 2020.12.14.422622.

574 Craig, H., J. P. Kennedy, D. J. Devlin, R. D. Bardgett, and J. K. Rowntree. 2020. Effects of
575 maternal genotypic identity and genetic diversity of the red mangrove *Rhizophora mangle*
576 on associated soil bacterial communities: a field-based experiment. *Ecology and Evolution*
577 10: 13957–13967.

578 Crutsinger, G. M., M. D. Collins, J. A. Fordyce, Z. Gompert, C. C. Nice, and N. J. Sanders.
579 2006. Plant genotypic diversity predicts community structure and governs an ecosystem
580 process. *Science* 313: 966–968.

581 Davies, C., C. J. Ellis, G. R. Iason, and R. A. Ennos. 2014. Genotypic variation in a foundation
582 tree (*Populus tremula* L.) explains community structure of associated epiphytes. *Biology*
583 *Letters* 10: 20140190.

584 Devaney, J. L., M. Lehmann, I. C. Feller, and J. D. Parker. 2017. Mangrove microclimates alter
585 seedling dynamics at the range edge. *Ecology* 98: 2513–2520.

586 Diniz-Filho, J. A. F., T. N. Soares, J. S. Lima, R. Dobrovolski, V. L. Landeiro, M. P. de C.
587 Telles, T. F. Rangel, and L. M. Bini. 2013. Mantel test in population genetics. *Genetics and*
588 *Molecular Biology* 36: 475–485.

589 Elamo, P., M. L. Helander, I. Saloniemi, and S. Neuvonen. 1999. Birch family and
590 environmental conditions affect endophytic fungi in leaves. *Oecologia* 118: 151–156.

591 Ellison, A. M., M. S. Bank, B. D. Clinton, E. A. Colburn, K. Elliott, C. R. Ford, D. R. Foster, et
592 al. 2005. Loss of Foundation Species: Consequences for the Structure and Dynamics of
593 Forested Ecosystems. *Frontiers in Ecology and the Environment* 3: 479–486.

594 Erickson, A. A., S. S. Bell, and C. J. Dawes. 2004. Does mangrove leaf chemistry help explain
595 crab herbivory patterns? *Biotropica* 36: 333–343.

596 Farnsworth, E. 1998. Issues of spatial, taxonomic and temporal scale in delineating links
597 between mangrove diversity and ecosystem function. *Global Ecology & Biogeography*
598 *Letters* 7: 15–25.

599 Gilbert, G. S., M. Mejía-Chang, and E. Rojas. 2002. Fungal diversity and plant disease in
600 mangrove forests: Salt excretion as a possible defense mechanism. *Oecologia* 132: 278–
601 285.

602 Giri, C., E. Ochieng, L. L. Tieszen, Z. Zhu, A. Singh, T. Loveland, J. Masek, and N. Duke. 2011.
603 Status and distribution of mangrove forests of the world using earth observation satellite
604 data. *Global Ecology and Biogeography* 20: 154–159.

605 Gloor, G. B., J. M. Macklaim, V. Pawlowsky-Glahn, and J. J. Egozcue. 2017. Microbiome
606 datasets are compositional: And this is not optional. *Frontiers in Microbiology* 8: 2224.

607 Goslee, S. C., and D. L. Urban. 2007. The ecodist Package for Dissimilarity-based Analysis of
608 Ecological Data. *Journal of Statistical Software* 22: 1–19.

609 Gossner, M. M., M. Brändle, R. Brandl, J. Bail, J. Müller, and L. Opgenoorth. 2015. Where is
610 the extended phenotype in the wild? The community composition of arthropods on mature
611 oak trees does not depend on the oak genotype. *PLoS ONE* 10: e0115733.

612 Goudet, J. 2002. FSTAT (Version 2.9.3.2): a program to estimate and test gene diversities and
613 fixation indices. URL: <http://www2.unil.ch/popgen/softwares>.

614 Griffiths, S. M., M. Galambao, J. Rowntree, I. Goodhead, J. Hall, D. O’Brien, N. Atkinson, and
615 R. E. Antwis. 2020. Complex associations between cross-kingdom microbial endophytes
616 and host genotype in ash dieback disease dynamics. *Journal of Ecology* 108: 291–309.

617 Hansson, B., and L. Westerberg. 2002. On the correlation between heterozygosity and fitness in
618 natural populations. *Molecular Ecology* 11: 2467–2474.

- 619 Hill, M. O. 1973. Diversity and evenness: a unifying notation and its consequences. *Ecology* 54:
620 427–432.
- 621 Hughes, A. R., and J. J. Stachowicz. 2009. Ecological impacts of genotypic diversity in the
622 clonal seagrass *Zostera marina*. *Ecology* 90: 1412–1419.
- 623 Johnson, M. T. J., M. J. Lajeunesse, and A. A. Agrawal. 2006. Additive and interactive effects of
624 plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters* 9:
625 24–34.
- 626 Johnston, C. A., and D. S. Gruner. 2018. Marine fauna sort at fine resolution in an ecotone of
627 shifting wetland foundation species. *Ecology* 99: 2546–2557.
- 628 Jormalainen, V., M. Danelli, K. Gagnon, H. Hillebrand, E. Rothäusler, J. P. Salminen, and J.
629 Sjöroos. 2017. Genetic variation of a foundation rockweed species affects associated
630 communities. *Ecology* 98: 2940–2951.
- 631 Kagiya, S., M. Yasugi, H. Kudoh, A. J. Nagano, and S. Utsumi. 2018. Does genomic variation in
632 a foundation species predict arthropod community structure in a riparian forest? *Molecular*
633 *Ecology* 27: 1284–1295.
- 634 Kangas, P. C., and A. E. Lugo. 1990. The distribution of mangroves and saltmarsh in Florida.
635 *Tropical Ecology* 31: 32–39.
- 636 Kelleway, J. J., K. Cavanaugh, K. Rogers, I. C. Feller, E. Ens, C. Doughty, and N. Saintilan.
637 2017. Review of the ecosystem service implications of mangrove encroachment into salt
638 marshes. *Global Change Biology* 23: 3967–3983.
- 639 Kembel, S. W., and R. C. Mueller. 2014. Plant traits and taxonomy drive host associations in
640 tropical Phyllosphere fungal communities. *Botany* 92: 303–311.
- 641 Kennedy, J. P., E. M. Dangremond, M. A. Hayes, R. F. Preziosi, J. K. Rowntree, and I. C. Feller.
642 2020. Hurricanes overcome migration lag and shape intraspecific genetic variation beyond a
643 poleward mangrove range limit. *Molecular Ecology* 29: 2583–2597.
- 644 Kennedy, J. P., L. Garavelli, N. K. Truelove, D. J. Devlin, S. J. Box, L. M. Chérubin, and I. C.
645 Feller. 2017. Contrasting genetic effects of red mangrove (*Rhizophora mangle* L.) range
646 expansion along West and East Florida. *Journal of Biogeography* 44: 335–347.
- 647 Kennedy, J. P., R. F. Preziosi, J. K. Rowntree, and I. C. Feller. 2020. Is the central-marginal
648 hypothesis a general rule? Evidence from three distributions of an expanding mangrove
649 species, *Avicennia germinans* (L.) L. *Molecular Ecology* 29: 704–719.
- 650 Kennedy, J. P., J. M. Sammy, J. K. Rowntree, and R. F. Preziosi. 2021. Mating system variation
651 in neotropical black mangrove, *Avicennia germinans*, at three spatial scales towards an
652 expanding northern distributional limit. *Estuarine, Coastal and Shelf Science* 248: 106754.
- 653 Koide, R. T., K. D. Ricks, and E. R. Davis. 2017. Climate and dispersal influence the structure of
654 leaf fungal endophyte communities of *Quercus gambelii* in the eastern Great Basin, USA.
655 *Fungal Ecology* 30: 19–28.
- 656 Lahti, L., S. Shetty, T. Blake, and J. Salojarvi. 2017. Tools for microbiome analysis in R.
657 Version 2.1.26. URL: <http://microbiome.github.com/microbiome>.
- 658 Lamit, L. J., M. K. Lau, R. Reese Næsborg, T. Wojtowicz, T. G. Whitham, and C. A. Gehring.
659 2015. Genotype variation in bark texture drives lichen community assembly across multiple
660 environments. *Ecology* 96: 960–971.
- 661 Lamit, L. J., M. K. Lau, C. M. Sthultz, S. C. Wooley, T. G. Whitham, and C. A. Gehring. 2014.
662 Tree genotype and genetically based growth traits structure twig endophyte communities.
663 *American Journal of Botany* 101: 467–478.
- 664 Lee, N. L. Y., D. Huang, Z. B. R. Quek, J. N. Lee, and B. J. Wainwright. 2020. Distinct fungal

665 communities associated with different organs of the mangrove *Sonneratia alba* in the Malay
666 Peninsula. *IMA Fungus* 11: 17.

667 Lee, N. L. Y., D. Huang, Z. B. R. Quek, J. N. Lee, and B. J. Wainwright. 2019. Mangrove-
668 associated fungal communities are differentiated by geographic location and host structure.
669 *Frontiers in Microbiology* 10: 2456.

670 Lee, S. Y., J. H. Primavera, F. Dahdouh-Guebas, K. McKee, J. O. Bosire, S. Cannicci, K. Diele,
671 et al. 2014. Ecological role and services of tropical mangrove ecosystems: A reassessment.
672 *Global Ecology and Biogeography* 23: 726–743.

673 Legendre, P., M. J. Fortin, and D. Borcard. 2015. Should the Mantel test be used in spatial
674 analysis? *Methods in Ecology and Evolution* 6: 1239–1247.

675 Lonard, R. I., F. W. Judd, K. Summy, H. DeYoe, and R. Stalter. 2017. The Biological Flora of
676 Coastal Dunes and Wetlands: *Avicennia germinans* (L.) L. *Journal of Coastal Research* 33:
677 191–207.

678 Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads.
679 *EMBnet.journal* 17: 10–12.

680 McMurdie, P. J., and S. Holmes. 2013. Phyloseq: An R Package for Reproducible Interactive
681 Analysis and Graphics of Microbiome Census Data. *PLoS ONE* 8: e61217.

682 McMurdie, P. J., and S. Holmes. 2014. Waste Not, Want Not: Why Rarefying Microbiome Data
683 Is Inadmissible. *PLoS Computational Biology* 10: e1003531.

684 Mori, G. M., M. I. Zucchi, I. Sampaio, and A. P. Souza. 2010. Microsatellites for the mangrove
685 tree *Avicennia germinans* (Acanthaceae): Tools for hybridization and mating system
686 studies. *American Journal of Botany* 97: 79–81.

687 Nagelkerken, I., S. J. M. Blaber, S. Bouillon, P. Green, M. Haywood, L. G. Kirton, J. O.
688 Meynecke, et al. 2008. The habitat function of mangroves for terrestrial and marine fauna:
689 A review. *Aquatic Botany* 89: 155–185.

690 Nettel, A., F. Rafii, and R. S. Dodd. 2005. Characterization of microsatellite markers for the
691 mangrove tree *Avicennia germinans* L. (Avicenniaceae). *Molecular Ecology Notes* 5: 103–
692 105.

693 Nguyen, N. H., D. Smith, K. Peay, and P. Kennedy. 2015. Parsing ecological signal from noise
694 in next generation amplicon sequencing. *New Phytologist* 205: 1389–1393.

695 Nilsson, R. H., K. H. Larsson, A. F. S. Taylor, J. Bengtsson-Palme, T. S. Jeppesen, D. Schigel, P.
696 Kennedy, et al. 2019. The UNITE database for molecular identification of fungi: Handling
697 dark taxa and parallel taxonomic classifications. *Nucleic Acids Research* 47: D259–D264.

698 Ochoa-Zavala, M., J. P. Jaramillo-Correa, D. Piñero, A. Nettel-Hernanz, and J. Núñez-Farfán.
699 2019. Contrasting colonization patterns of black mangrove (*Avicennia germinans* (L.) L.)
700 gene pools along the Mexican coasts. *Journal of Biogeography* 46: 884–898.

701 van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-
702 CHECKER: Software for identifying and correcting genotyping errors in microsatellite
703 data. *Molecular Ecology Notes* 4: 535–538.

704 Osland, M. J., L. C. Feher, K. T. Griffith, K. C. Cavanaugh, N. M. Enwright, R. H. Day, C. L.
705 Stagg, et al. 2017. Climatic controls on the global distribution, abundance, and species
706 richness of mangrove forests. *Ecological Monographs* 87: 341–359.

707 Osland, M. J., L. C. Feher, J. López-Portillo, R. H. Day, D. O. Suman, J. M. Guzmán Menéndez,
708 and V. H. Rivera-Monroy. 2018. Mangrove forests in a rapidly changing world: Global
709 change impacts and conservation opportunities along the Gulf of Mexico coast. *Estuarine,
710 Coastal and Shelf Science* 214: 120–140.

711 Palmer, J. M., M. A. Jusino, M. T. Banik, and D. L. Lindner. 2018. Non-biological synthetic
712 spike-in controls and the AMPtk software pipeline improve mycobiome data. *PeerJ* 6:
713 e4925.

714 Pan, J. J., A. M. Baumgarten, and G. May. 2008. Effects of host plant environment and *Ustilago*
715 *maydis* infection on the fungal endophyte community of maize (*Zea mays*). *New*
716 *Phytologist* 178: 147–156.

717 Pauvert, C., M. Buée, V. Laval, V. Edel-Hermann, L. Fauchery, A. Gautier, I. Lesur, et al. 2019.
718 Bioinformatics matters: The accuracy of plant and soil fungal community data is highly
719 dependent on the metabarcoding pipeline. *Fungal Ecology* 41: 23–33.

720 Peakall, R., and P. E. Smouse. 2012. GenA1Ex 6.5: Genetic analysis in Excel. Population genetic
721 software for teaching and research - an update. *Bioinformatics* 28: 2537–2539.

722 Pecl, G. T., M. B. Araújo, J. D. Bell, J. Blanchard, T. C. Bonebrake, I. C. Chen, T. D. Clark, et
723 al. 2017. Biodiversity redistribution under climate change: Impacts on ecosystems and
724 human well-being. *Science* 355: eaai9214.

725 Pil, M. W., M. R. T. Boeger, V. C. Muschner, M. R. Pie, A. Ostrensky, and W. A. Boeger. 2011.
726 Postglacial north-south expansion of populations of *Rhizophora mangle* (Rhizophoraceae)
727 along the Brazilian coast revealed by microsatellite analysis. *American Journal of Botany*
728 98: 1031–1039.

729 Piovia-Scott, J. 2011. Plant phenotype influences the effect of ant mutualists on a polymorphic
730 mangrove. *Journal of Ecology* 99: 327–334.

731 Pironon, S., G. Papuga, J. Villellas, A. L. Angert, M. B. García, and J. D. Thompson. 2017.
732 Geographic variation in genetic and demographic performance: new insights from an old
733 biogeographical paradigm. *Biological Reviews* 92: 1877–1909.

734 Pohjanmies, T., A. J. M. Tack, P. Pulkkinen, S. Elshibli, P. Vakkari, and T. Roslin. 2015.
735 Genetic diversity and connectivity shape herbivore load within an oak population at its
736 range limit. *Ecosphere* 6: 101.

737 Porrás-Alfaro, A., and P. Bayman. 2011. Hidden Fungi, Emergent Properties: Endophytes and
738 Microbiomes. *Annual Review of Phytopathology* 49: 291–315.

739 Quinn, T. P., I. Erb, M. F. Richardson, and T. M. Crowley. 2018. Understanding sequencing data
740 as compositions: An outlook and review. *Bioinformatics* 34: 2870–2878.

741 R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for
742 Statistical Computing, Vienna, Austria. URL: <https://R-project.org>.

743 Raffard, A., F. Santoul, J. Cucherousset, and S. Blanchet. 2019. The community and ecosystem
744 consequences of intraspecific diversity: a meta-analysis. *Biological Reviews* 94: 648–661.

745 Ricks, K. D., and R. T. Koide. 2019. Biotic filtering of endophytic fungal communities in
746 *Bromus tectorum*. *Oecologia* 189: 993–1003.

747 Rodriguez, R. J., R. S. Redman, and J. M. Henson. 2004. The role of fungal symbioses in the
748 adaptation of plants to high stress environments. *Mitigation and Adaptation Strategies for*
749 *Global Change* 9: 261–272.

750 Rodriguez, W., I. C. Feller, and K. C. Cavanaugh. 2016. Spatio-temporal changes of a
751 mangrove–saltmarsh ecotone in the northeastern coast of Florida, USA. *Global Ecology and*
752 *Conservation* 7: 245–261.

753 De Ryck, D. J. R., N. Koedam, T. Van der Stocken, R. M. van der Ven, J. Adams, and L. Triest.
754 2016. Dispersal limitation of the mangrove *Avicennia marina* at its South African range
755 limit in strong contrast to connectivity in its core East African region. *Marine Ecology*
756 *Progress Series* 545: 123–134.

757 Schwarz, G. 1978. Estimating the dimension of a model. *Annals of Statistics* 6: 461–464.

758 Silva, L. L., R. C. O. Santos, and M. E. B. Fernandes. 2017. Linking *Avicennia germinans*
759 (Acanthaceae) architecture to gall richness and abundance in Brazilian Amazon mangroves.
760 *Biotropica* 49: 784–791.

761 Smith, D. P., and K. G. Peay. 2014. Sequence depth, not PCR replication, improves ecological
762 inference from next generation DNA sequencing. *PLoS ONE* 9: e90234.

763 Smith, R. S., T. Z. Osborne, I. C. Feller, and J. E. Byers. 2019. Detrital traits affect
764 substitutability of a range-expanding foundation species across latitude. *Oikos* 128: 1367–
765 1380.

766 Smouse, P. E., and R. Peakall. 1999. Spatial autocorrelation analysis of individual multiallele
767 and multilocus genetic structure. *Heredity* 82: 561–573.

768 Sobrado, M. A., and E. D. Greaves. 2000. Leaf secretion composition of the mangrove species
769 *Avicennia germinans* (L.) in relation to salinity: A case study by using total-reflection X-ray
770 fluorescence analysis. *Plant Science* 159: 1–5.

771 Soldan, R., F. Mapelli, E. Crotti, S. Schnell, D. Daffonchio, R. Marasco, M. Fusi, et al. 2019.
772 Bacterial endophytes of mangrove propagules elicit early establishment of the natural host
773 and promote growth of cereal crops under salt stress. *Microbiological Research* 223–225:
774 33–43.

775 de Souza Sebastianes, F. L., A. S. Romão-Dumaresq, P. T. Lacava, R. Harakava, J. L. Azevedo,
776 I. S. De Melo, and A. A. Pizzirani-Kleiner. 2013. Species diversity of culturable endophytic
777 fungi from Brazilian mangrove forests. *Current Genetics* 59: 153–166.

778 Stevenson, C. R., C. Davies, and J. K. Rowntree. 2017. Biodiversity in agricultural landscapes:
779 The effect of apple cultivar on epiphyte diversity. *Ecology and Evolution* 7: 1250–1258.

780 Suárez, N., and E. Medina. 2008. Salinity effects on leaf ion composition and salt secretion rate
781 in *Avicennia germinans* (L.) L. *Brazilian Journal of Plant Physiology* 20: 131–140.

782 Szulkin, M., N. Bierne, and P. David. 2010. Heterozygosity-fitness correlations: A time for
783 reappraisal. *Evolution* 64: 1202–1217.

784 Tack, A. J. M., O. Ovaskainen, P. Pulkkinen, and T. Roslin. 2010. Spatial location dominates
785 over host plant genotype in structuring an herbivore community. *Ecology* 91: 2660–2672.

786 Tomlinson, P. B. 1986. *The Botany of Mangroves*. Cambridge University Press, Cambridge, UK.

787 Tovar-Sánchez, E., L. Valencia-Cuevas, E. Castillo-Mendoza, P. Mussali-Galante, R. V Pérez-
788 Ruiz, and A. Mendoza. 2013. Association between individual genetic diversity of two oak
789 host species and canopy arthropod community structure. *European Journal of Forest*
790 *Research* 132: 165–179.

791 U'Ren, J. M., J. M. Riddle, J. T. Monacell, I. Carbone, J. Miadlikowska, and A. E. Arnold. 2014.
792 Tissue storage and primer selection influence pyrosequencing-based inferences of diversity
793 and community composition of endolichenic and endophytic fungi. *Molecular Ecology*
794 *Resources* 14: 1032–1048.

795 UNITE Community. 2019. UNITE general FASTA release for Fungi 2. Version 18.11.2018.
796 UNITE Community. URL: <https://doi.org/10.15156/BIO/786353>.

797 Valencia-Cuevas, L., P. Mussali-Galante, Z. Cano-Santana, J. Pujade-Villar, A. Equihua-
798 Martínez, and E. Tovar-Sánchez. 2018. Genetic variation in foundation species governs the
799 dynamics of trophic interactions. *Current Zoology* 64: 13–22.

800 Valkama, E., J. Koricheva, J. P. Salminen, M. Helander, I. Saloniemi, K. Saikkonen, and K.
801 Pihlaja. 2005. Leaf surface traits: Overlooked determinants of birch resistance to herbivores
802 and foliar micro-fungi? *Trees - Structure and Function* 19: 191–197.

803 Vanegas, J., A. Muñoz-García, K. A. Pérez-Parra, I. Figueroa-Galvis, O. Mestanza, and J.
804 Polanía. 2019. Effect of salinity on fungal diversity in the rhizosphere of the halophyte
805 *Avicennia germinans* from a semi-arid mangrove. *Fungal Ecology* 42: 100855.

806 Vega, F. E., A. Simpkins, M. C. Aime, F. Posada, S. W. Peterson, S. A. Rehner, F. Infante, et al.
807 2010. Fungal endophyte diversity in coffee plants from Colombia, Hawai'i, Mexico and
808 Puerto Rico. *Fungal Ecology* 3: 122–138.

809 White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal
810 ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Geffland, J. J. Sninsky, and
811 T. J. White [eds.], PCR protocols: A guide to method and applications, 315–322. Academic
812 Press, San Diego, CA.

813 Whitham, T. G., J. K. Bailey, J. A. Schweitzer, S. M. Shuster, R. K. Bangert, C. J. Leroy, E. V.
814 Lonsdorf, et al. 2006. A framework for community and ecosystem genetics: From genes to
815 ecosystems. *Nature Reviews Genetics* 7: 510–523.

816 Whitham, T. G., C. A. Gehring, L. J. Lamit, T. Wojtowicz, L. M. Evans, A. R. Keith, and D. S.
817 Smith. 2012. Community specificity: life and afterlife effects of genes. *Trends in Plant
818 Science* 17: 271–281.

819 Whitham, T. G., W. P. Young, G. D. Martinsen, C. A. Gehring, J. A. Schweitzer, S. M. Shuster,
820 G. M. Wimp, et al. 2003. Community and ecosystem genetics: a consequence of the
821 extended phenotype. *Ecology* 84: 559–573.

822 Wimp, G. M., W. P. Young, S. A. Woolbright, G. D. Martinsen, P. Keim, and T. G. Whitham.
823 2004. Conserving plant genetic diversity for dependent animal communities. *Ecology
824 Letters* 7: 776–780.

825 Younginger, B. S., and D. J. Ballhorn. 2017. Fungal endophyte communities in the temperate
826 fern *Polystichum munitum* show early colonization and extensive temporal turnover.
827 *American Journal of Botany* 104: 1188–1194.

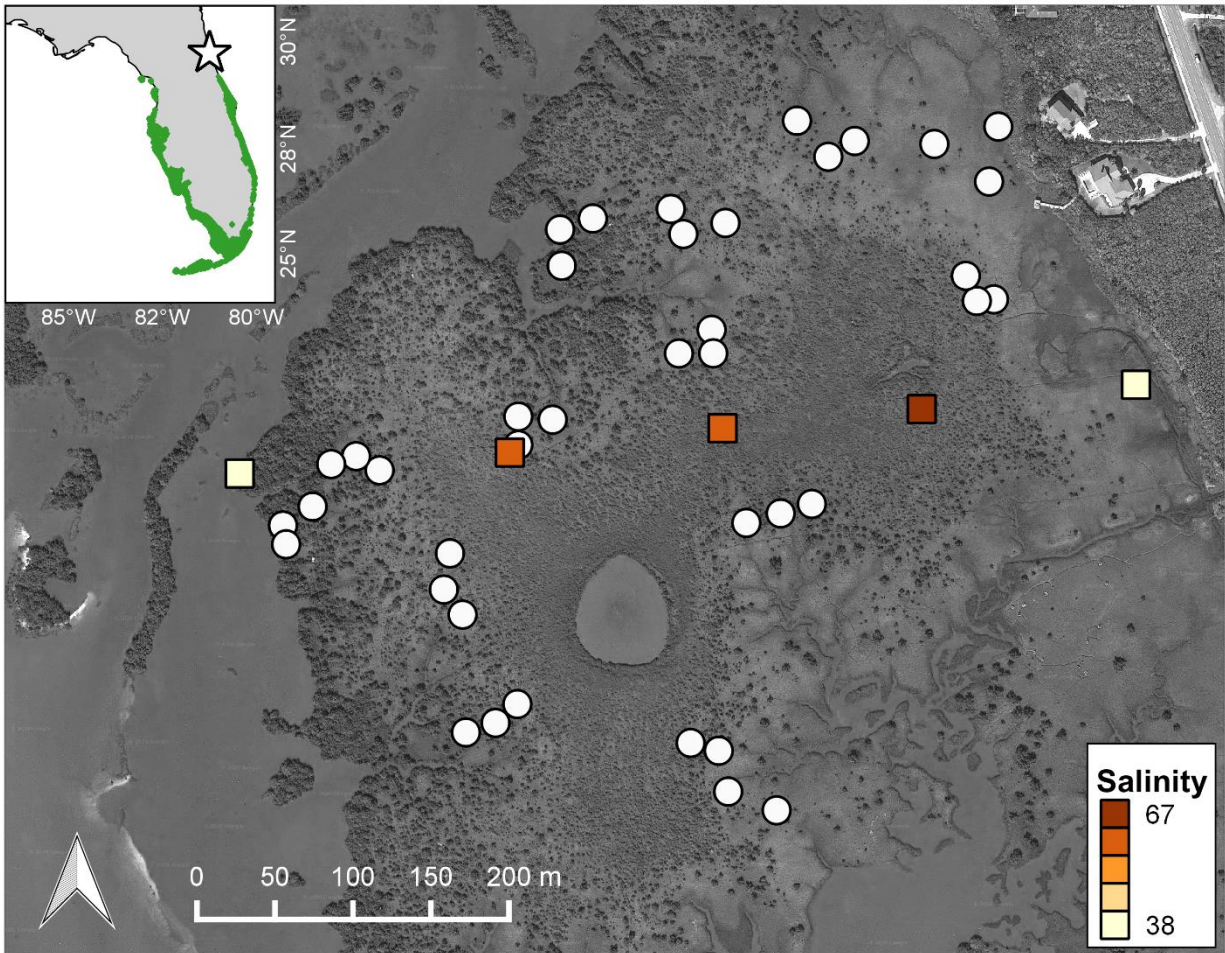
828 Zytynska, S. E., M. F. Fay, D. Penney, and R. F. Preziosi. 2011. Genetic variation in a tropical
829 tree species influences the associated epiphytic plant and invertebrate communities in a
830 complex forest ecosystem. *Philosophical Transactions of the Royal Society B: Biological
831 Sciences* 366: 1329–1336.

832

833 **Table 1.** Multiple linear regressions of alpha diversity of associated endophytic fungal
834 communities as a function of the heterozygosity and absolute spatial location of host trees. Alpha
835 diversity of associated communities was calculated with Hill numbers at the scales of $q=0$
836 (species richness), $q=1$ (exponential of Shannon index), and $q=2$ (inverse of Simpson index),
837 which put more weight on abundant species as the value of q increases. Bold values indicate
838 statistical significance ($p < 0.05$).

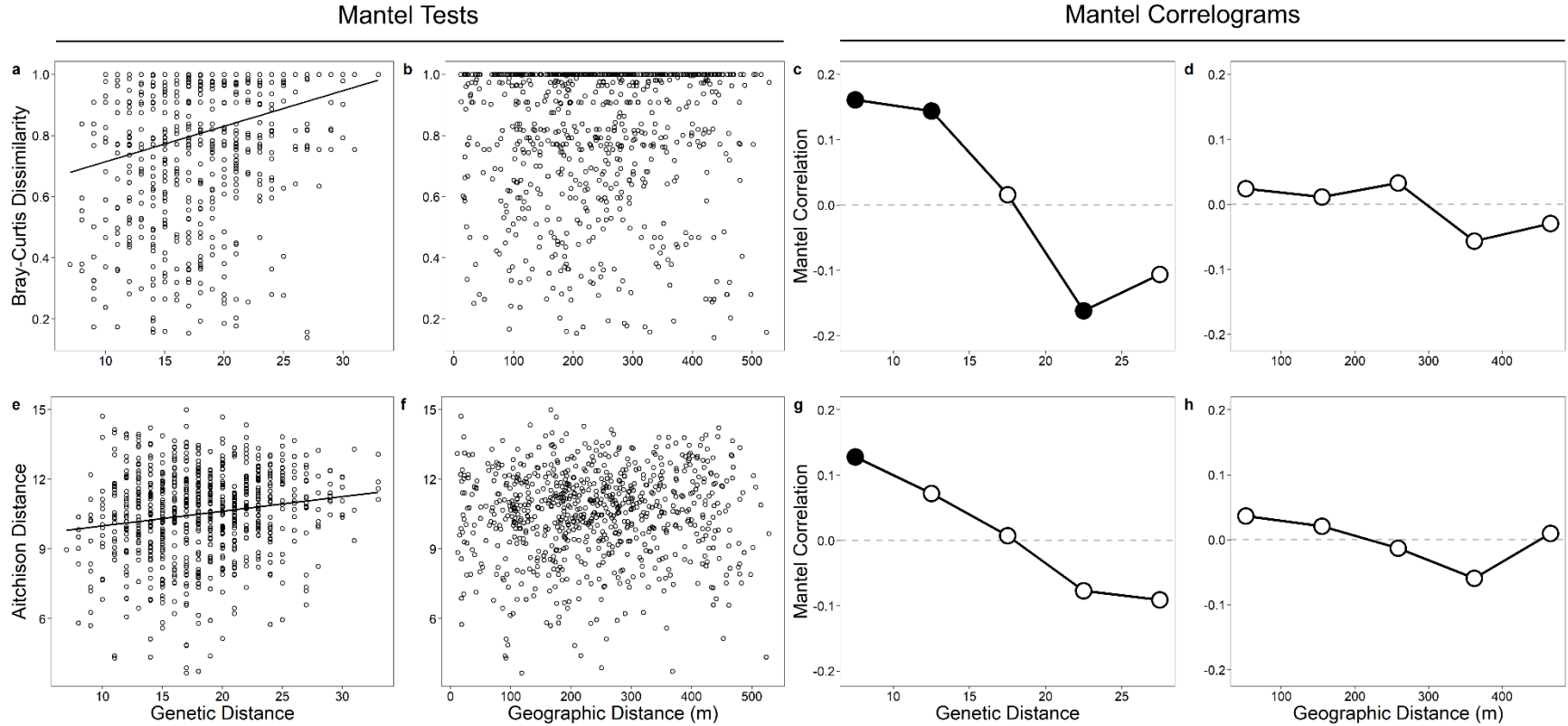
Response	Predictor	Estimate	SE	t	p
q=0	Heterozygosity	-0.01	0.16	-0.12	0.909
	Longitude	0.37	0.18	2.10	0.043
	Latitude	-0.26	0.18	-1.47	0.150
q=1	Heterozygosity	-0.08	0.15	-0.55	0.588
	Longitude	0.47	0.17	2.76	0.009
	Latitude	-0.11	0.17	-0.65	0.520
q=2	Heterozygosity	-0.10	0.15	-0.66	0.515
	Longitude	0.46	0.17	2.74	0.009
	Latitude	-0.05	0.17	-0.29	0.772

839



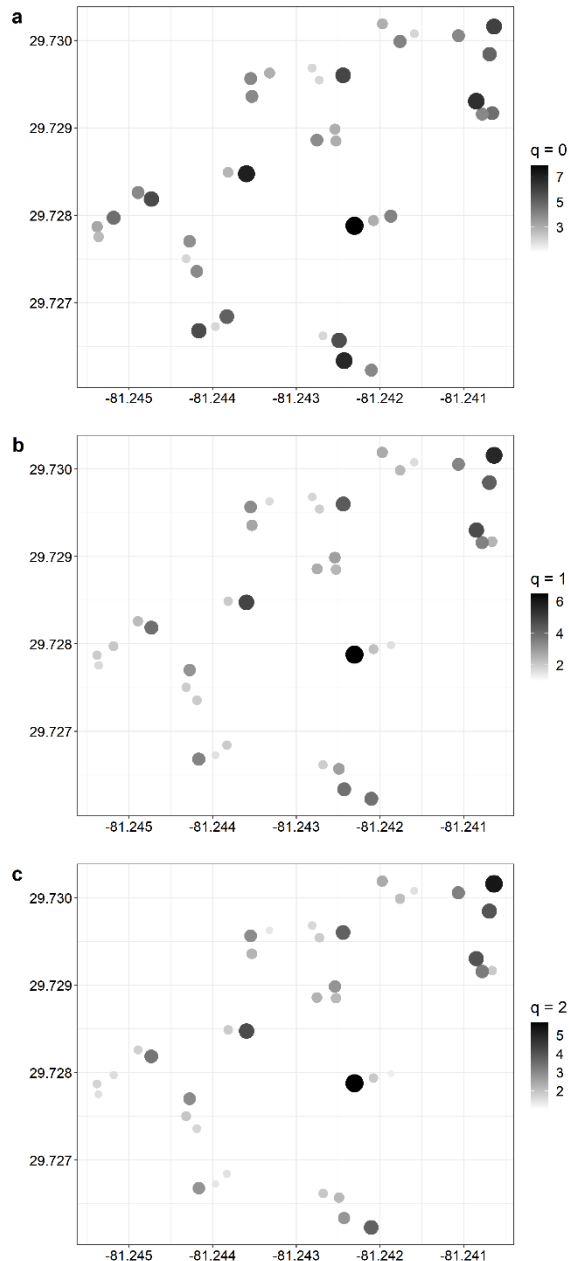
840

841 **Figure 1.** Collection site at the Atlantic Florida, USA, northern distributional limit of *Avicennia*
 842 *germinans* with locations of the 40 sampled *A. germinans* trees. This site is flanked by a brackish
 843 lagoon to the west and a fringe of terrestrial forest to the east. Soil salinities (‰) are mean values
 844 measured between September and November (2012–2017) (Guana Tolomato Matanzas National
 845 Estuarine Research Reserve, *unpublished data*). Upper panel shows the location of the collection
 846 site (with a star) and the Florida mangrove distribution in green (Giri et al., 2011).



847

848 **Figure 2.** Genetically-similar mangrove host trees harboured similar associated endophytic fungal communities, independent of
 849 geographic distances among these host trees. Panels show graphical representations of the relationships between fungal community
 850 dissimilarity (measured with Bray-Curtis dissimilarity and Aitchison distance) and each of the two predictor variables (inter-host
 851 genetic distance and inter-host geographic distance) across all mangrove host trees (Mantel tests) and at five distance classes (Mantel
 852 correlograms). Statistically significant ($p < 0.05$) correlations between fungal community dissimilarity and inter-host genetic
 853 distance(s) are depicted with solid lines for Mantel tests and with black circles for Mantel correlograms.



854

855 **Figure 3.** Spatial distribution of the alpha diversity of associated endophytic fungal communities
 856 within 40 *Avicennia germinans* trees across a collection site at the northern distributional limit of
 857 this species. Alpha diversity was calculated with Hill numbers at the scales of (a) $q=0$ (species
 858 richness), (b) $q=1$ (exponential of Shannon index), and (c) $q=2$ (inverse of Simpson index),
 859 which put more weight on abundant species as the value of q increases. In the figure, values of
 860 fungal alpha diversity for each tree increase with colour (from white to black) and with the size
 861 of the data point.