Double Lives: Transfer of fungal endophytes from leaves to woody substrates

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Fungal endophytes are a ubiquitous feature of plants, yet for many fungi the benefits of endophytism are still unknown. The Foraging Ascomycete (FA) hypothesis proposes that saprotrophic fungi can utilize leaves as both as dispersal vehicles and as resource havens during times of scarcity. The presence of saprotrophs in leaf endophyte communities has been previously observed but their ability to transfer to extra-foliar saprobic substrates has not been well-investigated. To assess this ability, we conducted a culture study by placing surface-sterilized leaves from single tropical angiosperm tree (*Nectandra lineatifolia* Mez) directly onto sterile wood fragments and incubating them for 6 weeks. Fungi from the wood were subsequently isolated in culture and identified by ITS sequences or morphology to the genus level. 477 fungal isolates comprising 24 distinct taxa were cultured from the wood. Of these, 70.8% (82.3% of isolates) belong to saprotrophic genera according to the FUNGuild database. Furthermore, 27% of OTUs (6% of isolates) were basidiomycetes, an unusually high proportion compared to typical endophyte communities. *Xylaria flabelliformis*, although absent in our original isolations, formed anamorphic fruiting structures on the woody substrates. We introduce the term *viaphyte* (literally, "by way of plant") to refer to fungi that undergo an interim stage as leaf endophytes and, after leaf senescence, colonize other sabrobic substrates via hyphal growth. Our results support the Foraging Ascomycete Hypothesis and suggest that viaphytism may play a significant role in the dispersal of fungal saprotrophs.

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ABSTRACT

Fungal endophytes are a ubiquitous feature of plants, yet for many fungi the benefits of

endophytism are still unknown. The Foraging Ascomycete (FA) hypothesis proposes that

saprotrophic fungi can utilize leaves as both as dispersal vehicles and as resource havens during

times of scarcity. The presence of saprotrophs in leaf endophyte communities has been

previously observed but their ability to transfer to extra-foliar saprobic substrates has not been

well-investigated. To assess this ability, we conducted a culture study by placing surface-

sterilized leaves from single tropical angiosperm tree (*Nectandra lineatifolia* Mez) directly onto

sterile wood fragments and incubating them for 6 weeks. Fungi from the wood were

subsequently isolated in culture and identified by ITS sequences or morphology to the genus

level. 477 fungal isolates comprising 24 distinct taxa were cultured from the wood. Of these,

70.8% (82.3% of isolates) belong to saprotrophic genera according to the FUNGuild database.

Furthermore, 27% of OTUs (6% of isolates) were basidiomycetes, an unusually high proportion

compared to typical endophyte communities. *Xylaria flabelliformis*, although absent in our

original isolations, formed anamorphic fruiting structures on the woody substrates. We introduce

the term *viaphyte* (literally, "by way of plant") to refer to fungi that undergo an interim stage as

leaf endophytes and, after leaf senescence, colonize other sabrobic substrates via hyphal growth.

Our results support the Foraging Ascomycete Hypothesis and suggest that viaphytism may play a

significant role in the dispersal of fungal saprotrophs.

KEYWORDS

Ascomycota; Basidiomycota; ecological theory; foraging ascomycete; fungi; life history;

saprotroph; viaphyte; *Xylaria*

36 **Introduction**

37 Endophytes are symptomless endosymbionts of living plants (Stone, Bacon & White, 2000) and

38 are ubiquitously present in terrestrial plant tissues worldwide (Arnold & Lutzoni, 2007).

39 Virtually every plant surveyed to date has turned up several to hundreds of species of fungal

40 endophytes per individual, and a single plant species may host thousands of these symbionts

41 across its entire range (Arnold & Lutzoni, 2007; Rodriguez et al., 2009). The effects of

42 endophytes on host plants are varied, and have attracted considerable attention (Carroll, 1988;

43 Rodriguez et al., 2009). Here, we examine a possible benefit of endophytic life histories for the

44 *fungal* partners.

45   The question of why fungi may adopt endophytic lifestyles has garnered a variety of

46 hypotheses, although few studies have directly addressed them.

47   Some endophytes may be *latent saprotrophs*, which benefit from priority effects (Chase,

48 2003; Osono, 2006) by being the first to colonize plant tissues after senescence or death of the

49 host (Promputtha et al., 2007; Parfitt et al., 2010; Porras-Alfaro & Bayman, 2011; Szink et al.,

50 2016). A majority of endophytes have been observed in the litter layer as decomposers (Osono,

51 2006), although most of these are restricted to the early stages of litter decomposition, when

52 there is higher availability of simple sugars and easily degradable compounds (Carroll & Petrini,

53 1983). Some endophytes also persist into the late stages of litter decomposition (Peršoh et al.,

54 2013). Certain endophytes have demonstrated an ability to degrade more complex substrates,

55 such as lignin, which further suggests that these may play a role during later stages of litter decay

56 (Osono & Takeda, 1999). Parfitt and colleagues (2010) suggest that most, if not all, trees carry

57 sapwood endophytes with the potential to degrade them, controlled by environmental and

58 biological conditions within the woody tissues themselves.

59   Some endophytes are considered *mutualists*, with their fitness directly tied to that of their

60   hosts, as is common in the clavicipitaceous grass endophytes, which benefit from direct vertical

61   transmission to their hosts' offspring (Clay, 1988; Hodgson et al., 2014). Finally, others may be

62   *latent pathogens* waiting to exploit a weakened state of their host (Carroll, 1988; Slippers &

63   Wingfield, 2007).

64   However, the vast majority of observed endophytic fungi do not fit neatly into one of

65   these categories. For instance, a number of major wood decomposers have been found to be

66   common endophytes in leaves (Promputtha et al., 2007), but the benefits of such leaf

67   colonization, for taxa that do not fruit on leaves, is obscure. It has been proposed that such

68   endophytic colonization represents an evolutionary "dead-end" (*i.e.*, saprotrophs found as

69   endophytes are unlikely to escape that state), an idea initially supported by the fact that many

70   endophyte infections occupy only one or a few host plant cells (Carroll, 1988; Bayman et al.,

71   1998; Arnold & Lutzoni, 2007), and more recently by observation that endophyte communities

72   are often different between twigs and leaves of the same host (Sun et al., 2012; Tateno et al.,

73   2015; Thomas et al., 2019), because the two tissues are likely colonized independently and

74   through different mechanisms (Peršoh, 2013).

75   Because the colonization of live plant tissues requires specialized chemical and physical

76   systems (Kusari, Hertweck & Spiteller, 2012), the construction of such cellular mechanisms

77   during development, along with with propagule loss, incurs evolutionary costs that are

78   unaccompanied by benefits if endophytism is truly a 'dead end' for these fungi (Carroll, 1988;

79   Thomas et al., 2016, 2019)(Thomas et al., 2016)(Carroll, 1988; Thomas et al., 2016, 2019). One

80   possible explanation for this discrepancy is the **Foraging Ascomycete (FA) hypothesis** (Carroll,

81   1999; Thomas et al., 2016; Thomas, Vandegrift & Roy, 2017)(Thomas et al., 2016) (Carroll,

82   1999; Thomas et al., 2016; Thomas, Vandegrift & Roy, 2017), which proposes that the function

83   of leaf endophytism for some fungi may be to increase dispersal to other substrates by helping to

84   bridge spatiotemporal gaps in prefered substrate. While some saprotrophic endophytes canfruit

85   directly from fallen leaves, the FA hypothesis proposes that after leaves senesce and fall, the

86   endophytes are capable of colonizing other, preferred substrates in the environment. Thus, during

87   times of environmental scarcity, endophytes may have an increased likelihood of survival

88   compared to spores or saprobic mycelia, because the highly buffered environment of leaves

89   provides a source of food and water regardless of surrounding environmental conditions

90   (Thomas et al., 2016). The investment of spores into leaf colonization is essentially a form of

91   evolutionary bet-hedging, a strategy that "reduces the temporal variance in fitness at the expense

92   of a lowered arithmetic mean fitness" (Ripa, Olofsson & Jonzén, 2010). Direct spore dispersal by

93   itself may result in a higher mean success rate in colonizing substrates, but it will be highly

94   variable depending on environmental conditions. When a subset of spores from each sporulation

95   event become endophytes, they decrease this variance of dispersal success (Thomas et al., 2016).

96   To encompass the processes described by the FA hypotheses, we introduce the new term

97   *viaphyte* to refer to fungi that undergo these lifestyle shifts. We do this because (1) referring to

98   such fungi as "foragers" is vague and leads to confusion, and (2) referring to them as "foraging

99   ascomycetes" (or "FA utilizing fungi" and other such permutations) is unwieldy and inaccurate,

100   as a subset of endophytes in the Basidiomycota likely utilize this dispersal strategy as well.

101   "Viaphyte" is a portmanteau that joins the word *via* — defined as "travelling through a place en

102   route to a destination" — with the suffix, *phyte,* which denotes a plant. Generally, the term refers

103   to fungi that colonize living leaves as endophytes and use those leaves to transfer to another

104   substrate when they fall. In this study, we use the term specifically to refer to fungi which

105   display the ability to transfer from an endophytic state (inhabiting living leaf tissue, necessarily

106   biotrophic) to a free-living state (inhabiting a dead woody substrate, necessarily saprotrophic).

107   For the FA hypothesis to be feasible —for viaphytism to occur — it must be shown that

108   transfer from living leaves to another substrate is possible. Thomas and Vandegrift et al.

109   (Thomas et al., 2016) observed this transfer, but they restricted the scope of their work to a single

110   fungal genus, *Xylaria*. It is unclear how prevalent this ability is among other fungal endophytes.

111   Here, we set out to conduct a more inclusive survey of the viaphytic abilities of endophytes

112   present in tropical leaves (a hotspot for endophyte diversity; Arnold & Lutzoni, 2007). We also

113   assessed (1) the overall diversity of observed viaphytes, (2) how populations of viaphytes vary

114   between leaves on the same host, and (3) the presumed ecological roles of each isolated

115   viaphytic fungus. Leaf endophytes are hyperdiverse and have a wide taxonomic breadth. As a

116   subset of the endophytic community, we expected that viaphytes would also represent a wide

117   taxonomic breadth. Despite the fact that source communities were likely to harbor many

118   biotrophs capable of facultative saprotrophy, based on the framework of the FA hypothesis we

119   hypothesized that the majority of viaphytes isolated would be taxa whose primary nutritional

120   mode is saprotrophy.

121

122

123 **Materials and Methods**

124 *Culture Methods*

125   Twelve evergreen leaves of a randomly selected tree (Lauraceae; *Nectandra lineatifolia*

126 (Ruiz & Pav.) Mez) were collected in an Ecuadorian cloud forest. The tree was within Reserva

127 Los Cedros, which is on the western slope of the Andes in northwestern Ecuador (00°18031.000

128 N, 78°46044.600 W), at 1200m above sea level. Eight 2-cm^2 sections were cut from each leaf

129 and surface-sterilized by successive immersion in 70% ethanol for one min, 5% sodium

130 hypochlorite (equivalent to full strength bleach) for two min, then rinsed in sterile water. The

131 leaf sections were placed onto twice-autoclaved white birch (*Betula papyrifera* Marshall) tongue

132 depressors (Puritan, Guilford, Maine, U.S.A.) as a standardized angiosperm woody substrate.

133 The sections from each leaf were split between two tongue depressors (4 sections each) resulting

134 in a total of 24 tongue depressors. These were incubated in three 95% EtOH-sterilized Ziploc

135 storage boxes (eight in each box) at the field station in ambient temperature for six weeks. Each

136 box contained an open container of twice-autoclaved water to maintain humidity. The incubation

137 period provided opportunity for the endophytic fungi in the leaves to colonize the wood. After

138 incubation, the sticks were placed into airtight, sterile bags and brought to our lab at the

139 University of Oregon.

140   Fungal cultures were isolated from the inoculated wood by breaking 15 small fragments

141 (~5 mm^2 each) of wood from each tongue depressor using flame-sterilized tools and dispersing

142 them evenly among five 100 mm water agar plates. The ends of growing hyphae were excised

143 from the agar using a dissecting microscope and a scalpel and transferred onto nutrient plates

144 (MEA, 2% maltose) over a two-month period. Cultures were also made from several fruiting

145 structures that grew directly from the birch substrate fragments. After a growth period of seven

146   or more days the isolates were grouped into morphotypes (Lacap, Hyde & Liew, 2003) at the

147   genus level based on macro- and microscopic features.

148   All field work was done with the approval of the Ecuadorian Ministry of the Environment

149   (Ministerio del Ambiente de Ecuador, Permit No. 03-2011-IC-FLO-DPAI/MA).

150   *Identification of Viaphytes*

151   A single representative of each morphotype was subcultured in liquid media (MEA) for

152   DNA extraction using the Qiagen DNeasy Plant kit following the manufacturer's instructions,

153   and the ITS region (the standard "barcode" gene for fungi; Schoch et al., 2012) was amplified

154   using the fungal-specific primer set ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and

155   ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990), or in cases where those

156   primers were ineffective, with ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and LR3 (5'-

157   CCGTGTTTCAAGACGGG-3') primers. DNA amplification was conducted with 12.5-μL

158   reaction volumes (2.5 μL of template, 6.25 μL of Sigma Aldrich JumpstartTM Taq

159   ReadymixTM, 2.75 μL sterile water, 0.5 μL 25 mM MgCl2, and 0.25 μL of each primer). PCR

160   amplification was done with an MJ Research PTC-200 DNA Engine thermal cycler with the

161   following parameters: initial denaturation at 95˚C for 2 min, five cycles of denaturation at 95˚C

162   for 30 s, annealing at 60˚C for 30 s, and extension at 72˚C for 1 min; followed by 25 cycles of

163   denaturation of 95˚C for 30 s, annealing at 55˚C for 30 s, and extension at 72˚C for 1 min; a final

164   extension at 72˚C for 10 min and a final step of indefinite duration at 4˚C. PCR products were

165   visualized on a 1 percent agarose gel. Samples were then frozen until shipping for sequencing at

166   Functional Biosciences, Inc (Madison, WI, U.S.A.) on ABI 3730xl instruments using Big Dye

167   V3.1.The ITS amplicons were sequenced in both directions, then paired and edited using

168   Geneious (v6.0.3; Biomatters Limited, Auckland, New Zealand); sequences were trimmed to

169   remove priming sites and consensus sequences were generated, with any mismatches checked

170   against the chromatograms. The consensus sequences were then compared to published

171   sequences in the UNITE database (v8.0; Kõljalg et al., 2013) using the *assign_taxonomy.py*

172   function from the Quantitative Insights into Microbial Ecology (QIIME) pipeline (Caporaso et

173   al., 2010). Taxa that returned species assignments as "unidentified" were further examined, and

174   the lowest E-Value match with greater than 97% sequence identity across the entire ITS region,

175   with taxonomic assignment at genus level or better, was used as the taxon assignment. If there

176   was no match above 97% sequence identity, sequences were left as "unidentified". Putative

177   *Xylaria* species were compared to our database of ITS sequences generated from authenticated

178   material within that genus at the same site (Thomas et al., 2016), and assigned to a taxon if the

179   there was greater than 98% sequence identity. Taxa with greater than 99% sequence identity

180   were assumed to be the same taxon (i.e., OTU); all taxa with identical assignments by UNITE

181   met this criteria.

182   Functional guilds were assigned to each genus by using the FUNGuild online tool

183   (Nguyen et al., 2016), which assigns functional information to taxa in DNA datasets. If

184   functional guilds were not available in FUNGuild, they were determined based on the literature

185   wherever possible.

186   *Statistical Methods*

187   Species richness was estimated using Chao2 and Jacknife1 estimators (Burnham &

188   Overton, 1978; Chao, 1984; Colwell & Coddington, 1994). Diversity was estimated between all

189   leaves, within leaves, and within boxes using Shannon's index (log base *e* was used; Shannon,

190   1948) and Simpson's index (1-*D*; Simpson, 1949), and community structure was visualized using

191   non-metric multidimensional scaling (NMDS) and differences assessed with permutational

192 multivariate analysis of variance (PerMANOVA). Data were analyzed using R Statistical

193 Software, v. 3.1.0 (R Core Team 2014), including the *vegan* package (Oksanen et al., 2013).

194   All scripts, data tables, and raw data (morphotype counts and sequence chromatograms)

195 is available via an open FigShare repository (Nelson et al., 2019). Edited sequences have been

196 upload GeneBank (Table S1).

197

198 **Results**

199 *Diversity and Abundance of Viaphytes*

200   Numerous endophytes were able to transfer from leaves into wood. 477 fungal cultures

201 were isolated after making the initial transfer from leaves to wood. These were grouped into 64

202 morphotypes, 62 of which were successfully identified to genus (59 by DNA, 3 by morphology;

203 Table S1). DNA identification resulted in the consolidation of the morphotypes into 24 unique

204 taxa at the genus level (Table S2). The number of isolates for each taxon varied widely, with

205 57% of the isolates represented by just 2 genera (*Trichoderma* and *Penicillium*) while 7 of the

206 taxa were isolated only once (Fig. 1). In addition to hyphal growth from the wood substrates,

207 anamorphic fruiting structures were observed growing out of five stick fragments originating

208 from two leaves (Fig. S1), which were identified as *Xylaria flabelliformis* using DNA extracted

209 from stromatic tissues. Including the *X. flabelliformis,* a total of 24 viaphytic taxa, identified to

210 the genus level, were observed (Fig. 1). Additionally, we observed that several of the woody

211 substrate fragments were consumed by apparent white-rot, as evidenced by a white appearance

212 and a dramatic decrease in substrate volume, though we did not attempt to determine which taxa

213 were responsible for this dramatic reduction in volume.

214   The species accumulation curve did not reach a saturation point, suggesting

215   that the full richness of viaphytes from these leaves was not isolated (Fig. 2). Estimates of actual

216   species richness ranged from 36.5 (first order jackknife, $SE = 4.1$) to 42.3 (chao2, $SE = 13.8$).

217   Viaphyte communities within incubation boxes were more similar to each other than to

218   communities from other boxes (PerMANOVA: $F_{1, 23} = 6.34$, $p = 0.001$) while communities

219   between sticks that were inoculated by the same leaves were *not* more similar to each other than

220   to other sticks (PerMANOVA: $F_{1, 23} = 1.04$, $p = 0.404$; Fig. 3). Isolates of the four most common

221   taxa were concentrated in common boxes, with 100% of *Neopestalotiopsis foedans* in Box 1 (44

222   total isolates across all boxes), 96% of *Paecilomyces formosus* in Box 1 (75 total isolates), 87%

223   of *Trichoderma spp.* in Box 2 (89 total isolates), and 61% of *Penicillium spp.* in Box 3 (179 total

224   isolates).

225   *Taxonomic Distribution*

226   The higher order taxonomic ranks in our samples included two phyla, five classes, twelve

227   orders, and nineteen families (Table S2). Both Ascomycota and Basidiomycota were present,

228   though Ascomycota was the dominant phylum, both in terms of number of taxa and total number

229   of isolates (73% and 94%, respectively). Sordariomycetes were the most common class in terms

230   of taxa (38.4%), while Eurotiomycetes, driven by the frequency of *Penicillium spp.*, had the most

231   isolates (55.7%). Xylariales and Polyporales were the most common orders in terms of taxa

232   (19.2% each), while Eurotiales, again driven by *Penicillium spp.*, had the most isolates (55.1%).

233   *Functional Guilds*

234   The FUNGuild database contained putative functional guilds for all but two of the genera

235   we isolated as viaphytes. The first unassigned genus, *Alloconiothyrium,* is newly described and

236   presently represented by a single species, *A. aptrootii*, which was isolated from a soil sample in

237   Papua New Guinea (Verkley et al. 2014). We therefore did not assign it to a functional guild

238   since so little information is available. The second, *Neopestalotiopsis,* we classified as a "plant

239   pathogen/saprotroph" based on substrates listed in species descriptions (Maharachchikumbura et

240   al. 2014). The viaphyte genera of our study fit into three distinct functional guilds: *saprotroph*,

241   *plant pathogen*, and *plant pathogen/saprotroph*. Saprotroph was the dominant functional guild in

242   terms of number of genera (70.8%; 17 out of 24) and number of isolates (82.3%, 389 out of 467).

243   Four of the genera were plant pathogens (16.7%) and three were plant pathogen/saprotrophs

244   (12.5%). Of the isolates, 64 were plant pathogen/saprotrophs (13.7%) and fourteen were plant

245   pathogens (3.0%).

246

247

248 **Discussion**

249 *Viaphyte Prevalence*

250   Here, we demonstrate for the first time that a diverse array of tropical leaf endophytes can

251 emigrate through direct contact to woody substrates of separate hosts, representing an ability to

252 alternate between endophytic and saprotrophic life stages. Our results show that viaphytes are

253 commonplace and multiple fungal species have a potential for viaphytic dispersal from within

254 each leaf, even though it is likely that we underestimate richness due to the biases of culture-

255 based studies (Schmit & Lodge, 2005) and the incompleteness of our sequencing efforts. The

256 high frequency of viaphytic colonizations suggests that the underlying mechanisms are probably

257 mechanistically straightforward (*i.e.*, as simple as hyphae extending from one substrate into the

258 other), although the enzymatic potential to successfully colonize woody substrates may be taxon-

259 dependant.

260   While our present viaphyte survey examined only a single tree, it seems unlikely that this

261 host is unique in allowing the transfer of endophytes to other substrates, or that the viaphytes

262 observed within its tissues are only able to transfer from this particular host. In other words, if

263 the host tree and its endophytic symbionts are taken to represent what is typical for a broad-

264 leaved tropical tree, it follows that viaphytes are likely commonplace symbionts in the leaves of

265 tropical forests. Other studies that have demonstrated the high abundance of endophytes in

266 tropical forests corroborate this potential (Arnold & Lutzoni, 2007; Rodriguez et al., 2009;

267 Thomas et al., 2016; Del Olmo-Ruiz & Arnold, 2017; Roy & Banerjee, 2018).

268   Even if fungi with viaphytic abilities are common, the extent to which viaphytic

269 colonization events occur in natural systems is unknown. While we placed leaves containing

270 endophytes on sterile wood substrates, viaphytes in nature would face competition from other

271   sources of colonization, such as spores or saprotrophs already present in the wood. Future

272   experiments should empirically test the ability of viaphytic fungi to successfully colonize such

273   woody substrates in the face of competition. It is likely that viaphytism and direct spore

274   colonization each have their own set of advantages. For instance, it is possible that the carbon

275   and water supplies inherent in leaf tissues give an advantage to viaphytic dispersal as compared

276   to spores, especially if conditions are dry or otherwise unsuitable for spore germination. In

277   addition, leaves could trap moisture between the leaf and substrate, and may act as barriers that

278   exclude competing spores from being deposited on the woody substrate surfaces (Thomas et al.,

279   2016). Certainly, direct spore dispersal has its own advantages in the form of reduced complexity

280   (*i.e.*, no intermediate colonization stage is required), increased potential travel distance via air

281   currents (McCartney & West, 2007; Calhim et al., 2018), and much greater abundances

282   compared to leaf-born colonies. These ideas are explored by (Thomas, Vandegrift & Roy, 2017)

283   using a simple agent-based model. As predicted by Thomas & Vandegrift et al. (Thomas et al.,

284   2016), in these simulations, viaphytism is advantageous under adverse conditions, given

285   retention of endophyte infections and at least some trees on the landscape.

286   The viaphyte community was characterized by a few taxa with high abundances and a

287   large number of taxa with low abundances (Fig. 2). While this pattern is typical for leaf

288   endophytes (Arnold et al., 2000, 2007; Vega et al., 2010; Gazis & Chaverri, 2010; Ikeda et al.,

289   2014; Del Olmo-Ruiz & Arnold, 2017), some patterns in the data suggest that they are partly due

290   to methodological biases. For instance, *Penicillium spp.* and *Trichoderma spp.* were both

291   observed to be fast growing in culture in this study, and culture-based studies are known to be

292   biased for faster-growing taxa (Kirk et al., 2004). Also, given that that each of the four most

293   dominant taxa had a disproportionately high number of isolates concentrated in a single box,

294 these dominant taxa seem to have colonized the sticks within their respective boxes via

295 sporulation during the inoculation period (Fig. 3). All four of these dominant taxa readily

296 produced a high quantity of conidia in culture. Therefore, the number of isolates for these

297 abundant taxa should be interpreted with caution as they likely do not reflect the actual

298 abundance in host leaves, but rather comparatively fast growth and within-box contamination. It

299 is also notable that our experiment did not have a true negative control, without an inoculation

300 source, to account for true contaminants (i.e., taxa that may have originated outside of the

301 leaves). While it is possible that some taxa detected may have been contaminants, there are

302 several factors which suggest relatively low rates of outside contamination: (1) the thorough

303 sterilization procedures we employed; (2) the high endophyte load in the tropics (Arnold et al.,

304 2000; Arnold & Lutzoni, 2007); (3) the near ubiquity of detected taxa being found in tropical

305 endophyte datasets; and (4) the restriction of common taxa to single boxes.

306 *Ecological Strategies*

307   It is well documented that many endophytes—e.g., Xylariaceae, the majority of which do

308 not typically reproduce in the litter—have a much broader host range in the endophytic state than

309 as saprotrophs (Davis et al., 2003; Peršoh et al., 2010). It is, in fact, apparently common for such

310 endophytes to be present in the leaves of hosts upon whose wood they never fruit (Carroll &

311 Carroll, 1978; Peršoh et al., 2010; Unterseher, Peršoh & Schnittler, 2013). This is evidence for a

312 Foraging Ascomycete ecology, as a broad host range in the endophytic stage is incompatible

313 with latent saprotrophic strategies for fungi with restricted host ranges in that life stage (Thomas

314 et al., 2016). It is interesting that many fungi which do not typically fruit on litter, such as

315 members of the Xylariaceae, are well knowns as highly competitive litter decay organisms

316 (Koide, Osono & Takeda, 2005; Osono, 2007; Osono et al., 2011) — it is logical that increased

317   substrate utilization in the litter, and therefor increased resource accumulation, translates to

318   increased ability to successfully compete for preferred substrates within range of the searching

319   fungus (Boddy, 2000).

320   Latent saprotrophism is a well-documented strategy of some leaf endophytes (Osono,

321   2006; Parfitt et al., 2010; Voříšková & Baldrian, 2013). An excellent example of this ecological

322   strategy is the fungus *Rhabdocline parkeri (Sherwood-Pike, Stone & Carroll, 1986)*, which

323   spends most of its lifecycle as endophyte in the needles of *Pseudotsuga menziesii*, waiting for the

324   needles to die (typically 4–5 years). After needle senescence, the fungus rapidly invades the

325   surrounding needle tissues (often before they are even shed), and then produces conidial states, a

326   small perithecial teleomorph early in the winter, soon after the leaves are shed (Stone, 1987). The

327   host specificity of *R. parkerii*, and other fungi like it, is explained by the role of priority effects

328   (Chase, 2003) in the latent saprotrophic habit: while priority effects may work to benefit

329   viaphytic fungi somewhat, they serve as a strong evolutionary filter for fungi utilizing a latent

330   saprotrophic strategy. Future studies examining viaphytic ecological strategies should focus on

331   exploring the boundaries between viaphytic and latent saprotrophic ecologies.

332   *Taxonomic Distribution*

333   The viaphytes in this study belong to a wide taxonomic breadth, consisting of both

334   Basidiomycota and Ascomycota. This implies that the benefits described by the FA hypothesis

335   are available to members of the Basidiomycota as well, though the original idea concerned only

336   the Ascomycota (Carroll, 1999). The taxonomic distribution of viaphytes from this study

337   resemble those of general tropical leaf-endophytes described in other work (Arnold & Lutzoni,

338   2007; Thomas et al., 2016; Roy & Banerjee, 2018). In particular, Arnold et al. (Arnold et al.,

339   2007) reported a similar pattern and proportion of Eurotiomycetes, Dothideomycetes, and

340   Sordariomycetes, also noting the dominance of Ascomycota.

341   The wide taxonomic distribution of viaphytes suggests that viaphytic dispersal may be a

342   deeply ancestral trait. This would parallel endophytes in general, which appear to have

343   associated with plants as long as 400 mya (Krings et al., 2007). Future taxonomic and

344   paleontological work may help inform when viaphytism emerged as a dispersal strategy within

345   the Fungi.

346   *Functional Guilds*

347   Most of the viaphytic taxa in our study (17 of 25) were classified by FUNGuild as having

348   saprotrophic abilities (Table S3). Many of these saprotrophic taxa are known wood-decay fungi,

349   including *Xylaria spp.* and *Phanerochaete spp.* (Nguyen et al., 2016). In addition, our host leaves

350   were harboring at least some species of physiological white-rot fungi, as evidenced by bleaching

351   of the wood and a substantial decrease in size in several of our substrate fragments. Even some

352   ascomyceteous molds are known to be outstanding degraders of lignin, including some

353   *Penicillium spp.*, *Trichoderma spp.*, and *Fusarium oxysporum*, all of which were present among

354   our isolates (Rodriguez et al., 1996; Ryazanova, Chuprova & Luneva, 2015). While the

355   prevailing explanation for the occurrence of saprotrophic fungi as endophytes is that they are

356   latent saprotrophs waiting to consume leaves upon senescence (Peršoh, 2013), many taxa we

357   observed here, and others commonly isolated as endophytes, are not known to reproduce on dead

358   leaves. Alternately, such endophytic saprotrophs may represent an evolutionary 'dead-end' if

359   they are unable to escape that state (Bayman et al., 1998), but our data suggests that it may be the

360   norm for such fungi to transfer out of an endophytic state. Additionally, the presence of several

361   taxa classified as primarily pathotrophs suggests that the facultative ability to access saprotrophic

362   lifestyles may serve as a functional bridge for certain biotrophic species. One might expect that if

363   biotrophs are cultivated on any given substrate, the resulting community would be dominated by

364   fungi that were typically biotrophic, but with facultative saprotrophic abilities. This, however, is

365   not what we find here, indicating that it is likely that a large proportion of endophytes isolated

366   here are not transitioning to saprotrophy in a facultative manner, but as a transition back to their

367   primary nutritional mode.

368   We observed several instances of fungi apparently thriving after colonizing wood. For

369   example, despite the fact that only very few, generally host-specific, *Xylaria* are capable of

370   fruiting from leaves (Rogers, 2000), *Xylaria flabelliformis* was observed fruiting directly from

371   the woody substrates after transfer from an endophytic state. Interestingly, this taxon was found

372   to be a common endophyte of forests in Taiwan (Vandegrift et al., 2019). Previously, we found

373   five *Xylaria* species both as endophytes and as stromata on woody substrates at Los Cedros

374   (Thomas et al., 2016). Emigration from leaves to wood is likely necessary for such endophytic

375   individuals to regain reproductive potential.

376

377   **Conclusion**

378   As an alternative to the latent saprotroph hypothesis, the FA hypothesis (viaphytism)

379   suggests that many saprotrophs use endophytism to modify dispersal to their primary (*i.e.,*

380   reproductive) substrates (Carroll, 1999; Thomas et al., 2016; Thomas, Vandegrift & Roy, 2017).

381   Here, we demonstrate for the first time that a diverse assemblage of foliar endophytes can

382   directly colonize woody substrates from leaves, and that a high proportion of these fungi are

383   ecological saprotrophs. This work provides new support for the FA hypothesis. While the

384   prevalence of viaphytic dispersal in nature is currently unknown, the diversity and abundance of

385   viaphytes observed here suggests that it may be commonplace. Viaphytic dispersal may have

386   ramifications not only for the dispersal and competition dynamics of fungi, but also for larger

387   scale processes, such as decomposition (Thomas, Vandegrift & Roy, 2017). These dynamics are

388   largely unexplored and represent a vast potential for future research (but see, *e.g.*, Osono, 2006).

389   One such research topic that is suggested by this work concerns the effects of viaphytic

390   dispersal on outcrossing (and thus evolutionary trajectories) of taxa utilizing this dispersal

391   strategy. Dispersal by viaphytism could lead to an increase in outcrossing by reducing the

392   chances of mating between spores of the same parent: spores released from the same fruiting

393   event have a relatively high likelihood of colonizing the same nearby substrates and mating.

394   However, if a subset of those spores delay their colonization of wood by becoming endophytes,

395   it is likely that they increase their chances of mating with a non-sibling.

396

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415

416

417 **Figures Legends**

418

419 **Figure 1: Summary of identified fungal endophytes that transferred from host leaves into a**

420 **woody substrate**. From 12 leaves, 25 taxa transferred to wood and were subsequently isolated.

421 Of a total of 472 identified isolates, 82% were represented by the four most common taxa. The

422 total isolates per taxa roughly corresponds to the number of leaves they were isolated from. The

423 numbers on the bars specify the number of cultures per taxon. [Note: the left axis is on a

424 logarithmic scale.] 5 isolates remained unidentified and are not included in the figure.

425

426 **Figure 2: Species accumulation curve for viaphytes**. The culturing did not achieve a saturation

427 of culturable viaphytic taxa.

428

429 **Figure 3: Non-metric Multidimensional Scaling (NMDS) plot of viaphyte communities.**

430 Each point represents an individual birch tongue depressor; lines connect sticks that were

431 inoculated with the same leaf; color indicates inoculation box.

432

433 **Figure S1: photos of saprotrophic *Xylaria flabelliformis* stromata**. Growing on wood

434 substrates inoculated by leaf endophytes.

435

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608

Figure 1

Summary of identified fungal endophytes that transferred from host leaves into a woody substrate.

From 12 leaves, 25 taxa transferred to wood and were subsequently isolated. Of a total of 472 identified isolates, 82% were represented by the four most common taxa. The total isolates per taxa roughly corresponds to the number of leaves they were isolated from. The numbers on the bars specify the number of cultures per taxon. [Note: the left axis is on a logarithmic scale.] 5 isolates remained unidentified and are not included in the figure.
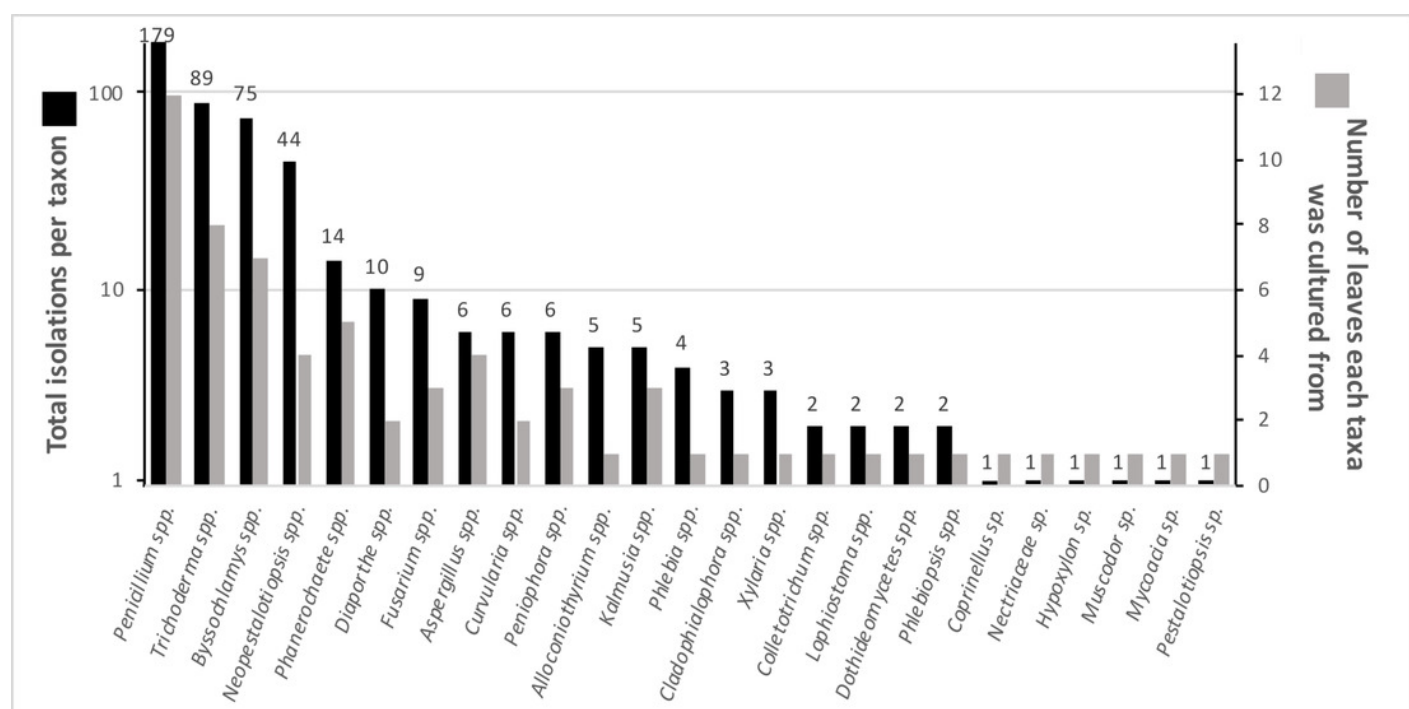
Figure 2

Species accumulation curve for viaphytes.

The culturing did not achieve a saturation of culturable viaphytic taxa.
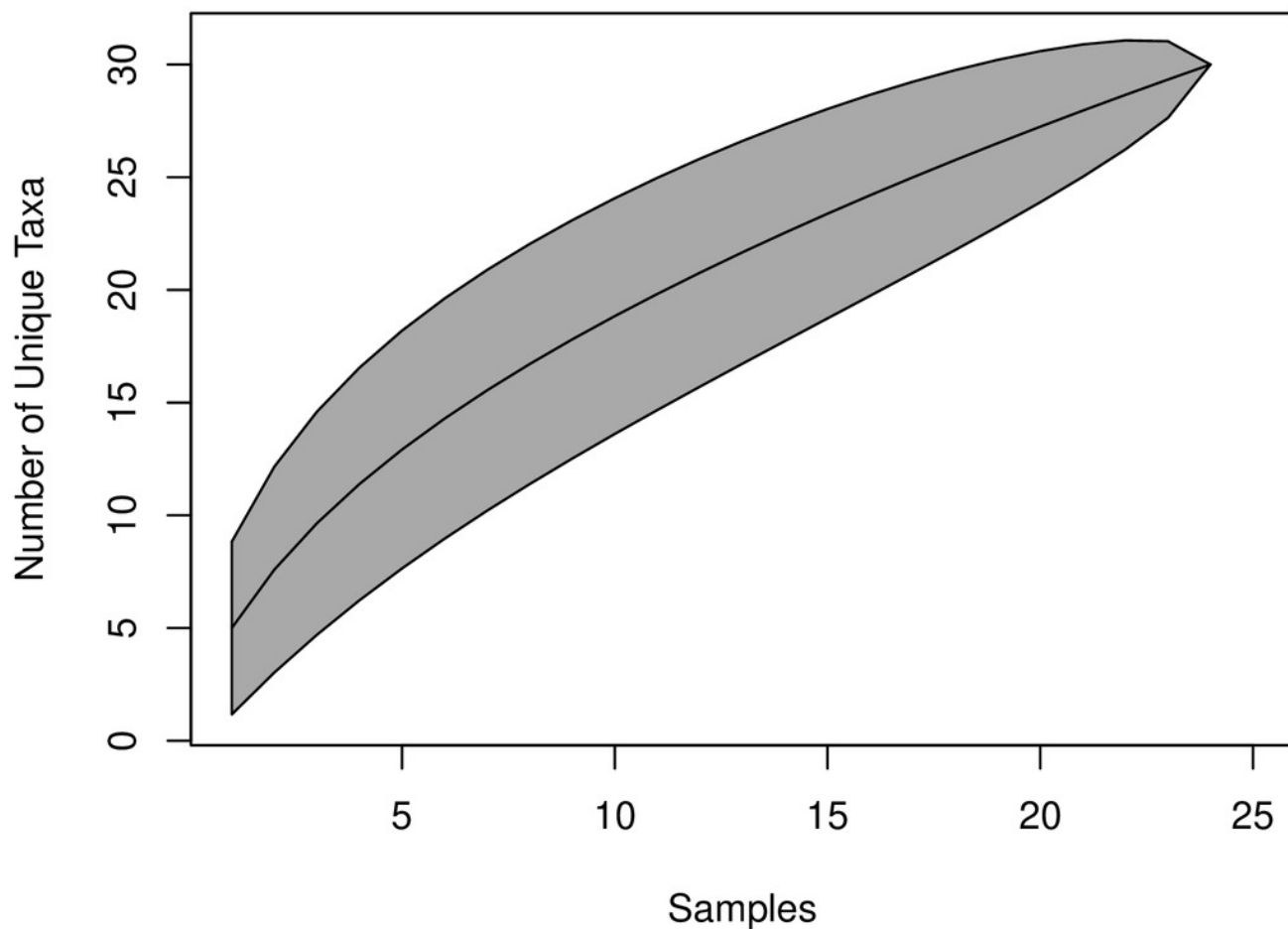
Figure 3

Non-metric Multidimensional Scaling (NMDS) plot of viaphyte communities.

Each point represents an individual birch tongue depressor; lines connect sticks that were inoculated with the same leaf; color indicates inoculation box.