

# 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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**13 ABSTRACT**

14 Fungal endophytes are a ubiquitous feature of plants, yet for many fungi the benefits of  
15 endophytism are still unknown. The Foraging Ascomycete (FA) hypothesis proposes that  
16 saprotrophic fungi can utilize leaves as both as dispersal vehicles and as resource havens during  
17 times of scarcity. The presence of saprotrophs in leaf endophyte communities has been  
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21 sterile wood fragments and incubating them for 6 weeks. Fungi from the wood were  
22 subsequently isolated in culture and identified by ITS sequences or morphology to the genus  
23 level. 477 fungal isolates comprising 24 distinct taxa were cultured from the wood. Of these,  
24 70.8% (82.3% of isolates) belong to saprotrophic genera according to the FUNGuild database.  
25 Furthermore, 27% of OTUs (6% of isolates) were basidiomycetes, an unusually high proportion  
26 compared to typical endophyte communities. *Xylaria flabelliformis*, although absent in our  
27 original isolations, formed anamorphic fruiting structures on the woody substrates. We introduce  
28 the term *viaphyte* (literally, “by way of plant”) to refer to fungi that undergo an interim stage as  
29 leaf endophytes and, after leaf senescence, colonize other saprobic substrates via hyphal growth.  
30 Our results support the Foraging Ascomycete Hypothesis and suggest that viaphytism may play a  
31 significant role in the dispersal of fungal saprotrophs.

32

**33 KEYWORDS**

34 Ascomycota; Basidiomycota; ecological theory; foraging ascomycete; fungi; life history;  
35 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; Porrás-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.





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<sup>2</sup> 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<sup>2</sup> 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- $\mu$ L  
158 reaction volumes (2.5  $\mu$ L of template, 6.25  $\mu$ L of Sigma Aldrich Jumpstart<sup>TM</sup> Taq  
159 Readymix<sup>TM</sup>, 2.75  $\mu$ L sterile water, 0.5  $\mu$ L 25 mM MgCl<sub>2</sub>, and 0.25  $\mu$ L 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 Jackknife1 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 known 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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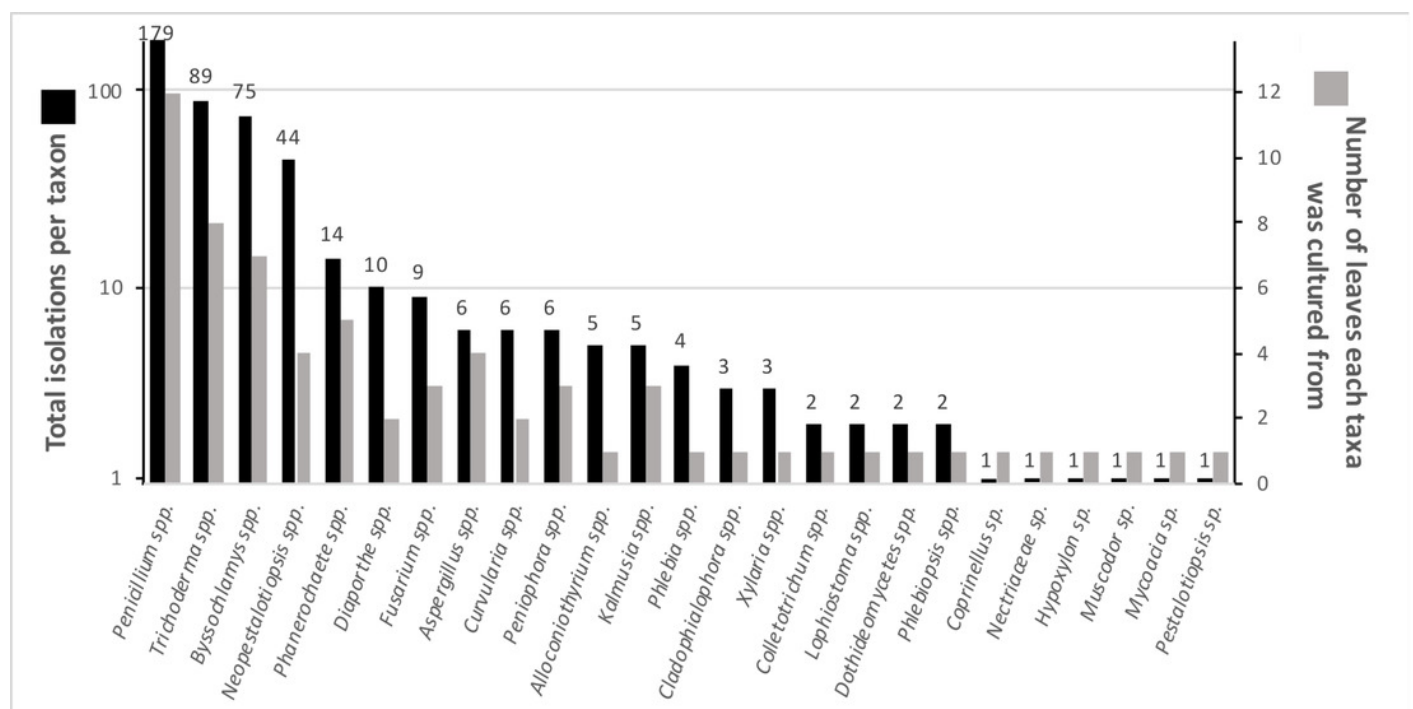
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607        barks. *Catalysis in Industry*. 7:82–9.

608

## Figure 1

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

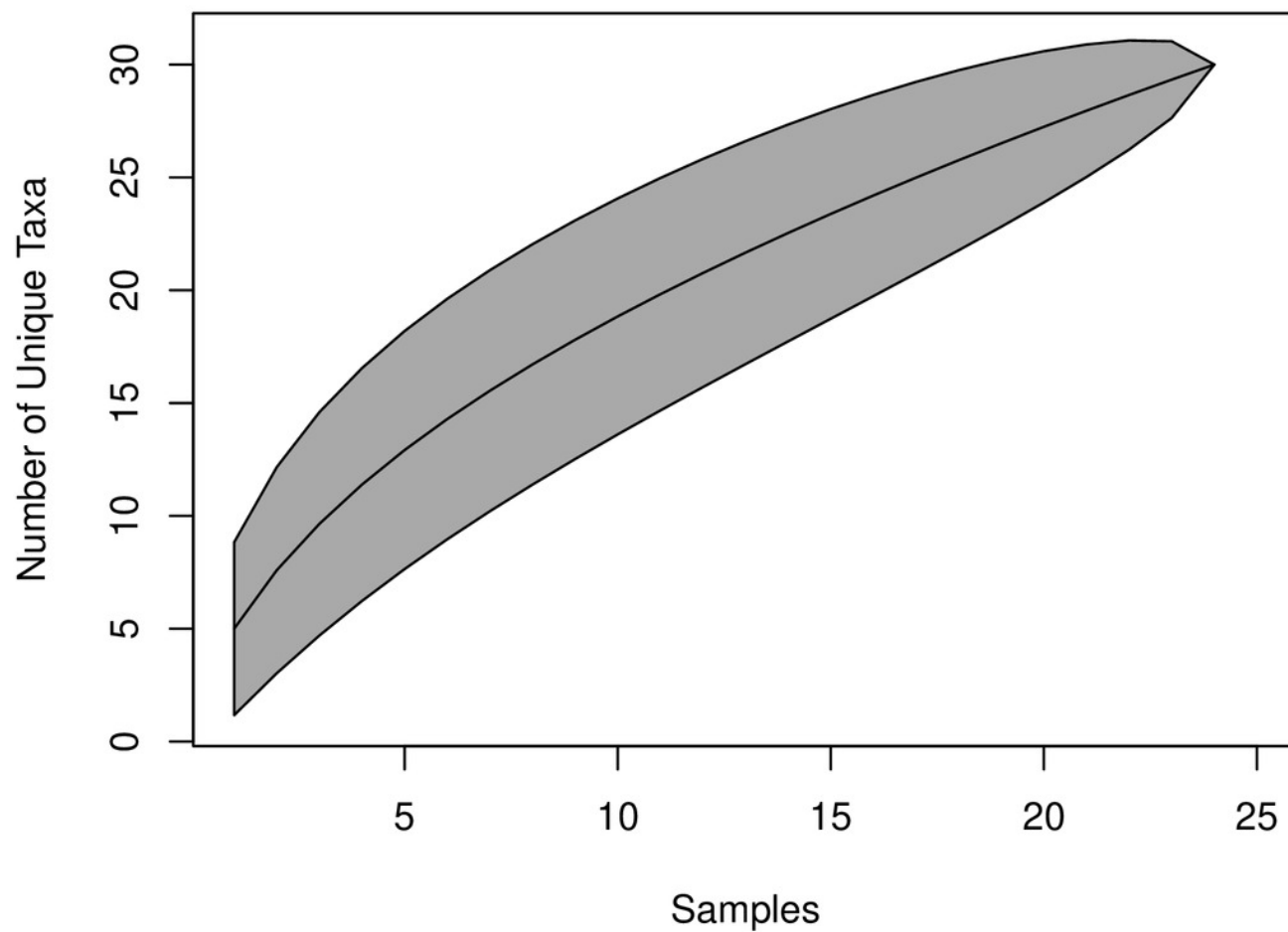
From 12 leaves, 25 taxa transferred to wood and were subsequently isolated. Of a total of 472 identified isolates, 82% were represented by the four most common taxa. The total isolates per taxa roughly corresponds to the number of leaves they were isolated from. The numbers on the bars specify the number 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.

