

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

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10 *indicates equal contribution

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12

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 both as dispersal vehicles and as resource havens during
17 times of scarcity. The presence of saprotrophs in leaf endophyte communities has been
18 previously observed but their ability to transfer to non-foliar saprobic substrates has not been
19 well investigated. To assess this ability, we conducted a culture study by placing surface-
20 sterilized leaves from a single tropical angiosperm tree (*Nectandra lineatifolia* Mez) directly
21 onto sterile wood fragments and incubating them for 6 weeks. Fungi from the wood were
22 subsequently isolated in culture and identified to the genus level by ITS sequences or
23 morphology. Four-hundred and seventy-seven fungal isolates comprising 24 taxa were cultured
24 from the wood. Of these, 70.8% of taxa (82.3% of isolates) belong to saprotrophic genera
25 according to the FUNGuild database. Furthermore, 27% of OTUs (6% of isolates) were
26 basidiomycetes, an unusually high proportion compared to typical endophyte communities.
27 *Xylaria flabelliformis*, although absent in our original isolations, formed anamorphic fruiting
28 structures on the woody substrates. We introduce the term *viaphyte* (literally, “by way of plant”)
29 to refer to fungi that undergo an interim stage as leaf endophytes and, after leaf senescence,
30 colonize other woody substrates via hyphal growth. Our results support the Foraging
31 Ascomycete hypothesis and suggest that viaphytism may play a significant role in the fungal
32 dispersal.

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41 **Introduction**

42 Endophytes are symptomless endosymbionts of living plants (Stone, Bacon & White, 2000) and
43 are ubiquitously present in terrestrial plant tissues worldwide (Arnold & Lutzoni, 2007).

44 Virtually every plant **genus** surveyed to date has **documented** several to hundreds of species of
45 fungal endophytes per individual, and a single plant species may host thousands of these
46 symbionts across its entire range (Martins et al., 2016; Barge et al., 2019). **Although variable, the**
47 effects of endophytes on host plants **have** attracted considerable attention (Carroll, 1988;
48 Rodriguez et al., 2009); **yet, the potential** benefit of endophytic life histories for the *fungal*
49 partners **is less well explored**.

50 The question of why fungi may adopt endophytic lifestyles has garnered a variety of
51 hypotheses. **In particular, a number of authors have hypothesized that** endophytes may be latent
52 saprotrophs **that** benefit from **being** the first to colonize plant tissues after senescence or death of
53 the host (Promputtha et al., 2007; Parfitt et al., 2010; Porras-Alfaro & Bayman, 2011; Szink et
54 al., 2016), **a phenomenon known as priority effects (Chase, 2003; Osono, 2006). Studies that**
55 **sampled living and decomposing leaves from the same plant individuals have observed the**
56 **majority of foliar** endophytes **can persist** in the litter layer as decomposers (Osono, 2006; U'Ren
57 & Arnold, 2016), **especially in the** early stages of litter decomposition, when **litter contains a**
58 higher availability of simple sugars and **other** easily degradable compounds (Carroll & Petrini,
59 1983; Voříšková & Baldrian, 2013). Endophytes **observed to** persist into the late stages of litter
60 decomposition (Peršoh et al., 2013) **often** have demonstrated an ability to degrade more complex
61 substrates, such as lignin, which **supports the hypothesis that some fungi with an endophytic life**
62 **stage** may **also** play a role during later stages of litter decay (Osono & Takeda, 1999). **Although**
63 **the majority of studies have focused on foliar endophytes**, Parfitt et al. (2010) suggest that most,

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87 if not all, trees carry sapwood endophytes with the potential to degrade ~~the woody tissues of their~~
88 ~~host when~~ environmental and biological conditions ~~are conducive to decay~~. ~~In contrast, other~~
89 ~~studies have suggested~~ endophytes are ~~primarily~~ mutualists, with their fitness directly tied to that
90 of their hosts. ~~This is exemplified best by~~ clavicipitaceous grass endophytes, which benefit from
91 direct vertical transmission to their hosts' offspring (Clay, 1988; Hodgson et al., 2014). Finally,
92 ~~it has been hypothesized that~~ endophytes may be latent pathogens waiting to exploit a weakened
93 state of their host (Carroll, 1988; Slippers & Wingfield, 2007). ~~However, the vast majority of~~
94 observed endophytic fungi do not fit neatly into one of these categories ~~and may in fact be~~
95 ~~capable of a variety of context-dependent interactions with their hosts (i.e., endophytic~~
96 ~~continuum; Schultz and Boyle, 2005).~~

97 ~~Regardless of ecological mode, the evolutionary benefits of endophytic leaf colonization~~
98 ~~for species that do not form fruiting bodies on leaves remains obscure~~. For instance, a number of
99 ~~genotypes closely related to~~ wood decomposers have been found to ~~also inhabit living~~ leaves as
100 ~~endophytes~~ (Promputtha et al., 2007), ~~yet these taxa have not been observed to also form fruiting~~
101 ~~bodies on leaves~~. ~~Thus, it~~ has been proposed that endophytic colonization ~~may~~ represent an
102 evolutionary "dead-end" (*i.e.*, saprotrophs found as endophytes are unlikely to ~~reproduce from~~
103 ~~leaves~~). ~~This idea appears logical since most~~ endophyte infections ~~in living leaves remain~~
104 ~~localized, occupying~~ only one or a few host plant cells (Carroll, 1988; Bayman et al., 1998;
105 Arnold & Lutzoni, 2007), and ~~endophytes do not usually colonize~~ woody stems ~~from the~~ leaves,
106 ~~where the infection could~~ ~~resulting in fruiting body formation~~ (Sun et al., 2012; Tateno et al.,
107 2015; Thomas et al., 2019). ~~Yet~~, the colonization of live plant tissues requires specialized
108 chemical and physical systems (Kusari, Hertweck & Spiteller, 2012) ~~and~~ the construction of such
109 cellular mechanisms during development, along with propagule loss, incurs evolutionary costs

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SomeOther endophytes are considered *mutualists*, with their fitness directly tied to that of their hosts, as is common in the clavicipitaceous grass endophytes, which benefit from direct vertical transmission to their hosts' offspring (Clay, 1988; Hodgson et al., 2014). Finally, others may be *latent pathogens* waiting to exploit a weakened state of their host (Carroll, 1988; Slippers & Wingfield, 2007). ¶
However, the vast majority of observed endophytic fungi do not fit neatly into one of these categories. In contrast, other [1]
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171 that are unaccompanied by benefits if endophytism is truly a ‘dead end’ for these fungi.

172 One possible explanation for this discrepancy is the Foraging Ascomycete (FA)

173 hypothesis (Carroll, 1999; Thomas & Vandegrift et al., 2016; Thomas et al., 2019; Thomas,

174 Vandegrift & Roy, 2017), which proposes that the function of leaf endophytism for some fungi

175 may be to increase dispersal to other substrates by helping to bridge spatiotemporal gaps in

176 preferred substrate. While some saprotrophic endophytes can fruit directly from fallen leaves

177 (Add Reference), the FA hypothesis proposes that after leaves senesce and fall, leaf endophytes

178 are capable of transferring to other substrates in their environment that are separate from their

179 original endophytic hosts. Thus, during times of suboptimal environmental conditions,

180 endophytes may have an increased likelihood of survival compared to spores or saprobic mycelia,

181 because the highly buffered environment of living leaves, which can provide a source of

182 nutrients regardless of surrounding environmental conditions (Thomas & Vandegrift et al.,

183 2016). We hypothesize that the ability of spores to colonize living leaves is essentially a form of

184 evolutionary bet-hedging that “reduces the temporal variance in fitness at the expense of a

185 lowered arithmetic mean fitness” (Ripa, Olofsson & Jonzén, 2010). Direct spore dispersal by

186 itself may result in a higher mean success rate in colonizing substrates suitable for fruiting body

187 production, but success will be highly contingent on suitable environmental conditions (Provide

188 Reference for this). Thus, when a subset of spores from each sporulation event colonize leaves as

189 endophytes, a species can decrease the variance of dispersal success (Thomas & Vandegrift et

190 al., 2016).

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

192 viaphyte to refer to fungi that undergo these lifestyle shifts: the subset of endophytic fungi that

193 are primarily saprotrophic, but which also occur as leaf endophytes and are capable of dispersal

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221 from their endophytic hosts to other substrates following leaf senescence. We create this term
222 because (1) referring to such fungi as “foragers” is vague and leads to confusion, and (2)
223 referring to them as “foraging ascomycetes” (or “FA utilizing fungi” and other such
224 permutations) is inaccurate, as endophytes in the Basidiomycota are likely to utilize this dispersal
225 strategy, as well (Add Reference). “Viaphyte” joins the word *via* — defined as “travelling
226 through a place en route to a destination” — with the suffix, *phyte*, which denotes a plant. In this
227 study, we use the term specifically to refer to fungi that display the ability to directly transfer
228 from an endophytic state (inhabiting living leaf tissue, necessarily biotrophic) to a free-living
229 state (inhabiting a dead woody substrate, necessarily saprotrophic) though hyphal growth.

230 While viaphytism is superficially similar to latent saprotrophism, it is a distinct and more
231 complex process. Latent saprotrophy presupposes that the purpose of a fungus being present as
232 an endophyte is to consume the tissue of its host after senescence. The idea that endophytism
233 may be a vehicle, rather than an end destination, is a distinct concept. As such, the use of the
234 term “viaphyte” helps to clarify this distinction and avoid confusion as the literature around these
235 topics evolves.

236 For the FA hypothesis to be feasible (i.e., for viaphytism to occur) it must be shown that
237 transfer from living leaves to another substrate is possible. Thomas and Vandegrift et al. (2016)
238 observed such transfer, but that study was restricted to a single fungal genus, *Xylaria*, and it is
239 unclear how prevalent this ability is among fungal endophytes of other taxonomic groups. Here,
240 we conducted a survey of the viaphytic abilities of endophytes present in leaves of the tropical
241 tree, *Nectandra lineatifolia*, as the tropics represent a hotspot for endophyte diversity (Arnold &
242 Lutzoni, 2007). We also assessed the overall diversity of observed viaphytes and the
243 hypothesized about the presumed ecological roles of each isolated viaphytic fungus. Leaf

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273 endophytes are hyperdiverse and have a wide taxonomic breadth ([Arnold et al., 2000](#);
274 [Bazzicalupo, Bálint & Schmitt, 2013](#); [Thomas et al., 2019](#)). As a subset of the endophytic
275 community, we expected that viaphytes would also represent a wide taxonomic breadth. Despite
276 the fact that source communities were likely to harbor many biotrophs capable of facultative
277 saprotrophy, based on the framework of [the](#) FA hypothesis we hypothesized that the majority of
278 viaphytes isolated would be taxa whose primary nutritional mode is saprotrophy.

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283 **Materials and Methods**

284 *Culture Methods*

285 Twelve evergreen leaves of a randomly selected tree (Lauraceae; *Nectandra lineatifolia*
286 (Ruiz & Pav.) Mez) were collected in an Ecuadorian cloud forest. The tree was within Reserva
287 Los Cedros, which is on the western slope of the Andes in northwestern Ecuador (00°18031.000
288 N, 78°46044.600 W), at 1200m above sea level. Eight 2-cm² sections were cut from each leaf
289 and surface-sterilized by successive immersion in 70% ethanol for one min, 5% sodium
290 hypochlorite (equivalent to full strength bleach) for two min, then rinsed in sterile water. The
291 leaf sections were placed onto twice-autoclaved white birch (*Betula papyrifera* Marshall) tongue
292 depressors (Puritan, Guilford, Maine, U.S.A.) as a standardized angiosperm woody substrate.
293 The sections from each leaf were split between two tongue depressors (4 sections each) resulting
294 in a total of 24 tongue depressors. These were incubated in three 95% EtOH-sterilized Ziploc
295 storage boxes (eight in each box) at the field station in ambient temperature for six weeks. Each
296 box contained an open container of twice-autoclaved water to maintain humidity. The incubation
297 period provided opportunity for the endophytic fungi in the leaves to colonize the wood. After
298 incubation, the sticks were placed into airtight, sterile bags and brought to the University of
299 Oregon.

300 Fungal cultures were isolated from the inoculated wood by breaking 15 small fragments
301 (~5 mm² each) of wood from each tongue depressor using flame-sterilized tools and dispersing
302 them evenly among five 100 mm water agar plates. The ends of growing hyphae were excised
303 from the agar using a dissecting microscope and a scalpel and transferred onto nutrient plates
304 (MEA, 2% maltose) over a two-month period. Cultures were also made from several fruiting
305 structures that grew directly from the birch substrate fragments. After a growth period of seven

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307 or more days the isolates were grouped into morphotypes (Lacap, Hyde & Liew, 2003) at the
308 genus level based on macro- and microscopic features.

309 All field work was done with the approval of the Ecuadorian Ministry of the Environment
310 (Ministerio del Ambiente de Ecuador, Permit No. 03-2011-IC-FLO-DPAI/MA).

311 *Identification of Viaphytes*

312 A single representative of each morphotype was subcultured in liquid media (~~malt~~
313 ~~extract: ME~~) for DNA extraction using the Qiagen DNeasy Plant kit following the
314 manufacturer's instructions, and the ITS region (the standard "barcode" ~~locus~~ for fungi; Schoch
315 et al., 2012) was amplified using the fungal-specific primer set ITS1F (5'-
316 CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3')
317 (White et al., 1990), or in cases where those primers were ineffective, ~~isolates were amplified~~
318 with ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and LR3 (5'-

319 CCGTGTTC AAGACGGG-3') primers. DNA amplification was conducted with 12.5- μ L
320 reaction volumes (2.5 μ L of template, 6.25 μ L of Sigma Aldrich JumpstartTM Taq

321 ReadymixTM, 2.75 μ L sterile water, 0.5 μ L 25 mM MgCl₂, and 0.25 μ L of each ~~primer~~). PCR
322 amplification was ~~performed~~ with an MJ Research PTC-200 DNA Engine thermal cycler ~~under~~
323 the following parameters: initial denaturation at 95°C for 2 min, five cycles of denaturation at
324 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min; followed by 25 cycles
325 of denaturation of 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min; a
326 final extension at 72°C for 10 min, and a final step of indefinite duration at 4°C. PCR products
327 were visualized on a ~~1%~~ agarose gel. Samples were then frozen until shipping for sequencing at
328 Functional Biosciences, Inc (Madison, WI, U.S.A.) on ABI 3730xl instruments using Big Dye
329 V3.1. ITS amplicons were sequenced ~~bi-directionally~~, then ~~assembled into contigs~~, and ~~manually~~

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339 edited in Geneious (v6.0.3; Biomatters Limited, Auckland, New Zealand) to remove priming
 340 sites and resolve mismatches. The consensus sequences were then compared to published
 341 sequences in the UNITE database (v8.0; Kõljalg et al., 2013) using the *assign_taxonomy.py*
 342 function from the Quantitative Insights into Microbial Ecology (QIIME) pipeline (Caporaso et
 343 al., 2010). Taxa that returned species assignments as “unidentified” were further examined,
 344 Taxonomic identities were assigned at genus level and lower if the hit with the lowest E-Value
 345 had greater than 97% sequence identity across the entire ITS region. Sequences whose hits did
 346 not match these criteria were categorized as “unidentified”. Putative *Xylaria* species were
 347 compared to our database of ITS sequences generated from authenticated material within that
 348 genus at the same site (Thomas & Vandegrift et al., 2016) and assigned to a taxon if sequences
 349 had greater than 98% sequence identity. Taxa with greater than 99% sequence identity were
 350 assumed to be the same taxon (i.e., OTU). All taxa with identical assignments by UNITE met
 351 this criterion.

352 Functional guilds were assigned to each genus by using the FUNGuild online tool
 353 (Nguyen et al., 2016), which assigns functional information to taxa in DNA datasets. If
 354 functional guilds were not available in FUNGuild, they were determined based on the literature
 355 wherever possible.

356 *Statistical Methods*

357 Species richness was estimated using Chao2 and Jackknife1 estimators (Burnham &
 358 Overton, 1978; Chao, 1984; Colwell & Coddington, 1994). Diversity was estimated between all
 359 leaves, within leaves, and within boxes using Shannon’s index (log base *e* was used; Shannon,
 360 1948) and Simpson’s index (1-*D*; Simpson, 1949) and community structure was visualized using
 361 non-metric multidimensional scaling (NMDS) and differences assessed with permutational

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380 multivariate analysis of variance (PerMANOVA). Data were analyzed using R Statistical
381 Software, v. 3.1.0 (R Core Team 2014), including the *vegan* package (Oksanen et al., 2013).
382 All scripts, data tables, and raw data (morphotype counts and sequence chromatograms)
383 is available via an open FigShare repository (Nelson et al., 2019). Edited sequences have been
384 [uploaded to GenBank](#) ([accession numbers provided in Table S1](#)).

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386 Results

387 Diversity and Abundance of Viaphytes

388 Numerous endophytes [from surface-sterilized leaves of *Nectandra lineatifolia*](#)
389 [successfully colonized the wood substrate](#); 477 fungal cultures were isolated after making the
390 initial transfer from leaves to wood. [Isolates](#) were grouped into 64 morphotypes, 62 of which
391 were successfully identified to genus (59 by DNA, [three](#) by morphology; Table S1). DNA
392 identification resulted in the consolidation of the morphotypes into 24 unique taxa at the genus
393 level (Table S2). The number of isolates for each taxon varied widely, [such that](#) 57% of the
394 isolates represented by just [two](#) genera ([i.e.](#), *Trichoderma* and *Penicillium*) while [seven](#) of the
395 taxa were isolated only [a single time](#) (Fig. 1). In addition to hyphal growth from the wood
396 substrates, anamorphic fruiting structures were observed growing out of five stick fragments
397 originating from two leaves (Fig. S1). [These isolates](#) were identified as *Xylaria flabelliformis*
398 using DNA extracted from stromatic tissues. Including *X. flabelliformis*, [we observed](#) a total of
399 24 viaphytic taxa, [which were](#) identified to the genus level (Fig. 1). [Additionally, we observed](#)
400 [that several of the woody substrate fragments displayed a dramatic decrease in substrate volume](#)
401 [that may be explained by high levels of cell wall degrading enzymes typical of white-rot fungi](#).
402 [However](#), we did not attempt to determine which taxa were responsible for this dramatic

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419 reduction in volume.

420 The species accumulation curve did not reach a saturation point, suggesting
421 that the full richness of viaphytes from these leaves was not isolated (Fig. 2). Estimates of actual
422 species richness ranged from 36.5 (first order jackknife, $SE = 4.1$) to 42.3 (chao2, $SE = 13.8$).

423 Viaphyte communities within incubation boxes were more similar to each other than to
424 communities from other boxes (PerMANOVA: $F_{1,23} = 6.34$, $p = 0.001$), ~~whereas communities~~
425 ~~from~~ sticks that were inoculated by the same leaves were ~~not more similar to each other than to~~
426 ~~sticks inoculated from different leaves~~ (PerMANOVA: $F_{1,23} = 1.04$, $p = 0.404$; Fig. 3). Isolates
427 ~~representing~~ the four most common taxa were concentrated in common boxes, with 100% of
428 *Neopestalotiopsis foedans* in Box 1 (44 total isolates across all boxes), 96% of *Paecilomyces*
429 *formosus* in Box 1 (75 total isolates), 87% of *Trichoderma spp.* in Box 2 (89 total isolates), and
430 61% of *Penicillium spp.* in Box 3 (179 total isolates).

431 Taxonomic Distribution

432 The higher order taxonomic ranks in our samples included two phyla, five classes, twelve
433 orders, and nineteen families (Table S2). ~~Although~~ Ascomycota was the dominant phylum, both
434 in terms of number of taxa and total number of isolates (73% and 94%, respectively), ~~isolates of~~
435 ~~Basidiomycota also were obtained in culture. Among Ascomycota fungi,~~ Sordariomycetes were
436 the most common class in terms of ~~number of~~ taxa (38.4% ~~of total taxa~~), ~~whereas fungi in the~~
437 Eurotiomycetes, driven by the frequency of *Penicillium spp.*, ~~represented more than half of the~~
438 isolates (55.7%). ~~At the ordinal level, the most common orders among all taxa were~~ Xylariales
439 ~~(Sordariomycetes, Ascomycota) and Polyporales (Basidiomycota) (each representing 19.2% of all~~
440 ~~orders). Isolates of Eurotiales (Eurotiomycetes, Ascomycota),~~ again driven by *Penicillium spp.*,

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452 ~~represented~~ the most isolates (55.1%).

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453 *Functional Guilds*

454 The FUNGuild database contained putative functional guilds for all but two of the genera
455 we isolated as viaphytes. The first unassigned genus, *Alloconiothyrium*, is newly described and
456 presently represented by a single species, *A. aptrootii*, which was isolated from a soil sample in
457 Papua New Guinea (Verkley et al. 2014). We therefore did not assign it to a functional guild
458 since so little information is available. The second, *Neopestalotiopsis*, we classified as a “plant
459 pathogen/saprotroph” based on substrates listed in species descriptions (Maharachchikumbura et
460 al. 2014). The viaphyte genera of our study fit into three distinct functional guilds: *saprotroph*,
461 *plant pathogen*, and *plant pathogen/saprotroph*. Saprotroph was the dominant functional guild in
462 terms of number of genera (70.8%; 17 out of 24) and number of isolates (82.3%, 389 out of 467).

463 Four of the genera were ~~classified as~~ plant pathogens (16.7%) and three ~~genera~~ were ~~classified as~~
464 plant pathogen/saprotrophs (12.5%). Of the isolates, 64 were ~~classified as~~ plant
465 pathogen/saprotrophs (13.7%) and fourteen were ~~classified as~~ plant pathogens (3.0%).

466

467

469 **Discussion**

470 *Viaphyte Prevalence*

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

472 ~~colonize~~ woody substrates ~~through direct contact with leaves, thus~~ representing an ability to

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

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

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

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

477 high frequency of viaphytic colonization ~~suggests that the underlying mechanisms are~~ ~~likely~~

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

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

480 ~~dependent.~~

481 While ~~the~~ present viaphyte survey examined only a single tree of *Nectandra lineatifolia*,

482 it seems unlikely that this host is unique in allowing the transfer of endophytes to ~~woody~~

483 substrates, or that the viaphytes observed within its tissues are only able to transfer from this

484 particular host. In other words, if the host tree and its endophytic symbionts are taken to

485 represent what is typical for a broad-leaved tropical tree, it follows that viaphytes are likely

486 commonplace symbionts in the leaves of tropical forests. Other studies that have demonstrated

487 the high abundance of endophytes in tropical forests corroborate this potential (Arnold &

488 Lutzoni, 2007; Rodriguez et al., 2009; ~~Thomas & Vandegrift~~ et al., 2016; Del Olmo-Ruiz &

489 Arnold, 2017; Roy & Banerjee, 2018).

490 ~~Yet even~~ if fungi with viaphytic abilities are common, the extent to which viaphytic

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

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500 endophytes on sterile wood substrates, viaphytes in nature would face competition from other
501 sources of colonization, such as spores or saprotrophs already present in the wood ([Add](#)
502 [References](#)). Future experiments should empirically test the ability of viaphytic fungi to
503 successfully colonize such [diverse](#) woody substrates in the face of competition. It is likely that
504 viaphytism and direct spore colonization each have their own set of advantages. For instance, it
505 is possible that the carbon and water supplies inherent in leaf tissues give an advantage to
506 viaphytic dispersal as compared to spores, especially if conditions are dry or otherwise
507 unsuitable for spore germination. In addition, leaves could trap moisture between the leaf and
508 substrate, and may act as barriers that exclude competing spores from being deposited on the
509 woody substrate surfaces (Thomas & Vandegrift et al., 2016). Certainly, direct spore dispersal
510 has its own advantages in the form of reduced complexity (*i.e.*, no intermediate colonization
511 stage is required), increased potential travel distance via air currents (McCartney & West, 2007;
512 Calhim et al., 2018), and much greater abundances compared to leaf-born colonies. These ideas
513 ~~were previously~~ explored by (Thomas, Vandegrift & Roy, 2017) using a simple agent-based
514 model. As predicted by Thomas & Vandegrift et al. (2016), in these simulations, viaphytism is
515 advantageous under adverse conditions, given retention of endophyte infections and at least some
516 trees on the landscape.

517 The viaphyte community [of *Nectandra lineatifolia*](#) was characterized by a few taxa with
518 high abundances and a large number of taxa with low abundances (Fig. 2). While this pattern is
519 typical for [culturable studies of](#) leaf endophytes (Arnold et al., 2000, 2007; Vega et al., 2010;
520 Gazis & Chaverri, 2010; Ikeda et al., 2014; Del Olmo-Ruiz & Arnold, 2017), some patterns in
521 the data suggest that they are partly due to methodological biases. For instance, *Penicillium spp.*
522 and *Trichoderma spp.* were both observed to be fast growing in culture in this study, and culture-

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527 based studies are known to be biased for faster-growing taxa (Kirk et al., 2004). Also, given that
528 each of the four most dominant taxa had a disproportionately high number of isolates
529 concentrated in a single box, these dominant taxa ~~likely~~ colonized the sticks within their
530 respective boxes via sporulation during the inoculation period (Fig. 3). All four of these
531 dominant taxa readily produced a high quantity of conidia in culture. Therefore, the number of
532 isolates for these abundant taxa should be interpreted with caution as they likely do not reflect
533 the actual abundance in host leaves, but rather comparatively fast growth and within-box
534 contamination. It is also notable that our experiment did not have a true negative control, without
535 an inoculation source, to account for true contaminants (*i.e.*, taxa that may have originated
536 outside of the leaves). While it is possible that some taxa detected may have been contaminants,
537 there are several factors which suggest relatively low rates of outside contamination: (1) the
538 thorough sterilization procedures we employed; (2) the high endophyte load in the tropics
539 (Arnold et al., 2000; Arnold & Lutzoni, 2007); (3) the near ubiquity of detected taxa being found
540 in tropical endophyte datasets; and (4) the restriction of common taxa to single boxes.

541 *Ecological Strategies*

542 It is well documented that many endophytes ~~have a much broader host range in the~~
543 ~~endophytic state than as saprotrophs — e.g., Xylariaceae, the majority of which do not typically~~
544 reproduce in the litter (Davis et al., 2003; Peršoh et al., 2010; U'Ren et al., 2016). It is, in fact,
545 apparently common for such endophytes to be present in the leaves of hosts upon whose wood
546 they never fruit (Carroll & Carroll, 1978; Peršoh et al., 2010; Unterseher, Peršoh & Schnittler,
547 2013). This is evidence for a Foraging Ascomycete ecology, ~~since latent saprotrophism is~~
548 ~~excluded as a strategy for species which are incapable of fruiting out of leaves~~ (Thomas &
549 Vandegrift et al., 2016). It is interesting that many fungi ~~that are~~ not typically ~~observed fruiting~~

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563 on litter, such as members of the Xylariaceae, are well known as highly competitive litter decay
564 organisms (Koide, Osono & Takeda, 2005; Osono, 2007; Osono et al., 2011). It is logical that
565 increased substrate utilization in the litter, and therefore increased resource accumulation,
566 translates to increased ability to compete for substrates external to the litter (Boddy, 2000).

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567 Latent saprotrophism is a well-documented strategy of some leaf endophytes (Osono,
568 2006; Parfitt et al., 2010; Voříšková & Baldrian, 2013). An excellent example of this ecological
569 strategy is the fungus *Rhaddocline parkeri* (Sherwood-Pike, Stone & Carroll, 1986), which
570 spends most of its lifecycle as an endophyte in the needles of *Pseudotsuga menziesii*, waiting for
571 the needles to die (typically 4–5 years). After needle senescence, the fungus rapidly invades the

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572 surrounding needle tissues (often before they are even shed), and then produces its conidial state,
573 followed by a small perithecial teleomorph early in the winter, soon after the leaves are shed
574 (Stone, 1987). The host specificity of *R. parkerii*, and other fungi like it, is explained by the role
575 of priority effects (Chase, 2003) in the latent saprotrophic habit: while priority effects may work
576 to benefit viaphytic fungi somewhat, they serve as a strong evolutionary filter for fungi utilizing
577 a latent saprotrophic strategy. Future studies examining viaphytic ecological strategies should
578 focus on exploring the boundaries between viaphytic and latent saprotrophic ecologies.

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579 *Taxonomic Distribution*

580 The viaphytes in this study belong to a wide taxonomic breadth, consisting of both
581 Basidiomycota and Ascomycota. This implies that the benefits described by the FA hypothesis
582 are available to members of the Basidiomycota as well, though the original idea concerned only
583 the Ascomycota (Carroll, 1999). The taxonomic distribution of viaphytes from this study
584 resemble those of general tropical leaf-endophytes described in other work (Arnold & Lutzoni,
585 2007; Thomas & Vandegrift et al., 2016; Roy & Banerjee, 2018). In particular, Arnold et al.

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594 (2007) reported a similar pattern and proportion of Eurotiomycetes, Dothideomycetes, and
595 Sordariomycetes, also noting the dominance of Ascomycota.

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596 The wide taxonomic distribution of viaphytes suggests that viaphytic dispersal may be a
597 deeply ancestral trait. This would parallel endophytes in general, which appear to have
598 associated with plants since at least 400 mya (Krings et al., 2007). Future taxonomic and
599 paleontological work may help inform when viaphytism emerged as a dispersal strategy within
600 the Fungi.

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601 *Functional Guilds*

602 Most of the viaphytic taxa in our study (17 of 24 taxa) were classified by FUNGuild as
603 having saprotrophic abilities (Table S3). Many of these saprotrophic taxa are known wood-decay
604 fungi, including *Xylaria* spp. and *Phanerochaete* spp. (Nguyen et al., 2016). In addition, our host
605 leaves were harboring at least some species capable of physiological white-rot fungi, as
606 evidenced by bleaching of the wood and a substantial decrease in size in several of our substrate
607 fragments. Even some ascomyceteous molds are known to be degraders of lignin, including
608 some *Penicillium* spp., *Trichoderma* spp., and *Fusarium oxysporum*, all of which were present
609 among our isolates (Rodriguez et al., 1996; Ryazanova, Chuprova & Luneva, 2015). While the
610 prevailing explanation for the occurrence of saprotrophic fungi as endophytes is that they are
611 latent saprotrophs waiting to consume leaves upon senescence (Peršoh, 2013), many taxa we
612 observed here, and others commonly isolated as endophytes, are not known to reproduce on dead
613 leaves. Alternately, such endophytic saprotrophs may represent an evolutionary ‘dead-end’ if
614 they are unable to escape that state (Bayman et al., 1998), but our data suggests that it may be the
615 norm for such fungi to transfer out of an endophytic state. Additionally, the presence of several
616 taxa classified as primarily pathotrophs suggests that the facultative ability to access saprotrophic

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621 lifestyles may serve as a functional bridge for certain biotrophic species. One might expect that if
622 biotrophs are cultivated on any given substrate, the resulting community would be dominated by
623 fungi that were typically biotrophic, but with facultative saprotrophic abilities. This, however, is
624 not what we find here, indicating that it is likely that a large proportion of endophytes isolated
625 here are not transitioning to saprotrophy in a facultative manner, but as a transition back to their
626 primary nutritional mode.

627 We observed several instances of fungi apparently thriving after colonizing wood. For
628 example, despite the fact that only very few, generally host-specific, *Xylaria* are capable of
629 fruiting from leaves (Rogers, 2000), *Xylaria flabelliformis* was observed fruiting directly from
630 the woody substrates after transfer from an endophytic state. Interestingly, this taxon was found
631 to be a common endophyte of forests in Taiwan (Vandegrift et al., 2019). Previously, we found
632 five *Xylaria* species both as endophytes and as stromata on woody substrates at Los Cedros
633 (Thomas & Vandegrift et al., 2016). Emigration from leaves to wood is likely necessary for such
634 endophytic individuals to regain reproductive potential.

635

636 **Conclusion**

637 As an alternative to the latent saprotroph hypothesis, the FA hypothesis (viaphytism)
638 suggests that many saprotrophs use endophytism to modify dispersal to their primary (*i.e.*,
639 reproductive) substrates (Carroll, 1999; Thomas & Vandegrift et al., 2016; Thomas, Vandegrift
640 & Roy, 2017). Here, we demonstrate for the first time that a diverse assemblage of foliar
641 endophytes can directly colonize woody substrates from leaves, and that a high proportion of
642 these fungi are ecological saprotrophs. This work provides new support for the FA hypothesis.
643 While the prevalence of viaphytic dispersal in nature is currently unknown, the diversity and

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644 abundance of viaphytes observed here suggests that it may be commonplace. Viaphytic dispersal
645 may have ramifications not only for the dispersal and competition dynamics of fungi, but also for
646 larger scale processes, such as decomposition (Thomas, Vandegrift & Roy, 2017). These
647 dynamics are largely unexplored and represent a vast potential for future research (but see, *e.g.*,
648 [Osono, 2006](#)).

649 One such research topic that is suggested by this work concerns the effects of viaphytic
650 dispersal on outcrossing (and thus evolutionary trajectories) of taxa utilizing this dispersal
651 strategy. Dispersal by viaphytism could lead to an increase in outcrossing by reducing the
652 chances of mating between spores of the same parent: spores released from the same fruiting
653 event have a relatively high likelihood of colonizing the same nearby substrates and mating.
654 However, if a subset of those spores delay their colonization of wood by becoming endophytes,
655 it is likely that they increase their chances of mating with a non-sibling.

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663 manuscript by [the editor](#), an anonymous reviewer, and Naupaka Zimmerman.

664 **Figures Legends**

665

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

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

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

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

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

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

672

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

674 of culturable viaphytic taxa.

675

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

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

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

679

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

681 substrates inoculated by leaf endophytes.

682

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