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.

Spell out numbers at the beginning of a sentence
I assume you mean species?
Clarify by saying ’70.8% of species’

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

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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*

Introduction

Endophytes are symptomless endosymbionts of living plants (Stone, Bacon & White, 2000) and are ubiquitously present in terrestrial plant tissues worldwide (Arnold & Lutzoni, 2007). Virtually every plant surveyed to date has turned up several to hundreds of species of fungal endophytes per individual, and a single plant species may host thousands of these symbionts across its entire range (Arnold & Lutzoni, 2007; Rodriguez et al., 2009). The effects of endophytes on host plants are varied, and have attracted considerable attention (Carroll, 1988; Rodriguez et al., 2009). Here, we examine a possible benefit of endophytic life histories for the *fungal* partners.

The question of why fungi may adopt endophytic lifestyles has garnered a variety of hypotheses, although few studies have directly addressed them.

Some endophytes may be *latent saprotrophs*, which benefit from priority effects (Chase, 2003; Osono, 2006) by being the first to colonize plant tissues after senescence or death of the host (Promputtha et al., 2007; Parfitt et al., 2010; Porras-Alfaro & Bayman, 2011; Szink et al., 2016). A majority of endophytes have been observed in the litter layer as decomposers (Osono, 2006), although most of these are restricted to the early stages of litter decomposition, when there is higher availability of simple sugars and easily degradable compounds (Carroll & Petrini, 1983). Some endophytes also persist into the late stages of litter decomposition (Peršoh et al., 2013). Certain endophytes have demonstrated an ability to degrade more complex substrates, such as lignin, which further suggests that these may play a role during later stages of litter decay (Osono & Takeda, 1999). Parfitt and colleagues (2010) suggest that most, if not all, trees carry sapwood endophytes with the potential to degrade them, controlled by environmental and biological conditions within the woody tissues themselves.

too jargony

These aren't the best citations. Cite studies that examine endophytes of a host (or closely related hosts) across it's range

Also cite U'Ren & Arnold, 2016

Again, there are more recent citations that would better support your point (e.g., Voříšková & Baldrian, 2013)

Some 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. For instance, a number of major wood decomposers have been found to be common endophytes in leaves (Promputtha et al., 2007), but the benefits of such leaf colonization, for taxa that do not fruit on leaves, is obscure. It has been proposed that such endophytic colonization represents an evolutionary "dead-end" (*i.e.*, saprotrophs found as endophytes are unlikely to escape that state), an idea initially supported by the fact that many endophyte infections occupy only one or a few host plant cells (Carroll, 1988; Bayman et al., 1998; Arnold & Lutzoni, 2007), and more recently by observation that endophyte communities are often different between twigs and leaves of the same host (Sun et al., 2012; Tateno et al., 2015; Thomas et al., 2019), because the two tissues are likely colonized independently and through different mechanisms (Peršoh, 2013).

It may be more accurate to state 'genotypes closely related to wood decomposers'

I'm not following your logic here. Why would these factors support evol. dead end?

Because the colonization of live plant tissues requires specialized chemical and physical systems (Kusari, Hertweck & Spiteller, 2012), the construction of such cellular mechanisms during development, along with with propagule loss, incurs evolutionary costs that are unaccompanied by benefits if endophytism is truly a 'dead end' for these fungi (Carroll, 1988; Thomas et al., 2016, 2019)(Thomas et al., 2016)(Carroll, 1988; Thomas et al., 2016, 2019). One possible explanation for this discrepancy is the **Foraging Ascomycete (FA) hypothesis** (Carroll, 1999; Thomas et al., 2016; Thomas, Vandegrift & Roy, 2017)(Thomas et al., 2016) (Carroll,

1999; Thomas et al., 2016; Thomas, Vandegrift & Roy, 2017), which proposes that the function of leaf endophytism for some fungi may be to increase dispersal to other substrates by helping to bridge spatiotemporal gaps in prefered substrate. While some saprotrophic endophytes canfruit directly from fallen leaves, the FA hypothesis proposes that after leaves senesce and fall, the endophytes are capable of colonizing other, preferred substrates in the environment. Thus, during times of environmental scarcity, endophytes may have an increased likelihood of survival compared to spores or saprobic mycelia, because the highly buffered environment of leaves provides a source of food and water regardless of surrounding environmental conditions (Thomas et al., 2016). The investment of spores into leaf colonization is essentially a form of evolutionary bet-hedging, a strategy that "reduces the temporal variance in fitness at the expense of a lowered arithmetic mean fitness" (Ripa, Olofsson & Jonzén, 2010). Direct spore dispersal by itself may result in a higher mean success rate in colonizing substrates, but it will be highly variable depending on environmental conditions. When a subset of spores from each sporulation event become endophytes, they decrease this variance of dispersal success (Thomas et al., 2016).

Add space

To encompass the processes described by the FA hypotheses, we introduce the new term *viaphyte* to refer to fungi that undergo these lifestyle shifts. We do this because (1) referring to such fungi as "foragers" is vague and leads to confusion, and (2) referring to them as "foraging ascomycetes" (or "FA utilizing fungi" and other such permutations) is unwieldy and inaccurate, as a subset of endophytes in the Basidiomycota likely utilize this dispersal strategy as well. "Viaphyte" is a portmanteau that joins the word *via* — defined as "travelling through a place en route to a destination" — with the suffix, *phyte,* which denotes a plant. Generally, the term refers to fungi that colonize living leaves as endophytes and use those leaves to transfer to another substrate when they fall. In this study, we use the term specifically to refer to fungi which

It would be helpful here to reiterate what you mean by 'lifestyle shifts'.

as a mechanism to disperse

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

Citation needed

Materials and Methods

Culture Methods

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

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

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

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

Identification of Viaphytes

A single representative of each morphotype was subcultured in liquid media (MEA) for DNA extraction using the Qiagen DNeasy Plant kit following the manufacturer's instructions, and the ITS region (the standard "barcode" gene for fungi; Schoch et al., 2012) was amplified using the fungal-specific primer set ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990), or in cases where those primers were ineffective, with ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and LR3 (5'-CCGTGTTTCAAGACGGG-3') primers. DNA amplification was conducted with 12.5-µL reaction volumes (2.5 µL of template, 6.25 µL of Sigma Aldrich JumpstartTM Taq ReadymixTM, 2.75 µL sterile water, 0.5 µL 25 mM MgCl2, and 0.25 µL of each primer). PCR amplification was done with an MJ Research PTC-200 DNA Engine thermal cycler with the following parameters: initial denaturation at 95˚C for 2 min, five cycles of denaturation at 95˚C for 30 s, annealing at 60˚C for 30 s, and extension at 72˚C for 1 min; followed by 25 cycles of denaturation of 95˚C for 30 s, annealing at 55˚C for 30 s, and extension at 72˚C for 1 min; a final extension at 72˚C for 10 min and a final step of indefinite duration at 4˚C. PCR products were visualized on a 1 percent agarose gel. Samples were then frozen until shipping for sequencing at Functional Biosciences, Inc (Madison, WI, U.S.A.) on ABI 3730xl instruments using Big Dye V3.1.The ITS amplicons were sequenced in both directions, then paired and edited using Geneious (v6.0.3; Biomatters Limited, Auckland, New Zealand); sequences were trimmed to

remove priming sites and consensus sequences were generated, with any mismatches checked against the chromatograms. The consensus sequences were then compared to published sequences in the UNITE database (v8.0; Kõljalg et al., 2013) using the *assign_taxonomy.py* function from the Quantitative Insights into Microbial Ecology (QIIME) pipeline (Caporaso et al., 2010). Taxa that returned species assignments as "unidentified" were further examined, and the lowest E-Value match with greater than 97% sequence identity across the entire ITS region, with taxonomic assignment at genus level or better, was used as the taxon assignment. If there was no match above 97% sequence identity, sequences were left as "unidentified". Putative *Xylaria* species were compared to our database of ITS sequences generated from authenticated material within that genus at the same site (Thomas et al., 2016), and assigned to a taxon if the there was greater than 98% sequence identity. Taxa with greater than 99% sequence identity were assumed to be the same taxon (i.e., OTU); all taxa with identical assignments by UNITE met this criteria.

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

Statistical Methods

Species richness was estimated using Chao2 and Jacknife1 estimators (Burnham & Overton, 1978; Chao, 1984; Colwell & Coddington, 1994). Diversity was estimated between all leaves, within leaves, and within boxes using Shannon's index (log base *e* was used; Shannon, 1948) and Simpson's index (1-*D*; Simpson, 1949), and community structure was visualized using non-metric multidimensional scaling (NMDS) and differences assessed with permutational

multivariate analysis of variance (PerMANOVA). Data were analyzed using R Statistical Software, v. 3.1.0 (R Core Team 2014), including the *vegan* package (Oksanen et al., 2013).

All scripts, data tables, and raw data (morphotype counts and sequence chromatograms) is available via an open FigShare repository (Nelson et al., 2019). Edited sequences have been upload 'to' GeneBank (Table S1).

Results

Diversity and Abundance of Viaphytes

Numerous endophytes were able to transfer from leaves into wood. 477 fungal cultures were isolated after making the initial transfer from leaves to wood. These were grouped into 64 morphotypes, 62 of which were successfully identified to genus (59 by DNA, 3 by morphology; Table S1). DNA identification resulted in the consolidation of the morphotypes into 24 unique taxa at the genus level (Table S2). The number of isolates for each taxon varied widely, with 57% of the isolates represented by just 2 genera (*Trichoderma* and *Penicillium*) while 7 of the taxa were isolated only once (Fig. 1). In addition to hyphal growth from the wood substrates, anamorphic fruiting structures were observed growing out of five stick fragments originating from two leaves (Fig. S1), which were identified as *Xylaria flabelliformis* using DNA extracted from stromatic tissues. Including the *X. flabelliformis,* a total of 24 viaphytic taxa, identified to the genus level, were observed (Fig. 1). Additionally, we observed that several of the woody substrate fragments were consumed by apparent white-rot, as evidenced by a white appearance and a dramatic decrease in substrate volume, though we did not attempt to determine which taxa were responsible for this dramatic reduction in volume.

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

that the full richness of viaphytes from these leaves was not isolated (Fig. 2). Estimates of actual species richness ranged from 36.5 (first order jackknife, *SE* = 4.1) to 42.3 (chao2, *SE* = 13.8).

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

Taxonomic Distribution

The higher order taxonomic ranks in our samples included two phyla, five classes, twelve orders, and nineteen families (Table S2). Both Ascomycota and Basidiomycota were present, though Ascomycota was the dominant phylum, both in terms of number of taxa and total number of isolates (73% and 94%, respectively). Sordariomycetes were the most common class in terms of taxa (38.4%), while Eurotiomycetes, driven by the frequency of *Penicillium spp.*, had the most isolates (55.7%). Xylariales and Polyporales were the most common orders in terms of taxa (19.2% each), while Eurotiales, again driven by *Penicillium spp.*, had the most isolates (55.1%).

Functional Guilds

The FUNGuild database contained putative functional guilds for all but two of the genera we isolated as viaphytes. The first unassigned genus, *Alloconiothyrium,* is newly described and presently represented by a single species, *A. aptrootii*, which was isolated from a soil sample in 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

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

The viaphyte community was characterized by a few taxa with high abundances and a large number of taxa with low abundances (Fig. 2). While this pattern is typical for leaf endophytes (Arnold et al., 2000, 2007; Vega et al., 2010; Gazis & Chaverri, 2010; Ikeda et al., 2014; Del Olmo-Ruiz & Arnold, 2017), some patterns in the data suggest that they are partly due to methodological biases. For instance, *Penicillium spp.* and *Trichoderma spp.* were both observed to be fast growing in culture in this study, and culture-based studies are known to be biased for faster-growing taxa (Kirk et al., 2004). Also, given that that each of the four most dominant taxa had a disproportionately high number of isolates concentrated in a single box,

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

Ecological Strategies

It is well documented that many endophytes—e.g., Xylariaceae, the majority of which do not typically reproduce in the litter—have a much broader host range in the endophytic state than as saprotrophs (Davis et al., 2003; Peršoh et al., 2010). It is, in fact, apparently common for such endophytes to be present in the leaves of hosts upon whose wood they never fruit (Carroll & Carroll, 1978; Peršoh et al., 2010; Unterseher, Peršoh & Schnittler, 2013). This is evidence for a Foraging Ascomycete ecology, as a broad host range in the endophytic stage is incompatible with latent saprotrophic strategies for fungi with restricted host ranges in that life stage (Thomas et al., 2016). It is interesting that many fungi which do not typically fruit on litter, such as members of the Xylariaceae, are well knowns as highly competitive litter decay organisms (Koide, Osono & Takeda, 2005; Osono, 2007; Osono et al., 2011) — it is logical that increased

This was examined more extensively in U'Ren et al., 2016 Molecular Phylogenetics and Evolution

Needs to be rewritten. It is confusing as written.

It would be more accurate to say 'fruiting bodies are not typically observed on litter'

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

Sordariomycetes, also noting the dominance of Ascomycota.

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

Functional Guilds

Most of the viaphytic taxa in our study (17 of 25) were classified by FUNGuild as having saprotrophic abilities (Table S3). Many of these saprotrophic taxa are known wood-decay fungi, including *Xylaria spp.* and *Phanerochaete spp.* (Nguyen et al., 2016). In addition, our host leaves were harboring at least some species of physiological white-rot fungi, as evidenced by bleaching of the wood and a substantial decrease in size in several of our substrate fragments. Even some ascomyceteous molds are known to be outstanding degraders of lignin, including some *Penicillium spp.*, *Trichoderma spp*., and *Fusarium oxysporum*, all of which were present among our isolates (Rodriguez et al., 1996; Ryazanova, Chuprova & Luneva, 2015). While the prevailing explanation for the occurrence of saprotrophic fungi as endophytes is that they are latent saprotrophs waiting to consume leaves upon senescence (Peršoh, 2013), many taxa we observed here, and others commonly isolated as endophytes, are not known to reproduce on dead leaves. Alternately, such endophytic saprotrophs may represent an evolutionary 'dead-end' if they are unable to escape that state (Bayman et al., 1998), but our data suggests that it may be the norm for such fungi to transfer out of an endophytic state. Additionally, the presence of several taxa classified as primarily pathotrophs suggests that the facultative ability to access saprotrophic lifestyles may serve as a functional bridge for certain biotrophic species. One might expect that if

biotrophs are cultivated on any given substrate, the resulting community would be dominated by fungi that were typically biotrophic, but with facultative saprotrophic abilities. This, however, is not what we find here, indicating that it is likely that a large proportion of endophytes isolated here are not transitioning to saprotrophy in a facultative manner, but as a transition back to their primary nutritional mode.

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

Conclusion

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

ramifications not only for the dispersal and competition dynamics of fungi, but also for larger scale processes, such as decomposition (Thomas, Vandegrift & Roy, 2017). These dynamics are largely unexplored and represent a vast potential for future research (but see, *e.g.*, Osono, 2006).

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

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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

436 **References**

437 Arnold AE, Henk DA, Eells RL, Lutzoni F, Vilgalys R. 2007. Diversity and phylogenetic affinities of
438 foliar fungal endophytes in loblolly pine inferred by culturing and environmental PCR. *Mycologia*
439 99:185–206.

440 Arnold AE, Lutzoni F. 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves
441 biodiversity hotspots? *Ecology* 88:541–549.

442 Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursar TA. 2000. Are tropical fungal endophytes
443 hyperdiverse? *Ecology letters* 3:267–274.

444 Bayman P, Angulo-Sandoval P, Báez-ortiz Z, Lodge DJ. 1998. Distribution and dispersal of Xylaria
445 endophytes in two tree species in Puerto Rico. *Mycological research* 102:944–948.

446 Boddy L. 2000. Interspecific combative interactions between wood-decaying basidiomycetes. *FEMS*
447 *microbiology ecology* 31:185–194.

448 Burnham KP, Overton WS. 1978. Estimation of the size of a closed population when capture probabilities
449 vary among animals. *Biometrika* 65:625–633.

450 Calhim S, Halme P, Petersen JH, Læssøe T, Bässler C, Heilmann-Clausen J. 2018. Fungal spore diversity
451 reflects substrate-specific deposition challenges. *Scientific reports* 8:5356.

452 Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG,
453 Goodrich JK, Gordon JI. 2010. QIIME allows analysis of high-throughput community sequencing
454 data. *Nature methods* 7:335–336.

455 Carroll G. 1988. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont.
456 *Ecology* 69:2–9.

457 Carroll GC. 1999. The foraging ascomycete. In: *16th International Botanical Congress, Abstracts*. Saint
458 Louis, MO, USA,.

459 Carroll GC, Carroll FE. 1978. Studies on the incidence of coniferous needle endophytes in the Pacific
460 Northwest. *Canadian journal of botany. Journal canadien de botanique* 56:3034–3043.

461 Carroll G, Petrini O. 1983. Patterns of substrate utilization by some fungal endophytes from coniferous
462 foliage. *Mycologia*:53–63.
463 Chao A. 1984. Nonparametric Estimation of the Number of Classes in a Population. *Scandinavian journal*
464 *of statistics, theory and applications* 11:265–270.
465 Chase JM. 2003. Community assembly: when should history matter? *Oecologia* 136:489–498.
466 Clay K. 1988. Fungal Endophytes of Grasses: A Defensive Mutualism between Plants and Fungi. *Ecology*
467 69:10–16.
468 Colwell RK, Coddington JA. 1994. Estimating terrestrial biodiversity through extrapolation.
469 *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 345:101–
470 118.
471 Davis EC, Franklin JB, Shaw AJ, Vilgalys R. 2003. Endophytic Xylaria (Xylariaceae) among liverworts
472 and angiosperms: phylogenetics, distribution, and symbiosis. *American journal of botany* 90:1661–
473 1667.
474 Del Olmo-Ruiz M, Arnold AE. 2017. Community structure of fern-affiliated endophytes in three
475 neotropical forests. *Journal of tropical ecology* 33:60–73.
476 Gazis R, Chaverri P. 2010. Diversity of fungal endophytes in leaves and stems of wild rubber trees
477 (Hevea brasiliensis) in Peru. *Fungal ecology* 3:240–254.
478 Hodgson S, de Cates C, Hodgson J, Morley NJ, Sutton BC, Gange AC. 2014. Vertical transmission of
479 fungal endophytes is widespread in forbs. *Ecology and evolution* 4:1199–1208.
480 Ikeda A, Matsuoka S, Masuya H, Mori AS, Hirose D, Osono T. 2014. Comparison of the diversity,
481 composition, and host recurrence of xylariaceous endophytes in subtropical, cool temperate, and
482 subboreal regions in Japan. *Population Ecology* 56:289–300.
483 Kirk JL, Beaudette LA, Hart M, Moutoglis P, Klironomos JN, Lee H, Trevors JT. 2004. Methods of
484 studying soil microbial diversity. *Journal of microbiological methods* 58:169–188.
485 Koide K, Osono T, Takeda H. 2005. Fungal succession and decomposition of Camellia japonica leaf
486 litter. *Ecological research* 20:599–609.

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM. 2013. Towards a unified paradigm for sequence-based identification of fungi. *Molecular ecology* 22:5271–5277.

Krings M, Taylor TN, Hass H, Kerp H, Dotzler N, Hermsen EJ. 2007. Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. *The New phytologist* 174:648–657.

Kusari S, Hertweck C, Spiteller M. 2012. Chemical ecology of endophytic fungi: origins of secondary metabolites. *Chemistry & biology* 19:792–798.

Lacap DC, Hyde KD, Liew ECY. 2003. An evaluation of the fungal "morphotype" concept based on ribosomal DNA sequences. *Fungal diversity* 12:53–66.

McCartney A, West J. 2007. Dispersal of fungal spores through the air. In: *Food Mycology*. CRC press, 79–96.

Nelson A, Vandegrift R, Carroll GC, Roy BA. 2019. Data from: Double Lives: Transfer of fungal endophytes from leaves to woody substrates. DOI: 10.6084/m9.figshare.9794699.v1.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal ecology* 20:241–248.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2013. Package "vegan." *R Packag ver* 254:20–8.

Osono T. 2006. Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. *Canadian journal of microbiology* 52:701–716.

Osono T. 2007. Ecology of ligninolytic fungi associated with leaf litter decomposition. *Ecological research* 22:955–974.

Osono T, Takeda H. 1999. Decomposing ability of interior and surface fungal colonizers of beech leaves with reference to lignin decomposition. *European journal of soil biology* 35:51–56.

513 Osono T, To-Anun C, Hagiwara Y, Hirose D. 2011. Decomposition of wood, petiole and leaf litter by
514 Xylaria species from northern Thailand. *Fungal ecology* 4:210–218.
515 Parfitt D, Hunt J, Dockrell D, Rogers HJ, Boddy L. 2010. Do all trees carry the seeds of their own
516 destruction? PCR reveals numerous wood decay fungi latently present in sapwood of a wide range of
517 angiosperm trees. *Fungal ecology* 3:338–346.
518 Peršoh D. 2013. Factors shaping community structure of endophytic fungi–evidence from the Pinus-
519 Viscum-system. *Fungal diversity* 60:55–69.
520 Peršoh D, Melcher M, Flessa F, Rambold G. 2010. First fungal community analyses of endophytic
521 ascomycetes associated with Viscum album ssp. austriacum and its host Pinus sylvestris. *Fungal*
522 *biology* 114:585–596.
523 Peršoh D, Segert J, Zigan A, Rambold G. 2013. Fungal community composition shifts along a leaf
524 degradation gradient in a European beech forest. *Plant and soil* 362:175–186.
525 Porras-Alfaro A, Bayman P. 2011. Hidden fungi, emergent properties: endophytes and microbiomes.
526 *Annual review of phytopathology* 49:291–315.
527 Promputtha I, Lumyong S, Dhanasekaran V, McKenzie EHC, Hyde KD, Jeewon R. 2007. A phylogenetic
528 evaluation of whether endophytes become saprotrophs at host senescence. *Microbial ecology*
529 53:579–590.
530 Ripa J, Olofsson H, Jonzén N. 2010. What is bet-hedging, really? *Proceedings. Biological sciences / The*
531 *Royal Society* 277:1153–1154.
532 Rodriguez A, Perestelo F, Carnicero A, Regalado V, Perez R, de la Fuente G, Falcon MA. 1996.
533 Degradation of natural lignins and lignocellulosic substrates by soil-inhabiting fungi imperfecti.
534 *FEMS microbiology ecology* 21:213–219.
535 Rodriguez RJ, White JF Jr, Arnold AE, Redman RS. 2009. Fungal endophytes: diversity and functional
536 roles. *The New phytologist* 182:314–330.
537 Rogers JD. 2000. Thoughts and musings on tropical Xylariaceae. *Mycological research* 104:1412–1420.
538 Roy S, Banerjee D. 2018. Diversity of Endophytes in Tropical Forests. In: Pirttilä AM, Frank AC eds.

Endophytes of Forest Trees: Biology and Applications. Cham: Springer International Publishing, 43–62.

Ryazanova TV, Chuprova NA, Luneva TA. 2015. Effect of Trichoderma fungi on lignin from tree species barks. *Catalysis in Industry* 7:82–89.

Schmit JP, Lodge DJ. 2005. Classical methods and modern analysis for studying fungal diversity. *Mycology Series*.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Bolchacova E, Voigt K, Crous PW, Miller AN, Wingfield MJ, Aime MC, An K-D, Bai F-Y, Barreto RW, Begerow D, Bergeron M-J, Blackwell M, Boekhout T, Bogale M, Boonyuen N, Burgaz AR, Buyck B, Cai L, Cai Q, Cardinali G, Chaverri P, Coppins BJ, Crespo A, Cubas P, Cummings C, Damm U, Beer ZW de, Hoog GS de, Del-Prado R, Dentinger B, Diéguez-Uribeondo J, Divakar PK, Douglas B, Dueñas M, Duong TA, Eberhardt U, Edwards JE, Elshahed MS, Fliegerova K, Furtado M, García MA, Ge Z-W, Griffith GW, Griffiths K, Groenewald JZ, Groenewald M, Grube M, Gryzenhout M, Guo L-D, Hagen F, Hambleton S, Hamelin RC, Hansen K, Harrold P, Heller G, Herrera C, Hirayama K, Hirooka Y, Ho H-M, Hoffmann K, Hofstetter V, Högnabba F, Hollingsworth PM, Hong S-B, Hosaka K, Houbraken J, Hughes K, Huhtinen S, Hyde KD, James T, Johnson EM, Johnson JE, Johnston PR, Jones EBG, Kelly LJ, Kirk PM, Knapp DG, Kõljalg U, Kovács GM, Kurtzman CP, Landvik S, Leavitt SD, Liggenstoffer AS, Liimatainen K, Lombard L, Luangsa-ard JJ, Lumbsch HT, Maganti H, Maharachchikumbura SSN, Martin MP, May TW, McTaggart AR, Methven AS, Meyer W, Moncalvo J-M, Mongkolsamrit S, Nagy LG, Nilsson RH, Niskanen T, Nyilasi I, Okada G, Okane I, Olariaga I, Otte J, Papp T, Park D, Petkovits T, Pino-Bodas R, Quaedvlieg W, Raja HA, Redecker D, Rintoul TL, Ruibal C, Sarmiento-Ramírez JM, Schmitt I, Schüßler A, Shearer C, Sotome K, Stefani FOP, Stenroos S, Stielow B, Stockinger H, Suetrong S, Suh S-O, Sung G-H, Suzuki M, Tanaka K, Tedersoo L, Telleria MT, Tretter E, Untereiner WA, Urbina H, Vágvölgyi C, Vialle A, Vu TD, Walther G, Wang Q-M, Wang Y, Weir BS, Weiß M, White MM, Xu J, Yahr R, Yang ZL, Yurkov A, Zamora J-C, Zhang N, Zhuang W-Y, Schindel D. 2012. Nuclear ribosomal

internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America* 109:6241–6246.

Shannon CE. 1948. A Mathematical Theory of Communication. *Bell System Technical Journal* 27:379–423.

Sherwood-Pike M, Stone JK, Carroll GC. 1986. Rhabdocline parkeri, a ubiquitous foliar endophyte of Douglas-fir. *Canadian journal of botany. Journal canadien de botanique* 64:1849–1855.

Simpson EH. 1949. Measurement of Diversity. *Nature* 163:688–688.

Slippers B, Wingfield MJ. 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal biology reviews* 21:90–106.

Stone JK. 1987. Initiation and development of latent infections by Rhabdocline parkeri on Douglas-fir. *Canadian journal of botany. Journal canadien de botanique* 65:2614–2621.

Stone JK, Bacon CW, White JF Jr. 2000. An overview of endophytic microbes: endophytism defined. In: *Microbial endophytes*. CRC Press, 17–44.

Sun Y, Wang Q, Lu X, Okane I, Kakishima M. 2012. Endophytic fungal community in stems and leaves of plants from desert areas in China. *Mycological progress* 11:781–790.

Szink I, Davis EL, Ricks KD, Koide RT. 2016. New evidence for broad trophic status of leaf endophytic fungi of Quercus gambelii. *Fungal ecology* 22:2–9.

Tateno O, Hirose D, Osono T, Takeda H. 2015. Beech cupules share endophytic fungi with leaves and twigs. *Mycoscience* 56:252–256.

Thomas DC, Vandegrift R, Ludden A, Carroll GC, Roy BA. 2016. Spatial Ecology of the Fungal Genus Xylaria in a Tropical Cloud Forest. *Biotropica* 48:381–393.

Thomas DC, Vandegrift R, Roy B. 2017. An agent-based model of the Foraging Ascomycete Hypothesis. *bioRxiv*.

Thomas D, Vandegrift R, Roy BA, Hsieh H-M, Ju Y-M. 2019. Spatial patterns of fungal endophytes in a subtropical montane rainforest of northern Taiwan. *Fungal ecology* 39:316–327.

Unterseher M, Peršoh D, Schnittler M. 2013. Leaf-inhabiting endophytic fungi of European Beech (Fagus

591 sylvatica L.) co-occur in leaf litter but are rare on decaying wood of the same host. *Fungal diversity*
592 60:43–54.
593 Vandegrift R, Thomas DC, Ju Y-M, Soukup H, Carroll GC, Roy BA. 2019. Spatial ecology of
594 endophytes in Taiwan: combining traditional collection and next-generation sequence-based
595 microbial survey techniques. *Mycologia*.
596 Vega FE, Simpkins A, Aime MC, Posada F, Peterson SW, Rehner SA, Infante F, Castillo A, Arnold AE.
597 2010. Fungal endophyte diversity in coffee plants from Colombia, Hawai'i, Mexico and Puerto Rico.
598 *Fungal ecology* 3:122–138.
599 Voříšková J, Baldrian P. 2013. Fungal community on decomposing leaf litter undergoes rapid
600 successional changes. *The ISME journal* 7:477–486.
601 White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal
602 RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* 18:315–322.

603 Rodriguez A, Perestelo F, Carnicero A, Regalado V, Perez R, De la Fuente G, Falcon MA. 1996.
604 Degradation of natural lignins and lignocellulosic substrates by soil-inhabiting fungi imperfecti.
605 *FEMS Microbiology Ecology*. 21:213–9.
606 Ryazanova TV, Chuprova NA, Luneva TA. 2015. Effect of *Trichoderma* fungi on lignin from tree species
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.

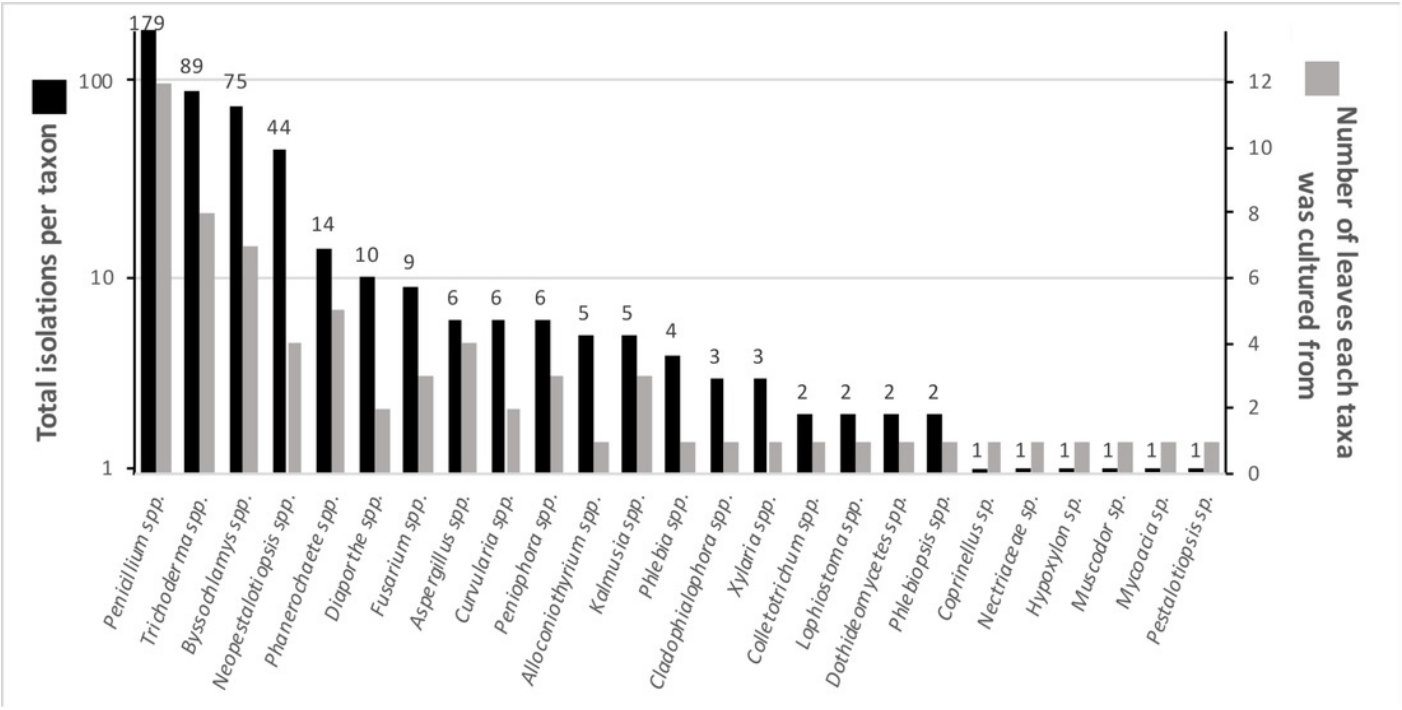

Figure 2

Species accumulation curve for viaphytes.

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

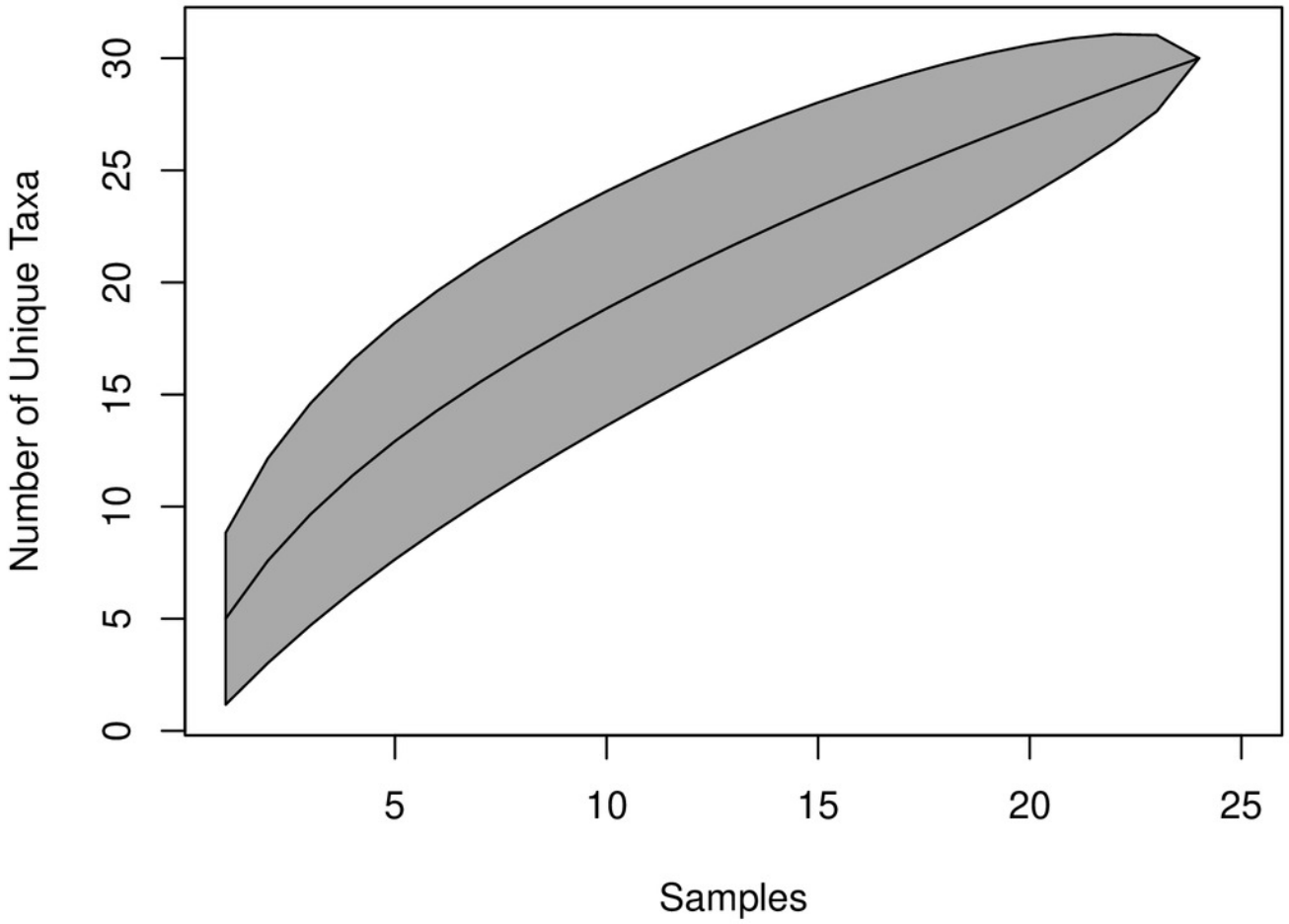

Figure 3

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.