The biogeography and biodiversity of endophytes—how far have we come and where do we go from here?

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Abstract

The interiors of plants are colonized by a diverse group of microorganisms. Many of these microbes do not harm their hosts in obvious ways for at least a portion of their life history and are referred to as endophytes. Because of their capacity to influence host phenotypes, endophytes have received a great deal of attention over the past few decades, yet basic questions of endophyte biogeography, ecology, and evolution remain unanswered. To determine the state of endophyte biodiversity exploration—at multiple spatial scales and across the plant phylogeny—we synthesized results from nearly 600 published studies. Our survey revealed a global interest in endophyte biology and highlighted several pressing gaps in knowledge. For instance, of the seventeen biomes encompassed by our survey, seven had fewer than 50 studies (including the boreal, alpine, and tropical grasslands biomes, among others) and together composed only 7% of the studies we considered. We found that fungal endophyte diversity has been characterized in at least one host from 31% of embryophyte families, while bacterial endophytes have been surveyed in hosts from only 10.5% of families. We complimented our broad survey with a meta-analysis and vote counting procedure to determine endophyte richness and diversity patterns at a small spatial scale—among plant tissue types. We found that variation in fungal endophyte richness and diversity among above-ground tissues differed as a function of host growth habit. Stems were the richest tissue in woody plants, whereas roots were the richest tissue in graminoids. For forbs, we observed no clear pattern of one tissue type harboring the most endophytic taxa. We propose a series of future directions and guidelines to fill the gaps in knowledge we uncovered and inspire further research.

Introduction

In 1887, Galippe reported that microbes could reside within the tissues of healthy plants. At the time, this work was unappreciated, perhaps because of the long-prevailing attitude that microbial assemblages solely comprised deleterious pathogens (Compant et al. 2012). Nevertheless, Galippe's observations set the stage for an exploration of the plant microbiome that took place during the early to mid 1900s. During those decades, knowledge began to accumulate regarding the diversity, prevalence, and ecological roles of so called "endophytes" (Box 1; Campbell 1908, Hyde and Soytong 2008), with most early work focused on the fungi living within grasses (e.g., Neill 1940, Sampson 1937). Seminal research in the 1970s and 80s led to widespread acknowledgement of the ubiquitous nature of non-pathogenic fungi and bacteria in plant tissues (Carroll and Carroll 1978, Carroll 1988, Petrini 1991). These studies have inspired intense and ever-growing interest from microbial ecologists (Fig. 1), yet answers to many basic questions regarding the natural history, biogeography, ecology, and evolution of endophytes remain elusive.

However, it is clear that fungal and bacterial endophytes are important—even critical—components of the world's ecosystems. Endophytes can affect plant phenotype, including decreasing disease susceptibility (Arnold et al. 2003, Busby et al. 2016, Christian et al. 2017, Compant et al. 2005, Herre et al. 2007), shaping phytochemical profiles (Kusari et al. 2012,

Panaccione et al. 2014), and mediating plant functional trait expression (Griffin et al. 2016, Friesen et al. 2011). Recent work has demonstrated how these various effects of endophytes can influence whole ecosystem level processes (Christian et al. 2019, Clay and Holah 1999, Griffin et al. 2017, Laforest-Lapointe et al. 2017b). Importantly, endophytes are often erroneously assumed to have predominantly mutualistic associations with their hosts. Reality is much more complex and the influence of endophyte taxa is highly context dependent (Carroll 1988), with interactions between hosts and endophytes ranging from mutualism through commensalism to latent or mild antagonism (Hardoim et al. 2008, Schulz and Boyle 2005, Saikkonen et al. 1998).

Much of the interest in endophytes has been driven by applied scientists interested in harnessing endophytes as a means to manipulate plant phenotype (e.g., increase growth; Doty 2008) and prevent pathogen colonization of crops (Busby et al. 2017). Endophytes have also attracted attention from natural products chemists who survey the world's organisms for useful compounds (Aly et al. 2010, Strobel and Daisy 2003). This is motivated by the capacity of various endophytes to synthesize an impressive array of bio-active small molecules (Newman et al. 2003, Strobel et al. 2004, Verma et al. 2009). Indeed, a number of endophyte-synthesized compounds are of medicinal value (Kharwar et al. 2011, Strobel et al. 1996).

Both basic and applied research regarding endophytes have been hampered by the lack of knowledge regarding endophyte biogeography. Biogeography is an inductive science that relies upon description of patterns in biodiversity to understand the forces that could have caused those patterns (Nemergut et al. 2013). Research by Higginbotham et al. (2013) provides an exemplar of how biogeographic knowledge can have both basic and applied implications. These researchers isolated over 3000 endophytic fungi from numerous tropical angiosperms and ferns and tested these cultures against common diseases, including malaria, Chagas disease, and cancer. They report that 30% of the fungi showed strong activity against at least one of the focal diseases and that bioactivity against a specific target was non-randomly distributed across the fungal phylogeny. Intriguingly, they also reported a generally higher degree of bioactivity in taxa sourced from cloud forests compared to lowland tropical forests—thus providing a biogeographic road-map for natural product discovery in tropical forests (also see Schulz et al. 2002).

Most of what is currently known regarding endophyte biogeography is limited to relatively-small spatial scales. For instance, many studies have confirmed that endophyte assemblages vary within hosts among tissue types (e.g., the endophyte assemblages in roots differ from those in the leaves; Coleman-Derr et al. e.g., 2016), though general patterns in endophyte richness among tissue types have not been described. Also, it is clear that endophyte assemblages shift among coexisting host species, at least to some extent (Griffin et al. 2019, Redford et al. 2010, Vincent et al. 2015). While these patterns may not seem to encompass a broad enough spatial scale to be "biogeographic" in the traditional sense, it must be remembered that the disparity in size between a single bacterium of $2\,\mu m^3$ and a large tree of $500\,m^3$ mirrors the ratio in scale between an automobile and a mid-sized country (as demonstrated in Fig. 1 of Remus-Emsermann and Schlechter 2018). Thus, the spatial scale at which endophytes are sampled—for example, the leaf or some portion thereof—encompasses significant biogeographical variation from a microbial perspective. Indeed, using traditional culturing and sequencing methodologies, we can only sample what are in effect whole "re-

gions" of endophytes that may include multiple assemblages that never directly interact. This complicates the study of endophyte biogeography because the scale of sampling is so much larger than many covariates that may affect membership of endophytes in a particular assemblage. For instance, microhabitat variation within leaves (such as proximity to upper or lower leaf surfaces, veins, etc.) may have affects on endophyte assemblages akin to the effects of shifting elevation on forest composition across a mountainside, and those forcings are unavailable for study when the unit of replication is an entire leaf, or even a leaf section (Herre et al. 2007, Lodge et al. 1996, Remus-Emsermann and Schlechter 2018, Vacher et al. 2016a). To further complicate matters, bacterial endophytes can live inside endophytic fungi (Shaffer et al. 2016), thus, for these bacterial endophytes, the habitat covariates most relevant for explaining inter-assemblage variation may be the traits of the host fungus, not the traits of the host plant.

At larger spatial scales, including across broad latitudinal and elevational gradients, and among biomes and continents, several patterns have emerged. Typically, endophyte assemblages are characterized by dramatically skewed rank abundance curves, where a few taxa are much more abundant than the numerous marginal taxa present (e.g., Davis and Shaw 2008, Shade and Handelsman 2012) and the similarity in assemblages declines with distance, though the causes of this decline are likely multifarious and poorly understood. This phenomenon is often referred to as "distance-decay" in assemblage similarity (Davis and Shaw 2008, Higgins et al. 2014, Nemergut et al. 2013, Vacher et al. 2016b). Moreover, seminal work by Arnold and Lutzoni (2007) showed that fungal endophyte diversity tended to increase at lower latitudes, thus mirroring the latitudinal gradient in biodiversity experienced by so many large, multicelluar taxa (Pianka 1966). Also, several studies have reported greater fungal endophyte richness in wetter locations (Lau et al. 2013, Zimmerman and Vitousek 2012), and, that more generally, endophyte biogeography is influenced by elevational and climatic variation. For example, Bowman and Arnold (2018) found that *Pinus ponderosa* hosted more diverse foliar fungal endophyte communities at mid-to-high elevations compared to lower elevations in southwestern Arizona (also see Giauque and Hawkes 2013). These patterns confirm that endophytes, like other microbes, do have meaningful biogeography that is shaped by contemporary circumstance (i.e, habitat variation; the Baas Becking hypothesis that "everything is everywhere, but the environment selects"; Baas Becking 1934). However, it remains unclear how historical factors and ecological drift influence endophyte distribution at any spatial scale (for a primer on the possible roles of these forces in community assembly see Nemergut et al. 2013 and Vellend 2010).

To understand the scope of research characterizing endophyte biodiversity and biogeography, we scoured the literature and extracted basic metadata from 596 studies characterizing endophyte assemblages. Our primary goal was to synthesize the foci of studies completed to date, ultimately with the hopes of highlighting particular portions of the plant phylogeny and specific biomes that need further exploration. Next, we paired this survey with a meta-analysis and vote counting procedure where we compared patterns of endophyte richness and diversity among tissue types. Our synthesis highlighted the challenges of pooling information among studies and, consequently, we offer specific guidelines for data sharing and research reproducibility moving forward.

Box 1. What, exactly, is an endophyte?

The term 'endophyte' is believed to have originated with de Bary (1866), who so dubbed pathogenic, plant-inhabiting microbes, because of their habitat. Since then, the term endophyte has been expanded to invoke both a habitat and a non-pathogenic lifestyle, and encompasses fungal (Rodriguez et al. 2009, Petrini 1991), bacterial (Griffin and Carson 2015, Ryan et al. 2008), and archael taxa (Moissl-Eichinger et al. 2018, Müller et al. 2015). In our experience, contemporary microbial ecologists most often use the term endophyte to refer to those taxa that live inside of plant tissues and, which over some portion of their life history, do not cause obvious harm to their hosts, such as inducing a hypersensitive response (Wilson 1995, Petrini 1991, Stone et al. 2000). The lack of precision in this definition is somewhat unsatisfying, but does hint at the complex life histories of many endophytic taxa (Rodriguez et al. 2009). Indeed, for perhaps the majority of endophytic taxa, individuals are horizontally transmitted among hosts and, consequently, may exist outside of the plant corpus for some time, for instance as spores or endospores, free living cells or colonies, or as epiphytic fruiting bodies on decaying tissue (Malloch and Blackwell 1992, Rodriguez et al. 2009). The term endophyte is particularly strained by the mycorrhizal fungi, which possess a mycelium that grows externally to the host but that also penetrates the root epidermis (Schulz and Boyle 2006, Jumpponen 2001). In some cases, mycorrhizae are found growing wholly within plant tissues, and their categorization as endophytes seems to be on an author-by-author basis (Schulz and Boyle 2006). These examples illustrate how the term endophyte is useful for communication, but not biologically well-delineated. For our purposes in this article, we do not consider obligate pathogens, epiphytes, or mycorrhizae; nor do we include a review of the large body of literature examining *Rhizobia* and their associations with legumes, as others have already done so (e.g., Peter et al. 1996, Willems 2006).

Methods

We searched Google Scholar and Web of Science for the term "endophyte" in conjunction with "fungal", "bacterial", "diversity", or "community". All publications in which the authors characterized endophyte assemblage biodiversity were collated. Studies of root endophytes were included, but those studies that focused on mycorrhizae were omitted from consideration. As we were primarily interested in studies characterizing endophyte biodiversity, we did not consider research involving manipulative experiments where no survey of microbial diversity was conducted. We also made the choice to omit studies that did not distinguish between epiphytes and endophytes through performing some form of surface sterilization. Searches were performed periodically from 2016–2018 and additional studies added to our database as we became aware of them until the beginning of 2019. We apologize to authors who have published their work in non-English language journals, which were inaccessible to us.

From each study, we collected information on host organism(s) studied, research location(s), tissue type(s) surveyed, and various metadata describing the nature of the survey conducted—for instance, if the endophyte assemblage was characterized via sequencing or culturing, if spatial or temporal replication was employed, host and culture vouchers deposited, and data made available. If the study location was not explicitly provided, we extrapolated an estimate based on the city or country reported by the authors. We assigned studies to biomes following the nomenclature of Olson et al. (2001). We found few studies conducted within dunes and on beaches and so combined them with those from the flooded grassland biome. Host plants collected from urban, agricultural, or areas that were otherwise managed, were classified as coming from "cultivated" landscapes, and these studies are not included in our estimates of the number of studies for each biome because managed areas experience ecological contingencies divergent from their surroundings (e.g., irrigation). We considered studies of "stems" as those involving sampling of woody branches, twigs, or grass shoots. Studies of "roots" included any survey of below-ground plant tissue, but excluded rhizosphere soil surveys. We considered studies of "leaves" to be those sampling leaf sections or whole leaves/leaflets (including needles), and did not consider studies that sampled petioles.

To understand the phylogenetic breadth of host plants surveyed, we calculated the total number of hosts examined for each plant family and plotted this information on a phylogeny of the Embryophyta (algal endophyte hosts were thus omitted from this portion of our analysis) generated using phyloT (online software accessible at `https://phylot.biobyte.de/`). The National Center for Biotechnology Information taxonomy database was used to generate the tree (database accessed March 15, 2019; Federhen 2012). iTOL v4.3.2 was used for tree visualization (Letunic and Bork 2016). All data wrangling was performed in the R statistical computing environment (R Core Team 2019).

Meta-analysis

In addition, we asked how endophyte richness shifted among tissue types, for both fungi and bacteria. We took two approaches to address this question—a formal meta-analysis and a simple vote counting approach. Because few studies used the same methods, comparing the effects of tissue type on richness *among* studies was inappropriate (this limitation precluded comparison of richness among taxa or across biomes, unfortunately). Thus, we only examined those studies that compared richness among multiple tissue types—thus all comparisons were made *within* studies. We omitted those studies that did not standardize observational effort among tissues by either mass or sample count (i.e., the number of samples from each tissue type). We also only considered studies that provided a table describing the counts of each microbial taxon observed within each sample (e.g., an operational taxonomic unit [OTU] table), because these data were required to calculate diversity and richness indices. Out of the 558 studies that examined multiple tissues, nine met these criteria for fungi. For bacteria, only a single study met these criteria, precluding a formal meta-analysis, thus for this taxon we only performed vote counting, which required a less stringent set of criteria for study selection (see below). We rarefied each OTU table by the minimum number of observations for a sample within that study and calculated richness and exponentiated Shannon's diversity

for each sample. Calculations were performed using the vegan R package v2.5-5(Oksanen et al. 2016). A random effects model was used to estimate differences in richness and diversity between tissue types while accounting for among-study variation. Models were implemented using the metafor v2.1-0 (Viechtbauer 2010) R package using a restricted maximum likelihood estimation approach.

Given the paucity of studies that met our criteria for meta-analysis, we decided to conduct a simple vote counting procedure where we considered each study independently and ranked tissue types by the relative richness reported in that study. We examined 243 studies in this way: 182 studies of fungal endophytes and 61 studies of bacterial endophytes (these studies met the aforementioned criteria, save the provision of an OTU table). After ranking tissues by relative richness separately for each study, we calculated, across studies, the proportion of times one tissue type had higher richness than another tissue (e.g., for what proportion of studies did leaves have higher richness than roots) and tested the significance of these proportions using a binomial sign test (Cooper and Hedges 1993). This test is simply the probability of observing a particular number, or more, of positive outcomes (in our case, one tissue type having higher richness than another) given a certain number of trials and assuming equal probability of positive and negative outcomes. For this vote counting approach, we focused on richness because fewer studies reported diversity metrics and, when not explicitly reported by authors, relative richness was simpler to calculate and extract from published summary tables and figures than were diversity entropies. To test how growth habit influenced relative microbial richness among tissues, we conducted vote counting separately for studies of hosts with the following growth habits: woody-stemmed trees and shrubs, forbs, and graminoids.

Results

Our survey highlighted the breadth of the endophyte biodiversity literature, as we extracted data from 596 unique publications. This level of research interest is all the more impressive given that few studies were included in our survey from before the mid 1970s. We report that interest in endophyte diversity is on the rise, with a sharp increase in studies per year since 2010 (Fig. 1). Fungi have received comparatively more attention than bacteria, though this disparity is diminishing (Figs. 1 & 2e). The majority of studies were of foliar endophytes (1694 unique combinations of study and host species), followed by root (577 combinations) and stem (540 combinations) endophytes. By comparison, floral tissues (39 combinations) and plant propagules were understudied (172 combinations; Fig. 2). Multiple-host studies were not the norm—approximately ~66% of studies focused on a single host taxon.

The global scale of endophyte biodiversity research

The geographical range encompassed by the studies we considered was impressive; endophytes, both fungal and bacterial, have been recovered from hosts across all major biomes and all continents (Fig. 3). Temperate mixed coniferous and deciduous forests were the best studied biomes, with 98 studies (16% of total). However, the most unique combinations of host and study were reported from tropical and subtropical wet forests (471, 21% of total).

This was due to several studies that surveyed many hosts within these forests (e.g., Rojas-Jimenez et al. 2016 with 92 hosts and Suryanarayanan et al. 2011 with 70 hosts). In terms of unique studies, research in tropical and subtropical forests composed a more modest 13% of studies in our survey. Many biomes were quite understudied. For instance, seven of the seventeen biomes that we considered had 50 or fewer studies (Fig. 2b). Together, studies from these biomes composed only 7% of the publications surveyed.

Across biomes, we found comparatively few studies of hosts growing in obvious wilderness, far from human development. Indeed, 33% of studies relied on hosts grown in cultivated environments, including urban locations, agricultural landscapes, and greenhouses (with university campuses being particularly well sampled). This estimate may be conservative as for some studies the exact collection location was difficult to determine and so we did not include them in the "cultivated" category, but sampling was likely not far from human development.

Much of the host phylogeny remains unsampled

The studies we surveyed encompassed 1702 unique taxa from 254 plant families. Poaceae was by far the most well-studied family (189 hosts studied), followed by Fabaceae (98 hosts), Pinaceae (82 hosts), and Asteraceae (79 hosts; Fig. 4). Fungal endophytes have been surveyed in hosts from 31% of plant families listed in the NCBI taxonomy database for Embryophyta. By comparison, bacterial endophytes have been characterized in only 10.5% of plant families. Of particular note, very few observations of foliar microbiota have been made among bryophyte and pteridophyte families (Fig. 4). Liverwort families have been comparatively well surveyed due to a single, excellent paper by Davis and Shaw (2008). Additionally, we observed a striking mismatch between host family species richness and sampling effort. For instance, only 29 Orchidaceae species have been surveyed out of the approximately 28,000 accepted orchid taxa occurring worldwide (The Plant List,Chase et al. 2015).

Replication and reproducibility could be improved

We also characterized details for each study regarding sampling scheme and reproducibility (Fig. 2de). We found that just over half of studies were spatially replicated (sampling areas were separated by at least a km) and fewer than a quarter of studies were temporally replicated. The majority of studies (~77% of both fungi and bacteria relied on culturing, however less than half of these studies reported accessioning cultures (Fig. 2e). By comparison, 37.6% of studies that relied on sequence data provided clear instructions for downloading raw data, though only 22% of these studies provided processed data (such as an OTU table). Surprisingly, fewer than 20% of studies mentioned accessioning host vouchers. For cultivated plants, we considered a description of the cultivar as equivalent to an accessioned voucher.

The effects of tissue type on endophyte richness and diversity

We performed vote counting and a meta-analysis to compare the relative richness and diversity of fungal endophyte assemblages in varying tissue types across plant taxa. Across all hosts considered via meta-analysis, we found no significantly supported differences among tissue types in richness or Shannon's diversity (Figs. S1 & S2). However, our vote counting approach allowed us to examine many more studies than the meta-analysis and clearly demonstrated that relative tissue richness was dependent upon host growth habit. For instance, stems had richer fungal endophyte assemblages than leaves for woody-stemmed

hosts, but this pattern was not observed for either forbs or graminoids (Table S1). By comparison, for graminoids, roots had richer fungal and bacterial endophyte assemblages than stems (Table S3). For forbs, no tissue type was clearly richer, on average, than other tissues (Table S2). Additionally, for fungal endophytes, we found that reproductive structures, including flowers and propagules, were relatively species poor, while bark was species rich (Table S1 & S2), though these results are tentative given the few studies that compared endophyte assemblages in these tissues to those in other portions of the plant corpus.

Discussion

We enthusiastically report a global interest in the study of endophyte biodiversity that is intensifying dramatically as awareness builds regarding the ecological importance of plant microbiomes (Fig. 1). Over just the past few decades, hundreds of studies have been published that demonstrate the ubiquity and taxonomic diversity of endophytes. This is heartening and confirms a rapid growth in understanding of endophyte biogeography and biodiversity. Our survey highlighted several gaps in knowledge that should be the target of focused effort as we build upon the existing body of work. Most importantly, we found that vast portions of the globe, including many important biomes, are understudied and the potential of the plant phylogeny to harbor novel endophyte lineages is only beginning to be explored. We also report that host growth habit influences the relative richness among tissue types for both fungi and bacteria.

Endophyte research spans the globe, but certain biomes and continents remain understudied

Endophyte biodiversity has been studied on every continent and within all biomes (Fig. 3). Given that widespread interest in endophytes did not occur until the 1970s, progress has been rapid and is worth celebration. However, great swathes of the globe still remain unsurveyed. Certain biomes have been particularly understudied—either due to their high biodiversity, which makes thorough sampling exceptionally difficult (i.e., tropical rainforests); large geographical area (the boreal forest); or because they are geographically restricted and simply have not received much attention (mangroves). For instance, we found only 17 studies from coastal dunes and flooded grasslands, when excluding studies from rice paddies. These habitats are challenging for plants, due to salinity, short intervals between disturbances, and, for flooded grasslands, the presence of anoxic soil. Surveys of understudied biomes will help define the scope of endophyte biodiversity. In particular, we suggest that surveys in flooded grasslands and mangroves may improve our understanding of archael endophyte biodiversity (Moissl-Eichinger et al. 2018), as this branch of life includes numerous halophiles and other extremophiles that may be able to cope with the harsh conditions characteristic of those locations. Similarly, studies in desert and alpine biomes may uncover endophytes with unique mechanisms for coping with the severe ultraviolet exposure, temperature swings, and desiccation that occurs in those habitats (Lopez et al. 2011, Massimo et al. 2015, Sangamesh et al. 2017).

A thorough characterization of inter-biome variation in endophyte biodiversity would further knowledge of how abiotic forces shape endophyte assemblages—a goal that has long been pursued by microbial ecologists (e.g., Nemergut et al. 2013, Zimmerman and Vitousek

2012). For instance, we still do not have a robust understanding of the relative importance of various abiotic gradients for broad patterns of endophyte richness and diversity or how these gradients might affect specific taxa (e.g., fungi versus bacteria or Ascomycetes versus Basidiomycetes). Such knowledge will be of critical practical importance as the climate continues to change, given that we wish to predict how endophyte assemblages will respond to shifts in precipitation, temperature, and disturbance regimes that come with global warming (Bálint et al. 2015, Giauque and Hawkes 2013). Importantly, endophyte assemblages can include latent pathogens that do not become symptomatic until times of host stress, including, stress due to drought or heat (Carroll 1988, Slippers and Wingfield 2007, Stanosz et al. 2001), thus it is likely that climate change related stressors will have profound effects on endophyte assemblages. Indeed, in an experimental warming and relocation experiment of *Populus balsamifera*, Bálint et al. (2015) reported that transplantation to northern latitudes led to an decrease in the relative abundance of *Mycosphaerella* fungi, a group that includes many pathogens, but that this effect was counteracted to a degree by experimental warming. Coupling manipulative experiments of this kind with data from large-scale surveys will be critical to disentangle the often confounded effects of abiotic forcings, geography, and climate change.

We also reported a lack of studies from Africa, west and north Asia, and the interiors of Australia and South America (Fig. 3). These areas hold some of the most biodiverse and charismatic landscapes on the planet; for instance, the Congo basin is the second largest tropical rainforest in the world, with thousands of endemic plant taxa (Brenan 1978, Linder 2001), and it has experienced less deforestation than other rainforests (Koenig 2008). Similarly, the Cape Floristic province in Africa has some of the highest levels of plant endemism in the world. Because these regions have evolutionary histories that have facilitated endemism, it seems likely that they harbor unique endophyte taxa and would be prime locations to study coevolution and codivergence between plants and endophytes. More generally, the lack of sampling outside of North American, Europe, and portions of Asia precludes a robust knowledge of endophyte biogeography, and sampling a variety of host taxa from poorly surveyed areas should be a priority moving forward.

The influence of human development on endophyte biodiversity

We acknowledge the logistical challenges of sampling the more remote locations that remain understudied. Indeed, we report an imprint of this challenge in even relatively well-studied regions, where we found that few studies were conducted more than a few kilometers from roadways, townships, and other human development. The lack of sampling in wilderness areas likely biases our nascent understanding of endophyte biodiversity. Human development is associated with pollution, habitat fragmentation, ecosystem disturbance frequency, and the abundance of introduced hosts (Crowl et al. 2008, Dietz et al. 2007)—all of which likely affect plant microbiomes. Evidence for this hypothesis is sparse, however Laforest-Lapointe et al. (2017a) reported many phyllosphere bacterial taxa shift in relative abundance along an urbanization gradient, with an overall decline in dominant Alphaproteobacteria with more urbanization. Similarly, Lappalainen et al. (1999) reported a decline in endophyte colonization of *Betula* trees with proximity to copper-nickel smelter. Variation in heavy metal concentrations (Tóth et al. 2009, Jurc et al. 1996), acid rain (Helander et al. 1994), and air pollution (Wolfe et al. 2018), have all been associated with shifts in endophyte assemblages—

thus, it seems likely that the effects of pollution and urbanization are multifarious and have effects which depend upon the endophytic taxon examined and the ecological context.

In addition to pollution, habitat fragmentation also increases in proximity to human development. Very little is known regarding how habitat fragmentation affects microbial assemblages or, more generally, how metacommunity processes manifest within microbiomes (Christian et al. 2015). However, classic island biogeography theory (MacArthur and Wilson 2001) suggests that human-caused habitat fragmentation likely shapes endophyte assemblages through determining proximity to inoculum sources. In a survey spanning islands of various sizes, Helander et al. (2007) reported that endophyte colonization of *Betula* spp. trees was greater on larger islands and islands closer to the mainland (also see Oono et al. 2017). This result, coupled with work documenting dispersal limitation in non-endophyte, microbial systems (Golan and Pringle 2017, Peay et al. 2010, 2007, Andrews et al. 1987) suggests that it is reasonable to expect variation in endophyte assemblages routinely follows the predictions of island biogeography, regardless of whether habitat fragmentation and patch size is caused by geological processes or human influence.

Another way in which endophyte assemblages may be affected by proximity to human development is through the influence of invasive plant taxa, which are often much more abundant near development than in wilderness areas. Invasive host taxa could influence endophytes in a variety of ways—from changing the inoculum pool within an area (i.e. "neighborhood" effects; Moeller et al. 2015), bringing along endophyte taxa or genotypes from the ancestral range of the host (Dickie et al. 2017), or affecting many other aspects of the local ecology (e.g. shifting fire regimes [Brooks et al. 2004], determining litter deposition rate and elemental composition [Allison and Vitousek 2004], influencing herbivore assemblages [Forister 2009], etc.).

All these anecdotes support the idea that endophyte assemblages in relatively undisturbed areas, such as portions of the Amazon or the Siberian forest, are likely to be different from those in conspecific hosts growing near human habitation or that are being actively cultivated (Coleman-Derr et al. 2016). Even if different microbial taxa are not observed in remote environs, study of the shifts in relative abundances among endophyte assemblages along urbanization and pollution gradients could provide insight into how endophytes interact and communities assemble (e.g., Gazis and Chaverri 2015).

Much of the host phylogeny remains unexplored—what might we be missing?

We found that members of about a third of plant families have been surveyed for fungal endophytes and only about a tenth of plant families are represented among bacterial studies. These results demonstrate how large the gap is in our understanding, and suggest we are likely missing the majority of the scope and distribution of endophytes among all plants. It is true that many endophytic taxa are known to have broad host ranges (e.g., Arnold and Lutzoni 2007), thus one could argue that an understanding of endophyte biodiversity does not hinge on thorough sampling of potential host taxa. However, we note that, in the majority of multivariate studies of endophyte biogeography, host taxon is an important predictor of assemblage variation (Griffin et al. 2019, Kivlin et al. 2019)—albeit a sometimes modest one (Vincent et al. 2015). Moreover, we know almost nothing regarding the host range of those rare endophyte taxa that compose the bulk of most assemblages (Arnold and Lutzoni 2007). An additional justification for surveying broadly across the plant phylogeny is the discovery

of specialist endophyte taxa—indeed, many of the most interesting and well known interactions between plants and endophytes involve relatively specialized, vertically-transmitted endophytes; for instance, the seed-borne fungal endophytes of grasses and locoweeds (Clay and Schardl 2002, Ralphs et al. 2008).

Comparative studies of host breadth among endophytes, including among rare taxa and also widespread, apparently generalized taxa (e.g. *Colletotrichum tropicale*; see Griffin and Carson 2018), would facilitate studies of the physiological mechanisms associated with plant-endophyte interactions. For example, generalist endophytes must possess mechanisms for dealing with a variety of host defences, despite those endophytes possessing common traits targeted by plant immune systems (e.g, molecules such as flagellin and chitin; Chisholm et al. 2006, Jones and Dangl 2006). Understanding the nature of those mechanisms would be streamlined through delimitation of endophyte host ranges, because comparative genomics and cellular biology studies could be more expeditiously directed. For instance, host taxa that are closely related but that differ in suitability for a particular endophyte could be targeted for study of the molecular and genetic basis of endophyte symbiosis.

Though studies clearly delineating endophyte host range are desperately needed, given the daunting scale of the sampling required, where then should we begin? First, we suggest that information sharing among studies will be critical. We must be able to compare data among studies to build checklists of where certain sequences, corresponding to specific taxa, have been found. Such efforts have been hampered by the constraints of PCR as the choice of primer inevitably biases against certain endophyte taxa and complicates comparison of studies that relied on differing primers (Nilsson et al. 2018). In the near future, such constraints will be reduced or eliminated through the use of PCR-free sequencing technology (Jones et al. 2015). Meanwhile, we suggest a shift from *de novo* operational taxonomic delineation (OTU) to the use of exact sequence variants (ESVs) to allow information sharing among studies (see further discussion below; Callahan et al. 2017).

Additionally, we suggest targeting those plant lineages with unique traits, such as production of specific secondary metabolites, or preferences for restricted or harsh habitats (e.g. halophiles and extremophiles). As an example, certain *Astragalus* taxa can hyperaccumulate selenium, and recent research has suggested that these plants may harbor unusual endophytic taxa that could influence selenium uptake (Sura-de Jong et al. 2015, Lindblom et al. 2018, 2013). Following a similar rationale, we also suggest surveying those plant families that are phylogenetically distinctive. If coevolution or codivergence has occurred between hosts and their endophytes, than unusual endophytic taxa could occur in hosts characterizing remote portions of the plant phylogeny (Hassani et al. 2019). Non-vascular plants, in particular, deserve more attention as these plants have dramatically different evolutionary histories, physiology, growth habits, and preferred habitats than vascular plants.

Within lineages (i.e., plant families or genera), we suggest focusing on those taxa with unique range sizes—whether large or small. Hosts with large ranges offer the opportunity to study the effects of abiotic gradients on endophyte assemblages without confounding host taxonomic variation with covariates of interest, because the same host taxon spans the entire gradient. Moreover, plant taxa with large ranges are often ecologically important, thus understanding the role of their microbiomes could provide insight into how endophytes could mediate ecosystem-level processes. Plants with small range sizes also represent profitable

opportunities for study. First, many geographically restricted taxa prefer unique soil types, or otherwise harsh conditions that few plants can survive (Rundel et al. 2015, Rabinowitz 1981). Such contingencies could favor adaptations by both hosts and endophytes that distinguish them from sister taxa and their study could thus improve knowledge of endophyte biodiversity and facilitate insights into endophyte evolution. The study of geographically restricted hosts could also help delineate the influence of host macroevolution on endophyte assemblages, because these host taxa include both species (or infrataxa) which have recently diverged from their sister taxon and those taxa that are in the process of going extinct (of course, these two categories are not mutually exclusive). Recently diverged taxa could offer insight into how endophyte-host interactions form and are maintained. On the other hand, host lineages that are in the process of extinction are worth surveying to ensure that interesting specialist endophytic taxa do not disappear along with their hosts before they are even described.

An example of small-scale biogeography: the effects of tissue type on endophyte assemblages

Our meta-analysis revealed no significant differences in richness and diversity between leaves, stems, and roots (Figs. S1 & S2). However, we acknowledge that the meta-analysis suffered from a lack of power. This led us to pursue a vote counting procedure whereby we could consider more studies (a total of 243) because criteria for consideration were less restrictive. Results from vote counting were similar to those from the meta-analysis (Tables S1–S3), though our vote counting approach more clearly suggested that in woody plants stems had higher richness than other tissues, for both fungi and bacteria. However, for graminoids, roots were the richest tissue. For forbs, inter-tissue patterns in richness were less clear. These results seem conflicting but may suggest that tissues with greater lifetime inocula exposure have the highest richness across plant life histories. Indeed, several studies have demonstrated that older leaves typically harbor richer microbial assemblages than younger leaves, presumably because of greater exposure to inoculum and increased time for microbial growth (Arnold et al. 2003, Ercolani 1991). Stems and bark of woody plants are exposed to inocula in air, water, and dust year round and have long lifespans, whereas leaves, even for evergreen trees, do not persist for nearly as long. Similarly, roots are the longest-lived tissues of many perennial forbs and graminoids, as above-ground tissues of these hosts often senesce annually. It is true that roots of woody-stemmed plants can be quite long-lived, however roots are primarily encountering inoculum from the surrounding soil matrix, thus it is possible that there is greater variation in the inoculum encountered by stems than by roots over the lives of those tissues. Alternatively, perhaps the resources available to microbes within stems of woody-plants favored higher richness compared to leaves, particularly of latent saprotrophs that catabolize lignin or other structural carbohydrates (Oses et al. 2006, 2008). These hypotheses are not mutually exclusive and await experimental testing.

Our meta analysis and vote counting survey come with several caveats. First, it is possible that the efficacy of surface sterilization may vary with tissue type; thus, for instance, the high fungal richness in bark that we report could be because it was more difficult to surface sterilize than leaves. Also, while we chose those studies that had the same sample size between each tissue type, it was not always apparent that the same mass was used for each sample. Moreover, for culture-based assays, the surface area exposed to growth media may be confounded with tissue type. For instance it can be difficult to cut stems or roots into

slivers as thin as a leaf. However, one would expect this bias would lead to higher richness in leaves, which we generally did not observe (Tables S1–S3). Additionally, both culture and sequence-based surveys suffer from taxonomic biases (Carini 2019, Nilsson et al. 2018) and if those biases coincide with taxonomic variation among tissue types, then richness estimates will be incorrect. Nevertheless, our analysis demonstrates the existence of clear patterns in richness among tissue types and suggests several hypotheses for those patterns that deserve further study.

How can we best share information among studies?

The breadth of literature pertaining to endophytes is remarkable and ripe for meta-analysis and synthesis (e.g., Meiser et al. 2014). As we catalogued the reproducibility of studies, we were pleased to find that many studies provided access to data. For instance, in 37.6% of studies reliant upon sequence-dependent methods the unprocessed DNA sequences were deposited in an online repository (e.g., GenBank). These data should be invaluable to future research aimed at defining the scope of endophyte biodiversity. However, it was quite rare for sufficient detail to be provided regarding sequence processing—including options and versions for software used and date accessed for taxonomy training databases, which are in constant flux. Given the challenge in reprocessing data and the influence different bioinformatic pipelines can have on results (e.g., Pauvert et al. 2019), we suggest that publication of polished data and scripts should be considered to facilitate information sharing among studies. Those data that would be most amenable to meta-analysis include replicate by taxon tables, sequences of ESVs, and the taxonomic hypotheses for those sequences.

Most of the sequence-based surveys that we encountered relied on a traditional definition of OTUs where sequences that were similar to one another (typically a 97% similarity threshold) were collapsed into a consensus sequence and counted. There has been an ongoing dialogue regarding if OTUs should be done away with in favor of exact sequence variants (ESVs), which provide single nucleotide resolution when determining sequence divergence. Callahan et al. (2017) have argued that ESVs should replace OTUs because the former provides benefits for sharing of information across studies since an ESV is a fixed, defined sequence and an OTU is not. An OTU often encompasses multiple genetic variants and the consensus sequence depends upon the data analyzed, at least when performing *de novo* OTU delineation, as is typical. While other authors (e.g., Nilsson et al. 2018) have questioned the suitability of ESVs given that the standard barcoding loci (i.e., 16s and ITS portions of the ribosomal operon) can have paralogs scattered throughout the genome (Brewer et al. 2019, Lofgren et al. 2019, Louca et al. 2018), thus one could obtain multiple ESVs from the same organism. Indeed, this poses challenges to the calculation of relative abundances, but this issue is only partially ameliorated through the use of traditionally delineated OTUs, which would sill be subject to biases imposed by copy number variation in marker loci. A long-standing criticism of OTUs is that their designation does not reliably correspond to any level within the taxonomic hierarchy. For instance, OTUs can be either above or below the species level (Nilsson et al. 2008, Gazis et al. 2011). In contrast, ESVs have a very well defined biological meaning as they are simply a genotype at a locus. The level of resolution afforded by ESVs could thus allow much more accurate estimates of endophyte host and geographic ranges than OTUs and even occasionally provide insight into ecologically-relevant genetic variation within an endophytic taxon (e.g., Harrison et al. 2018).

As a final suggestion for improving information sharing, we suggest that authors consider depositing vouchers of host taxa studied and cultures obtained in an herbarium whenever possible (Fig. 2d). This suggestion is motivated by fascinating new work by Daru et al. (2019) who have shown that endophytes within herbarium specimens can be sequenced, and, in some cases, even cultured. Thus, vouchers could act as "time capsules" that preserve endophyte genotypes and could afford unprecedented insight into endophyte evolution and shifts in host and geographic range over time. Deposited cultures could provide many of the same benefits, but would also allow researchers to grow endophytes of interest to meet various experimental goals. Finally, the plant taxonomy is ever-changing, thus as future researchers interpret published work, they may wish to examine vouchers to determine the most current taxonomic placement of the focal host. In sum, we see herbaria as tremendous resources for the study of the plant microbiome, and, consequently, we urge participation in their continued development.

Conclusion

To understand the evolutionary forces and ecological pressures that define endophyte assemblages, the delineation of biogeographic patterns in endophyte biodiversity is required. The enthusiasm among microbial ecologists for endophyte biology paired with the tools we now have at our collective disposal, suggests that such patterns are within grasp. We hope that our survey inspires others to fill the gaps in knowledge that we report. To that end, we have made the metadata from each study that we consider available (see supplemental material) in hopes that other researchers mine them for additional insights.

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Data availability

All scripts and processed data are available at:
https://bitbucket.org/harrisonjg/endophytereview/src/master/

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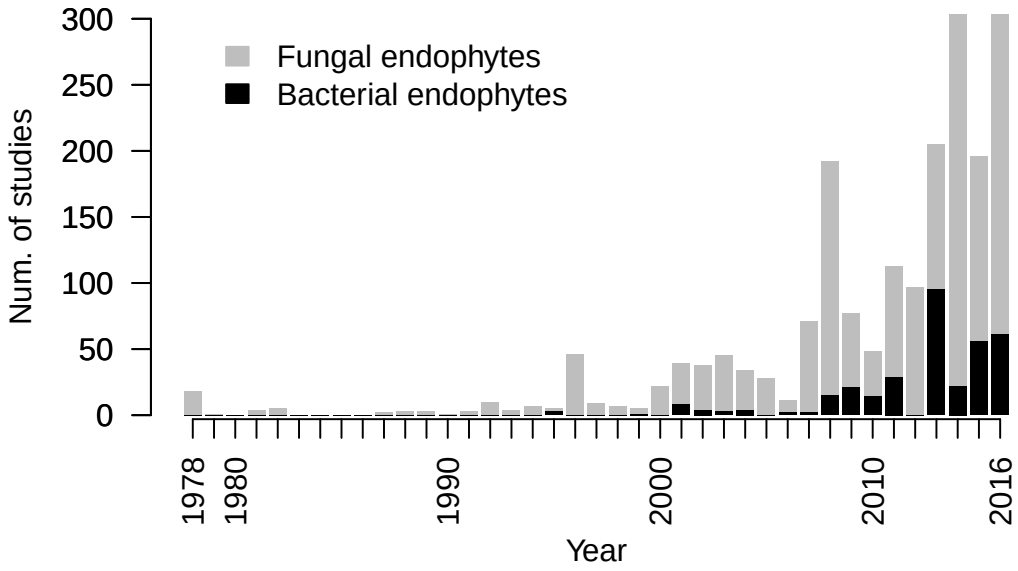

Figure 1: The number of studies characterizing endophyte biodiversity published each year since the late 1970s. Studies are parsed by taxonomy with fungal studies in gray and bacterial studies in black.

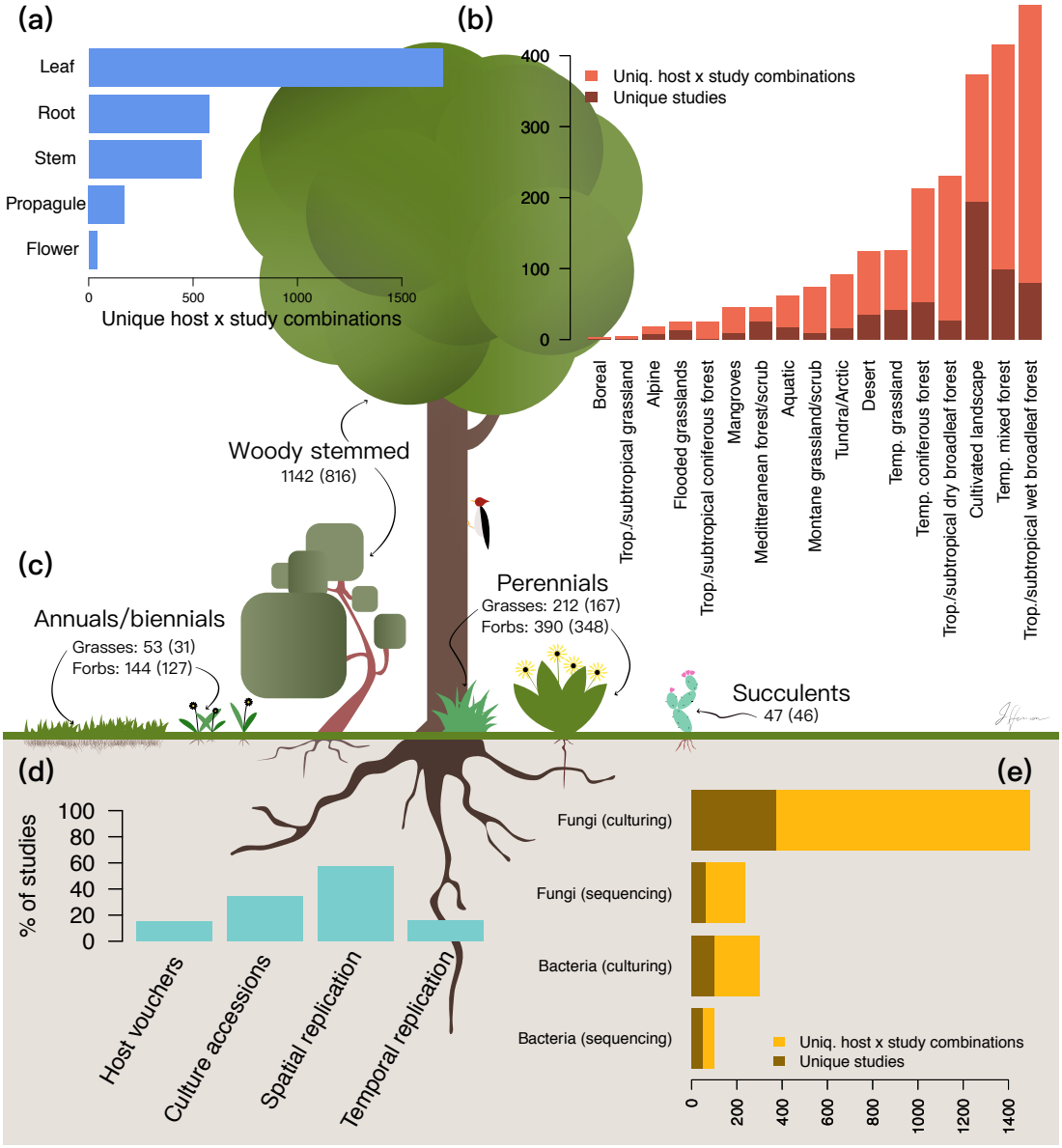

Figure 2: Summary of 596 publications characterizing endophyte biodiversity. Because many studies surveyed multiple hosts, we report both number of studies and number of unique host by study combinations. We counted the number of studies surveying each plant compartment (a), biome (b), and host life history category (c; values in parentheses are unique hosts). We also extracted information pertaining to study design and reproducibility (d). Finally, we determined the endophytic taxon characterized and the methodology employed (e).

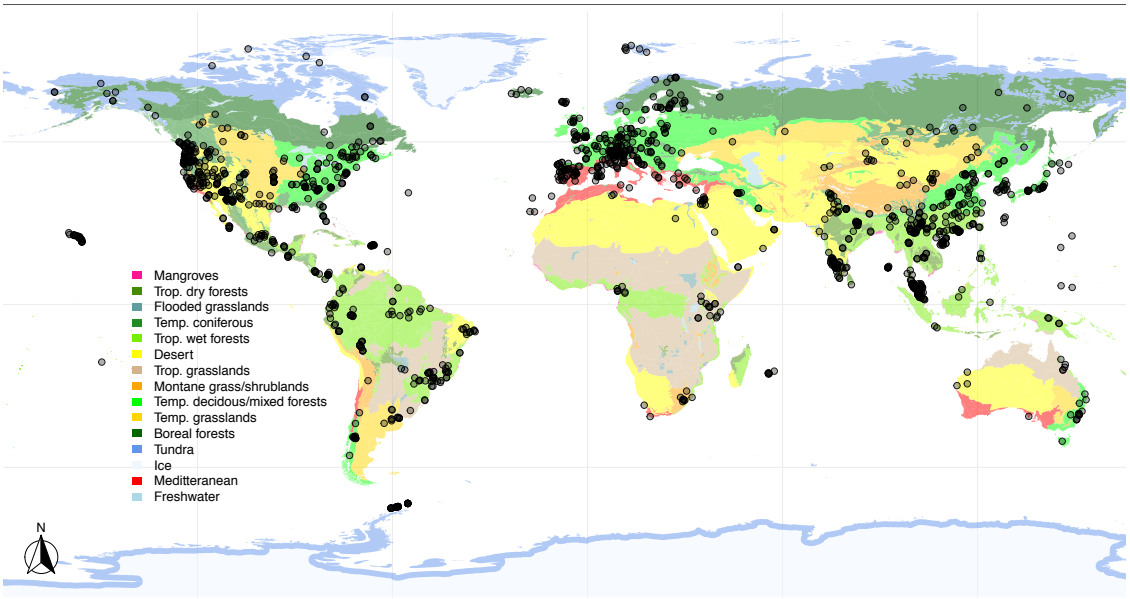

Figure 3: Locations of endophyte biodiversity studies considered. An interactive, zoomable version of this map can be found at: `https://jharrisonecoevo.github.io/EndophyteMap/`. Black points represent studies. Biomes are color coded and delineated in accordance with (Olson et al. 2001). In some cases, multiple, proximal locations were surveyed and a single point was used to graphically represent these locations.

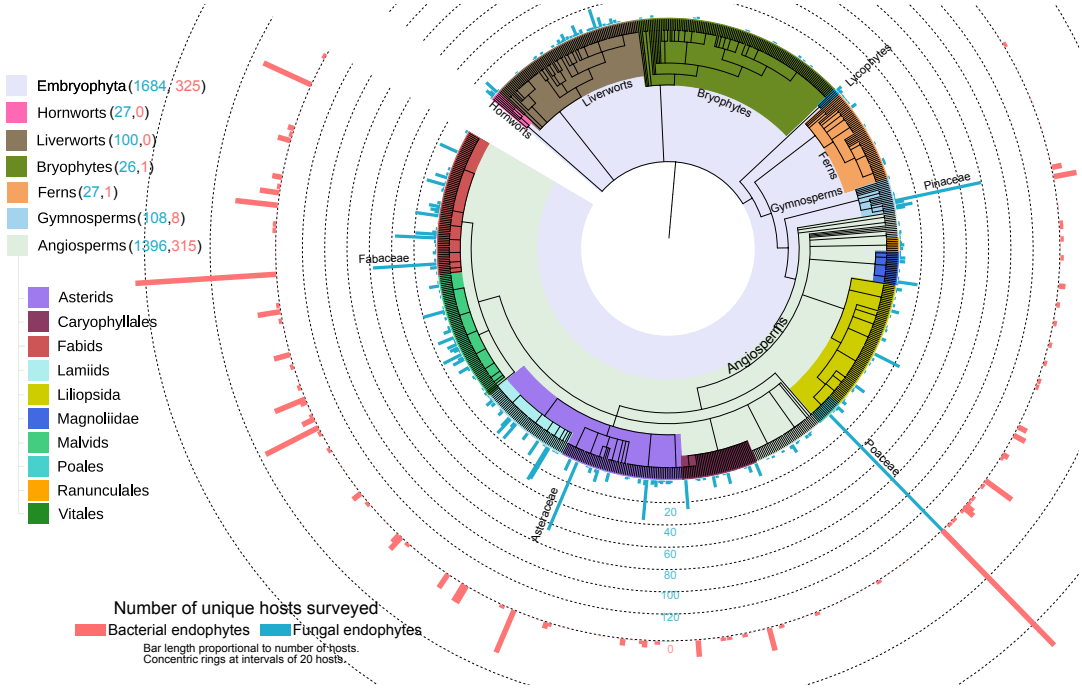

Figure 4: Survey effort across Embryophyta. Number of studies surveying fungal (blue) and bacterial (red) endophytes are shown extending outwards from the tips of the phylogeny. Tips are families. Notable taxa within Embryophyta are labeled and color-coded. Numbers in parentheses denote unique hosts surveyed. Very few surveys of bacterial endophytes have been conducted in bryophyte hosts, therefore this portion of the figure has been abbreviated.

Supplementary Material

Table S1: Differences among host tissues in fungal (top panel) and bacterial (bottom panel) endophyte richness in woody plants. Each cell in the table provides the number of times the tissue type on that *row* (the focal tissue) had higher richness than the tissue type in that *column* (the comparison tissue) followed by the number of studies reviewed for each comparison in parentheses. Significance was determined using a binomial sign test. For results from herbaceous plants see Table S2, for results from graminoids see Table S3

		Comparison tissue (Fungi)					
		Leaf	Root	Stem	Propagule	Flower	Bark
Focal tissue	Leaf >	–	4 (7)	10 (43)***	3 (4)	2 (3)	0 (4)
	Root >	3 (7)	–	1 (7)	0 (2)	1 (2)	0 (1)
	Stem >	33 (43)***	6 (7)	–	3 (3)	2 (3)	1 (2)
	Propagule >	1 (4)	2 (2)	0 (3)	–	0 (1)	0 (0)
	Flower >	1 (3)	1 (2)	1 (3)	1 (1)	–	0 (0)
	Bark >	4 (4)	1 (1)	1 (2)	0 (0)	0 (0)	–

		Comparison tissue (Bacteria)		
		Leaf	Root	Stem
Focal tissue	Leaf >	–	2 (7)	3 (8)
	Root >	5 (7)	–	4 (6)
	Stem >	5 (8)	1 (6)	–

***p<0.01, **p<0.05, *p<0.1

Table S2: Differences among host tissues in fungal (top panel) and bacterial (bottom panel) endophyte richness in herbaceous plants. Each cell in the table provides the number of times the tissue type on that *row* (the focal tissue) had higher richness than the tissue type in that *column* (the comparison tissue) followed by the number of studies reviewed for each comparison in parentheses. Significance was determined using a binomial sign test. For results from woody plants see Table S1, for results from graminoids see Table S3

		Comparison tissue (Fungi)					
		Leaf	Root	Stem	Propagule	Flower	Bark
Focal tissue	Leaf >	–	10 (22)	10 (21)	4 (5)	1 (1)	0 (1)
	Root >	9 (22)	–	10 (19)	4 (5)	0 (1)	0 (1)
	Stem >	9 (21)	7 (19)	–	3 (4)	0 (0)	0 (1)
	Propagule >	1 (5)	1 (5)	1 (4)	–	0 (0)	0 (0)
		Comparison tissue (Bacteria)					
			Leaf	Root	Stem		
Focal tissue	Leaf >	–	1 (6)	1 (5)			
	Root >	5 (6)	–	6 (8)			
	Stem >	4 (5)	1 (8)*	–			

***p<0.01, **p<0.05, *p<0.1

Table S3: Differences among host tissues in fungal (top panel) and bacterial (bottom panel) endophyte richness in graminoids. Each cell in the table provides the number of times the tissue type on that *row* (the focal tissue) had higher richness than the tissue type in that *column* (the comparison tissue) followed by the number of studies reviewed for each comparison in parentheses. Significance was determined using a binomial sign test. For results from woody plants see Table S1, for results from forbs see Table S2

		Comparison tissue (Fungi)		
		Leaf	Root	Stem
Focal tissue	Leaf >	–	2 (6)	2 (4)
	Root >	4 (6)	–	9 (11)**
	Stem >	2 (4)	2 (11)**	–

		Comparison tissue (Bacteria)		
		Leaf	Root	Stem
Focal tissue	Leaf >	–	1 (6)	1 (6)
	Root >	5 (6)	–	9 (9)***
	Stem >	4 (6)	0 (9)***	–

***p<0.01, **p<0.05, *p<0.1

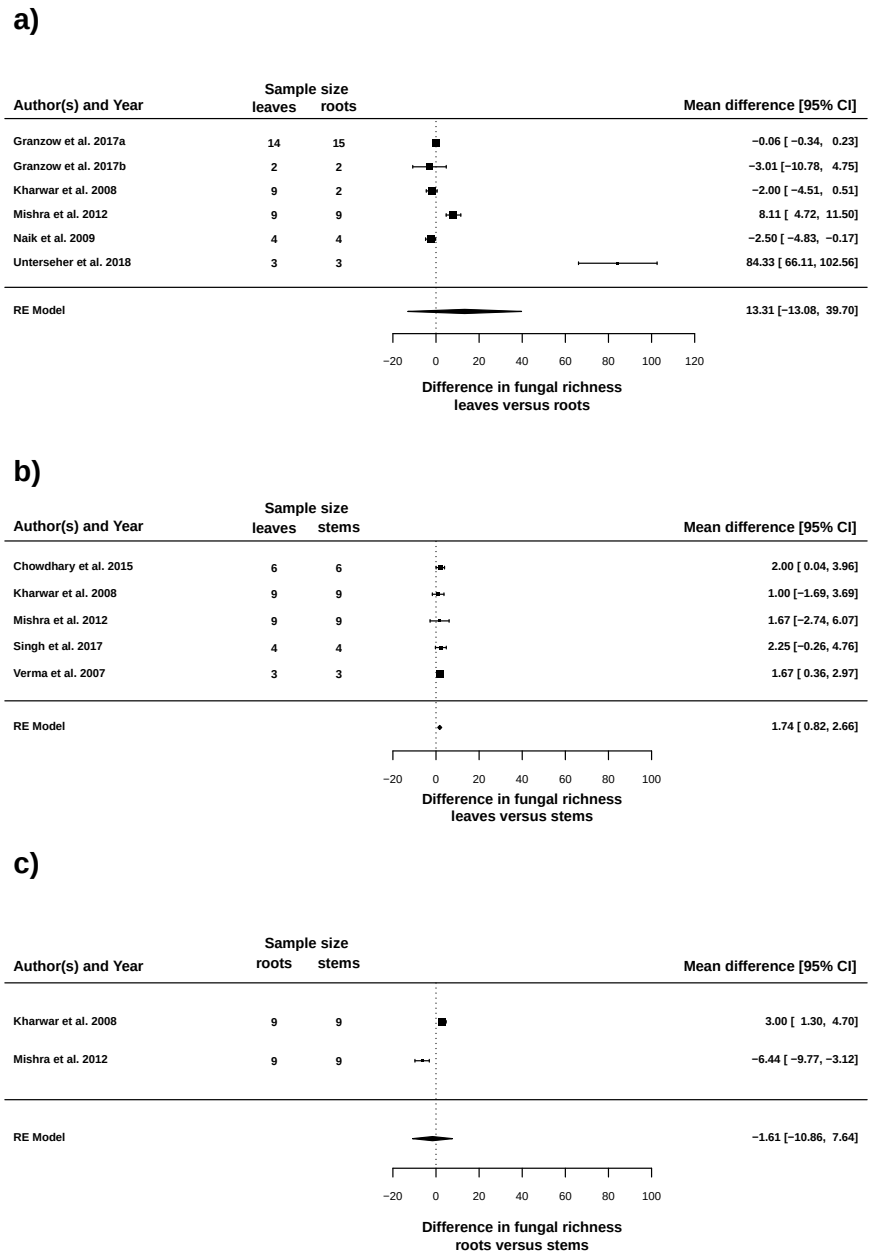

Figure S1: Differences in fungal endophyte richness among host tissues as determined through meta-analysis. Each panel depicts pairwise comparisons between two tissue types. Panel (a) depicts leaves versus roots, panel (b) leaves versus stems, and panel (c) roots versus stems. Mean differences between tissues for each study are shown in the right margins of each plot, with confidence intervals. No model was significantly supported at $p \leq 0.05$. Results were very similar for Shannon's diversity and can be seen in Fig. S2. Richness for Unterseher et al. (2018) was higher than the other studies because those authors relied on sequencing data whereas the other studies considered relied on culturing data. Two hosts were studied by Granzow et al. (2017) and results from each host are denoted by letters a and b.

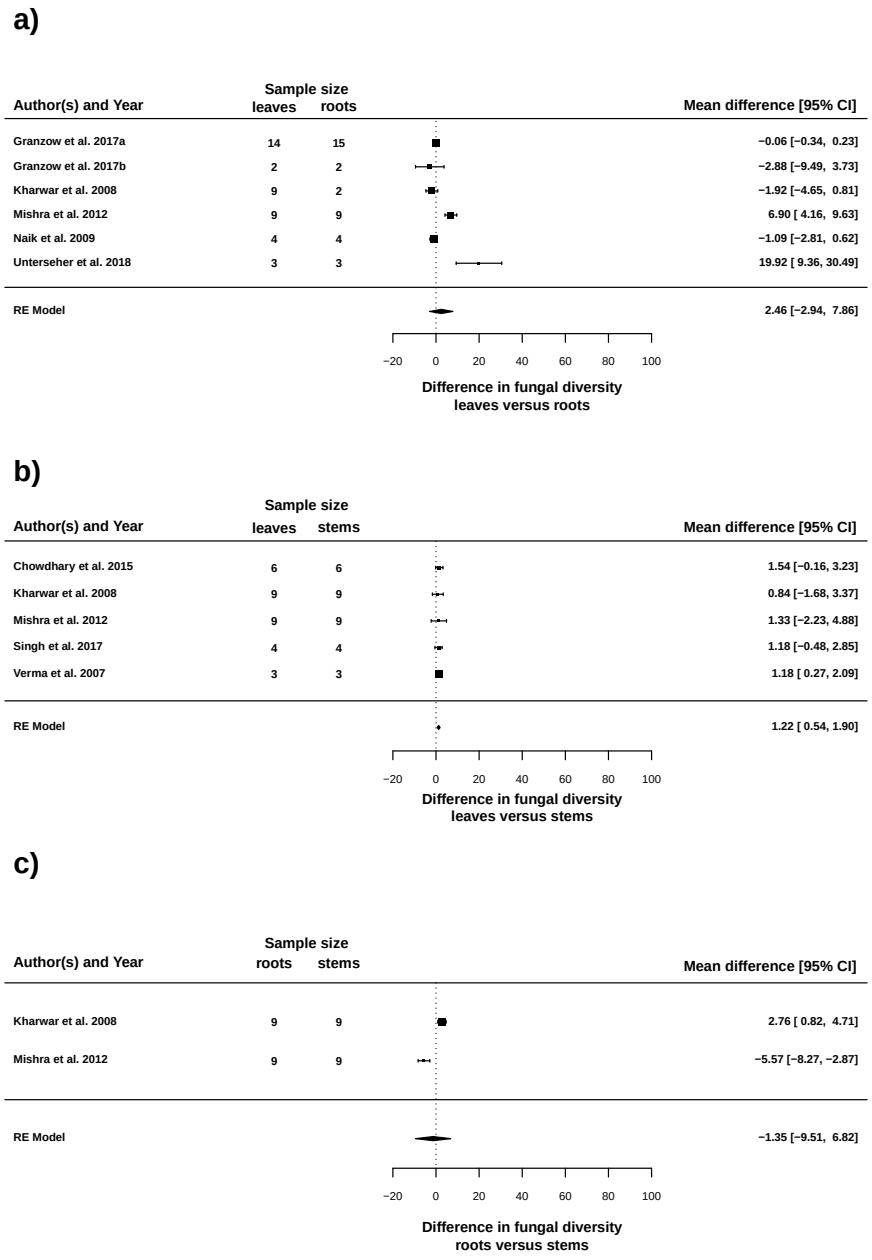

Figure S2: Differences in fungal endophyte diversity (exponentiated Shannon's entropy) among host tissues as determined through meta-analysis. Each panel depicts pairwise comparisons between two tissue types. Panel (a) depicts leaves versus roots, panel (b) leaves versus stems, and panel (c) roots versus stems. Mean differences between tissues for each study are shown in the right margins of each plot, with confidence intervals. Results were very similar for richness and can be seen in Fig. S1. Diversity for Unterseher et al. (2018) was higher than the other studies because those authors relied on sequencing data whereas the other studies considered relied on culturing data. Two hosts were studied by Granzow et al. (2017) and results from each host are denoted by letters a and b.