Manuscript Title: Fungal Endophytes

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Summary

Organisms are commonly grouped into ecological guilds that reflect their shared resource use and similar ecological roles. The guild concept has been used to categorize the vast diversity of the fungal kingdom (estimated at 2.2-5 million species) into groups of fungi with common lifestyles, such as mycorrhizal symbionts, pathogens of animals and plants, and the group that is the focus of this primer - fungal endophytes of plants. Fungal endophytes have resisted scientists' attempts to silo organisms into neat assemblages. Scattered across the fungal tree of life and lacking few diagnostic characteristics, they are defined less by what they are than by what they are not.

Endophytes were first formally recognized by the German botanist Heinrich Link in 1809, although at the time were classified as “entophytae”. The term “Endophyte” was likely first coined by the founder of fungal developmental biology, Anton de Bary, in 1866 and was originally applied to pathogenic fungi that colonize internal plant tissues. It was not until 1887, when Victor Galippe reported that non-disease causing soil-borne fungi could be found within healthy aboveground plant tissues, that endophytes were distinguished from pathogens. The definitions of “endophyte” and “endophytism” have been revised many times, but today they generally refer to fungi that asymptomatically colonize living plant tissues without causing obvious disease and without obviously forming the characteristic structures of mycorrhizal symbionts. In effect, endophytes are the “other” plant-associated fungi. This catch-all now encompasses thousands to potentially hundreds of thousands of species distributed across nearly every fungal phylum. Endophytes may therefore represent one of the most ecologically diverse fungal guilds, and are armed with a rich array of life strategies, molecular toolkits, and functional roles within the plant microbiome. Here we synthesize current findings on their ecology, evolutionary history, and modes of colonization.

Main text

Cryptic World of Fungal Endophytes

Despite being found in nearly all plants and prevalent throughout plant tissues, silently colonizing leaves, stems, and roots, the ecology and functional roles of endophytic fungi remain poorly understood. A single host plant often contains multiple endophytic fungal species simultaneously. These fungi usually colonize plant tissue without causing visible symptoms, thus rendering them hard to identify based on morphology alone. Identification and classification of fungal endophytes is challenging because of their evasive morphological characteristics, and in some cases resistance to isolation. Endophytic fungi are exceedingly flexible in their lifestyle as they can be mutualists, commensals, or latent pathogens depending on environmental conditions and host physiological status. Such ecological plasticity combined with the subtlety of their interactions has made it difficult to assign consistent functional roles to endophytes as well.

Researchers have effectively classified them into four operative classes based on host range, colonization, transmission patterns, and tissue specificity. Interestingly, all these classes of endophytes can confer different types of fitness benefits to their hosts, reflecting their distinct ecological roles and interactions. Thus, in endophytic interactions, the lines between mutualism, commensalism, parasitism, and saprotrophy are often blurred. Endophytic fungi embody a "mutualism-parasitism continuum" and reflect how context defines function in fungal-plant relationships. While this Primer focuses on plant-associated fungal endophytes in terrestrial systems, endophytic fungi have been found in aquatic plants, macroalgae, bryophytes, lichens, and even insect hosts. These associations reflect the broad functional and evolutionary capacity of endophytes to inhabit living tissues across ecosystems. The complex interplay of genetic, environmental, and host factors enabling these symbiotic interactions offers an exciting frontier in biological research.

Foundations of Endophytic Function

Fungal endophytes colonize plant tissues asymptomatically, inhabiting inter- and intracellular spaces without triggering host defenses. Their morphology varies greatly, from unicellular yeasts to multicellular septate hyphae found in Ascomycota and Basidiomycota, and coenocytic (i.e. non-septate) hyphae in Mucoromycota. While endophytes generally lack differentiated nutrient-exchange structures common in mycorrhizal (e.g. mantles, Hartig nets) or pathogenic (e.g. haustoria) associations, some exhibit internal hyphal networks, pseudo-mycorrhizal characteristics, or microsclerotia (i.e. dense hyphal masses), that form within host tissues. Through seemingly subtle physiological manipulations, endophytic fungi demonstrate diverse functional roles, enhancing nutrient uptake, promoting plant growth, and conferring stress tolerance. Their ecological strategies range from brief plant colonization to enduring systemic partnerships, often shifting dynamically in response to environmental conditions. Representative examples of beneficial endophytes include *Epichloë festucae*, improving phosphorus and nitrogen uptake in grasses, and *Serendipita indica*, known to enhance drought and salinity tolerance of plant hosts following root colonization. However, *Hypoxylon* and *Fusarium* spp. are capable of transitioning from asymptomatic or even beneficial colonizers to pathogens causing disease under certain stress conditions like drought or injury. Inversely, *Colletotrichum spp.*, typically reside asymptomatically in plant tissues without affecting growth but switch to perform a mutualistic role by enhancing phosphate uptake under phosphate deficient conditions.

Endophytes are generally divided into two broad groups, clavicipitaceous endophytes (Class I) and non-clavicipitaceous endophytes (Classes II-IV) (Fig 1). The non-clavicipitaceous endophytes are further distinguished by their colonization patterns and dispersal mechanisms.

Class II endophytes colonize both shoots and roots or rhizomes, transmitting both vertically and horizontally. Class III are horizontally transmitted foliar endophytes, seemingly restricted to above-ground tissues where they establish highly localized infections across diverse hosts. Class IV are root-associated "dark septate" endophytes, characterized by melanized inter- and intracellular hyphae.

Dispersal strategies mirror these ecological niches. Foliar and shoot endophytes transmit vertically through seeds, ensuring host-specific continuity, or horizontally through airborne spores, insect vectors, and rain splashes. Entry often occurs through natural openings like stomata or following mechanical injury, providing direct access to internal plant tissues. Once inside, foliar endophytes may occupy specialized plant tissue microhabitats such as the cuticle, epidermis, and mesophyll. These dispersal strategies are particularly relevant to Class I, II, and III endophytes, which inhabit aerial plant parts with either systemic or localized persistence.

In contrast, root-associated endophytes (i.e. Class II and IV) primarily disperse horizontally via soil-borne spores or hyphal fragments, colonizing the epidermis, cortex, or root tips. Some root-associated endophytes, such as *Hyaloscypha*, previously thought to disperse only via hyphae, have been shown to form true fruiting bodies in vitro revealing latent reproductive modes that blur the line between passive dispersion and asexual and sexual propagation. Although Class IV endophytes appear restricted to root tissues, non-clavicipitaceous fungi within Classes II and III can colonize multiple plant organs, including roots, shoots/stems, and leaves. As literature describing shoot/stem-specific endophytes remains sparse, we use the broader aboveground/belowground distinction in Fig. 1 for clarity.

In classic plant-fungal symbioses, such as arbuscular mycorrhiza (AM) and ectomycorrhiza (ECM), beneficial fungi typically induce specialized nutrient exchange structures. In contrast, endophytic fungi establish mutualistic relationships without forming distinct organs or causing visible disease symptoms, instead colonizing host tissues asymptomatically through inter- and intracellular spaces. Colonization is often taxa and tissue-specific: *Colletotrichum* and *Xylaria* are frequently isolated from leaves or shoots, *Piriformospora* or *Cadophora* inhabit roots in most documented cases, and grass-associated clavicipitaceous fungi such as Epichloë exhibit systemic colonization.

Endophytic colonization relies on sophisticated molecular dialogue between fungi and their host plants, though many specific pathways remain unclarified. Plants are believed to initiate these interactions by releasing signaling molecules like strigolactones, sugars, and flavonoids that help recruit compatible fungal partners. Recent studies in *Arabidopsis*, tomato, and raspberry suggest small- or microRNAs may actively regulate host responses during colonization, particularly through post-transcriptional control of defense and signaling pathways. Endophytes, in turn, release small secreted proteins, lipases, and cell wall-modifying enzymes that enable cell wall entry without provoking plant defenses. Conceptually, these molecular cues resemble those used by pathogens but may differ in composition, timing, or intensity. Unlike pathogens, endophytes establish under a state of low immune alert that permits colonization without compromising mutualistic benefits. These subtle exchanges, while not fully understood, could help plants discriminate beneficial endophytes from harmful pathogens, or perhaps overlook endophytes entirely.

Once established, some root-endophytic partnerships are thought to enhance host nutrient acquisition, especially under nutrient-limited conditions, by extending into surrounding soil. Fungal mycelia facilitate the uptake of essential nutrients by secreting organic acids, carbohydrate degrading enzymes, and siderophores to solubilize otherwise inaccessible soil nutrients, notably phosphate and iron, making them available to plants. Dark septate endophytes (DSEs), which dominate organic-rich soils where decomposition is slow, are particularly effective at mobilizing nitrogen and phosphorus from organic soil pools. In exchange plants typically reciprocate by supplying endophytes with photosynthetically derived carbon, closing a supposedly mutualistic feedback loop that underpins these cryptic interactions. Moreover, endophytes modify plant growth and stress tolerance by inducing phytohormones, including auxins, gibberellins, cytokinins, and ethylene.

Collectively, these intricate interactions not only impact plant health but ultimately contribute broadly to how ecosystems function by influencing plant community dynamics, supporting host resilience, and mediating nutrient cycles. However, understanding the extent to which endophytes influence the surrounding environment will require careful examination of how these relationships scale in space and time.

Endophytic Ecology

Fungal endophytes are present in virtually all ecosystems (Fig. 2). However, individual fungal endophytes exhibit specific environmental preferences and host affinities which constrain their ranges. Considering the life history of fungal endophytes as plant symbionts, their biogeographic distribution is determined jointly by their ability to withstand abiotic conditions, and by the distribution of the host plants they colonize. Additionally, spatial factors such as geographic isolation may play a role in structuring endophyte communities. Endophyte diversity varies widely across scales. At the scale of a single stem, shoot, leaf, or root segment, there are often multiple endophytic colonizers in proximity. Scaling up, an individual plant may harbor dozens or even hundreds of fungal taxa. Finally, local and regional plant species pools can host thousands of coexisting fungal endophytes.

While some of these fungal endophytes are obligate members of a plant's microbiome, endophytic communities can also vary widely across a plant's range, turning over in response to abiotic and biotic conditions. Foliar endophyte communities vary even within a single plant host, influenced by leaf age and position within the canopy. Evidence suggests that individual perennial hosts harbor unique endophytic communities that are retained throughout their lifespans. Global surveys indicate foliar endophyte diversity and colonization rates typically decline with increasing distance from the equator and at higher elevations, consistent with broader patterns observed in plant and soil fungal diversity. Root endophyte diversity, however, may show opposite latitudinal patterns compared to foliar endophytes. High latitude ecosystems, often hosting greater proportions of non-mycorrhizal plants, might rely more extensively on DSEs, which are particularly widespread at high elevations and latitudes (Fig. 3). Climate significantly impacts endophyte distribution, with foliar endophyte diversity positively correlated with increased precipitation. DSE colonization is most prevalent in acidic, nutrient-limited soils. Emerging evidence also suggests variations in plant functional traits influence endophytic distribution and diversity.

Temperature strongly shapes the diversity and composition of above and belowground fungal endophytes, though above-ground endophytes may be disproportionately affected by temperature extremes. Despite identifying broad environmental patterns, endophyte taxa exhibit diverse functional and phylogenetic characteristics, implying varied responses to climatic change. Predictive modeling of endophyte responses to global changes remains limited. Contrary to historical assumptions that microbial dispersal is unlimited, modern studies show mixed evidence for dispersal limitation in fungal endophytes; some support clear distance-decay relationships, while others find a limited role for distance-based community structuring and a stronger response to climatic or host factors. The mixed evidence for spatial structure of endophytes suggests that the group has broad dispersal capability, although it remains to be seen whether spatial patterns vary amongst endophytes with different dispersal strategies (e.g. vertical vs. horizontal).

Plant Residents

Although the evolutionary origins of other plant-associated fungi, such as mycorrhizae, have been well-studied across diverse lineages, the general principles that guide endophyte evolution remain unclear. Some have hypothesized that asymptomatic foliar endophytes are primarily saprotrophs, where endophytic colonization allows these fungi to be the first colonizers of senesced leaves, enabling unfettered access to the carbon and nutrients locked in organic leaf tissues. Indeed, foliar endophytic and saprotrophic isolates are often found to exhibit high sequence similarity and phylogenetic proximity, suggesting they mirror the same taxa. Furthermore, endophytic fungi appear to have developed independently on numerous occasions from both saprophytic and pathogenic progenitors.

Given their broad distribution across the fungal tree of life (Fig. 2) there are likely dozens to potentially hundreds of independent origins of endophytism. In contrast to the ectomycorrhizal lifestyle, generally regarded as highly evolutionarily stable, with few transitions to other lifestyles, endophytic lineages often include fungi with diverse biotrophic capabilities. For example, foliar fungal endophytes in Xylariales are interspersed among pathogens and saprotrophs, and in Sebacinales, root endophytes are thought to have arisen from saprotrophic ancestors, with later transitions to orchid-, ericoid-, and ectomycorrhizal symbioses. In some of these cases, diverse lifestyles within endophytic lineages are attributable to the plasticity of individual isolates to inhabit multiple lifestyles. However, in other cases, this phylogenetic pattern is likely attributable to frequent evolutionary transitions between lifestyles. Nevertheless, these transitions demonstrate that endophytism is not an evolutionary dead end, but instead a flexible and dynamic condition.

The “Waiting Room Hypothesis” (WRH) posits that below-ground endophytism serves as an evolutionary precursor enabling the development of mycorrhizal lifestyles that require more elaborate nutrient-exchange structures. This idea is strongly supported by the polyphyletic “rhizoctonias,” in which repeated transitions from endophytes to orchid, ericoid, and ectomycorrhizal symbionts have occurred. Although endophytic ancestors have not been identified for most mycorrhizal fungal lineages, the WRH provides a compelling hypothesis for the evolutionary steps that preceded the development of highly coordinated mycorrhizal associations.

At the same time coordinated associations do not necessarily end up with endophytic behavior. . Recent work has suggested that well studied groups of ectomycorrhizal fungi (such as *Russula* and *Tuber* spp.), which might be expected to have “left the waiting room”, can still endophytically colonize non-ectomycorrhizal plants. More broadly, many taxa display dramatic lifestyle plasticity. For instance, numerous DSE genera like *Phialophora*, *Cadophora*, *Exophiala*, *Leptodontidium*, and *Periconia* exhibit context-dependent lifestyles, acting as mild pathogens under benign conditions but shifting towards mutualists under stress by aiding in nutrient uptake, metal detoxification, and phytohormone regulation. This lifestyle spectrum is likely widespread across endophytes as it has been documented in diverse groups across most of the fungal tree of life. Whether such lifestyle plasticity is a transitory evolutionary relic consistent with the WRH, or instead an evolutionarily stable ecological strategy, remains an open question.

Along with the WRH model, the theory of by-product mutualism describes how plant benefits can arrise incidentally from microbial metabolism. For example, fungi may secrete auxins or antibiotics as routine metabolic by-products that indirectly promote plant growth or suppress competitors. Consistent with this view, DSE fungi associated with *Vaccinium roots have been shown to* promote plant growth and modulate stress-related gene expression, without evidence of active, reciprocal exchange. Instead, these benefits appear to arise as a by-product of soil organic matter decomposition. Because these benefits do not require costly, partner-specific investment, by-product mutualisms offer a low-risk evolutionarily stable rout to symbiosis with reduced opportunities for “cheating”. Many endophytes may therefore never evolve active mutualistic traits. Instead, they confer plant benefits as a byproduct of their own metabolism, resource acquisition, detoxification, or competition with other microbes. Collectively, these patterns underscore the evolutionary potential and ecological versatility of fungal endophytes and suggest the WRH and by-product mutualism as compelling complementary frameworks for understanding how simple endophytic associations can give rise to complex, coordinated mutualisms.

Research Toolkit

Fungal endophytes are inherently challenging to study due to their cryptic lifestyles and functional plasticity. Traditional culture-based methods, while informative, may only isolate a small fraction of endophytes as their growth requirements are relatively unknown. Thus, relying solely on traditional isolation can significantly underestimate the complexity and diversity of endophytic communities, accidentally omitting ecologically significant organisms. Surface sterilization remains a foundational step in distinguishing true endophytes from epiphytes in both molecular and culture-based studies, yet this approach alone may not fully resolve the hidden diversity that resides within plants.

An “Endophytic-toolkit” approach that integrates microscopy, fluorescent in-situ hybridization, isotopic tracing, physiological measurements and omics-based techniques may aid in resolving these challenges. Modern culture-free "omics" methodologies including genomics, transcriptomics, proteomics, and metabolomics have proven pivotal in addressing these challenges. Genomics helps identify phylogenetic similarities and evolutionary adaptations enabling colonization and persistence within host tissues. Transcriptomic analyses reveal dynamic gene expression responses to host and environmental signals. Proteomics and metabolomics supply an avenue for identifying key proteins and metabolites central to fungal-host interactions, illuminating complex biochemical dialogues. Emerging tools such as single-cell sequencing, metagenomics, and spatial transcriptomics now allow fine-scale resolution of endophytic population dynamics, relative distribution, and ecological roles within host tissues. This integrated strategy allows researchers to monitor molecular crosstalk between the plant and fungus, track shifts in gene expression during colonization, and quantify nutrient and carbon exchange across conditions.

Integrating molecular tools with ecological research, including controlled laboratory experiments and field observations, provides a more complete view of endophyte-host interactions. High throughput sequencing now enables researchers to bypass cultivation biases and directly detect complex microbial communities within plant tissues. By combining multiple complementary methods this research framework deepens our ecological understanding of endophytes and their hidden but influential roles in plant systems.

Future considerations

Endophytic fungi are vital to plant health and ecosystem function, but their interactions within multi-layered plant and microbial communities remain only partially understood. Future efforts should aim to uncover how endophytes mediate plant responses under changing climatic conditions. Equally important is deciphering how fungal endophytes interact with other plant-associated microbes including bacteria, mycorrhizal fungi, and pathogens to shape plant performance and ecosystem dynamics. At the molecular level, little is known about how endophytes engage with diverse hosts or influence plant metabolism, immunity, and development.

Answering these questions will require experimental systems that integrate endophyte-host-environment interactions. Progress will also depend on methodological enhancements and consensus around standards for endophyte isolation, characterization, and functional evaluation. Building such a framework will be essential for reproducibility and cross-study comparison. The blurred lines between endophytic, pathogenic, saprotrophic, and mutualistic lifestyles reveal that traditional fungal guilds may oversimplify their dynamic, context-dependent relationships. As evidence grows, fungal biologists are increasingly called to revisit how we define symbiotic strategies across the fungal tree of life.

Further Reading (10 max)

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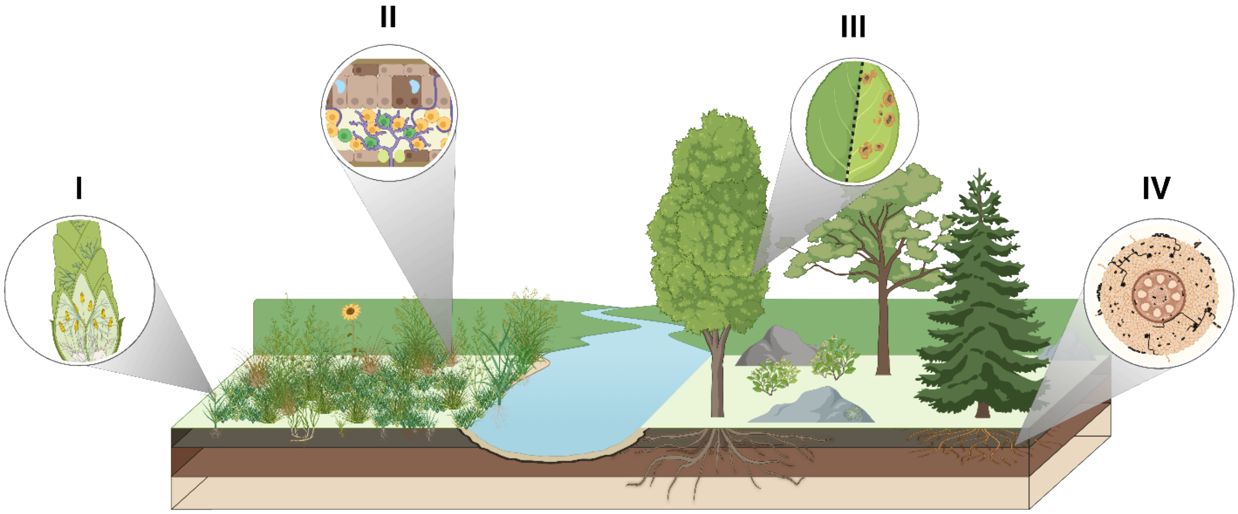
Figure 1 – Diversity of endophytic fungi



DISTRIBUTION	NARROW	BROAD	UNIVERSAL	BROAD
TISSUES	SYSTEMIC ABOVEGROUND	SYSTEMIC	ABOVEGROUND	BELOWGROUND
TRANSMISSION	VERTICAL	VERTICAL & HORIZONTAL	HORIZONTAL	HORIZONTAL
BENEFICIAL ROLE	RESILIENCE & DEFENSE	GROWTH PROMOTION	VARIED & UNCLEAR	RESILIENCE & UNCLEAR
GROUPING BASIS	TAXONOMIC	FUNCTIONAL	ECOLOGICAL	MORPHOLOGICAL
	I	II	III	IV

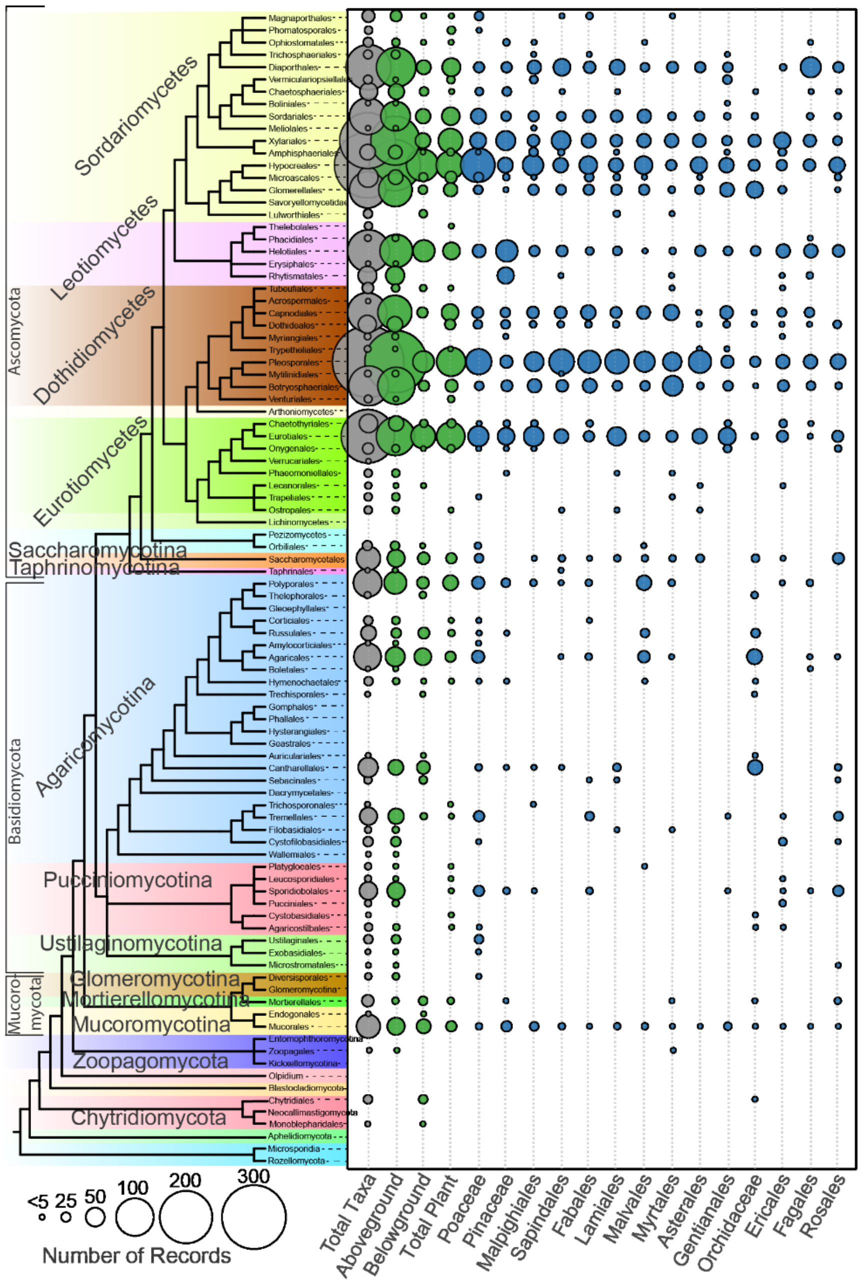
CLAVICIPITACEOUS

NON-CLAVICIPITACEOUS

CLASS PARADIGMS OF FUNGAL ENDOPHYTES

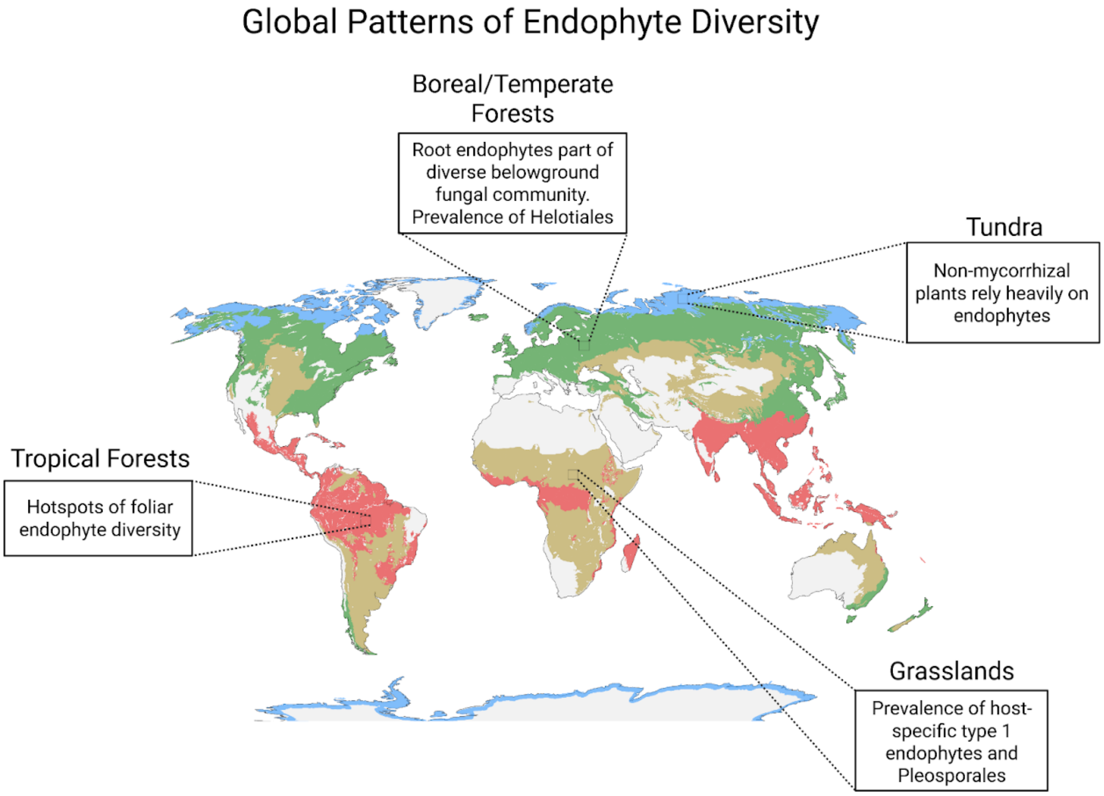
a. **Full caption** – Conceptual schematic for categories of endophytic fungi, characterized by (Rodriguez et al. 2009, Liao et al. 2025). Classification is based on host distribution, tissue colonization, transmission mode, plant-host benefits, and class-grouping criteria, highlighting the distinct paradigms of Clavicipitaceous (Class I) and Non-Clavicipitaceous (Classes II–IV) endophytes in terrestrial ecosystems. Each inset represents a microscopic illustration of tissue-level localization associated with each endophyte class.

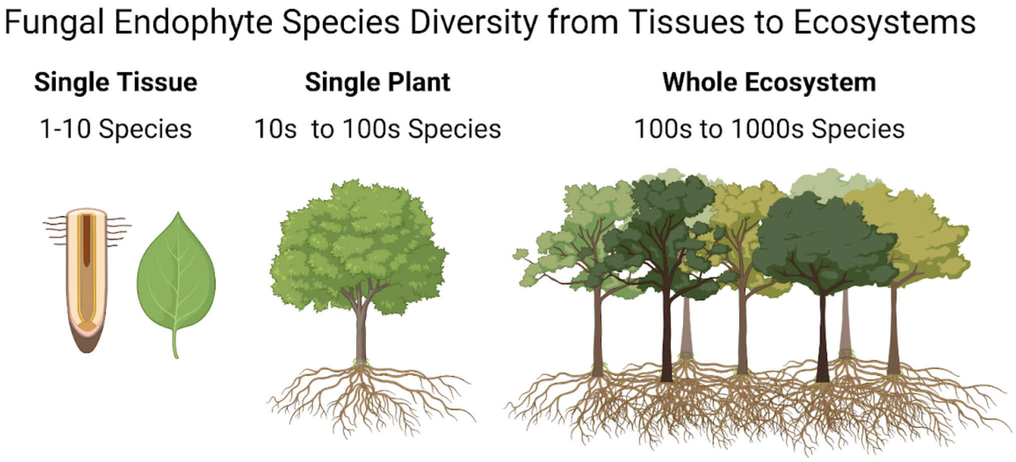
Figure 2 – Distribution of endophytes and their host associations across the fungal tree of life



b. **Full caption** – Endophyte records were compiled from Rashmi *et al.* (2019; doi:10.5943/mycosphere/10/1/19) and summarized by fungal and plant orders. “Aboveground” denotes endophytes reported from leaves, stems, or other aerial tissues; “Belowground” from roots or rhizomes; and “Total plant” indicates taxa documented in both above- and belowground tissues.

Figure 3 – Ecological Distribution of Endophytic Fungi





c. **Full Caption** – A summary of global patterns of fungal endophyte diversity and distribution. WWF biomes that have received high levels of attention from fungal endophyte researchers are highlighted. Hypothetical estimates of fungal endophyte species diversity across levels of biological systems ranging from tissues, to individual plants, to whole ecosystems.