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Exploring Ectomycorrhizal Communities: A Comparative Study of Forest Types in Costa Rica

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Waterfall at Cloudbridge Nature Reserve, Source: Benjamin Luke

Declaration of authorship

I confirm that this bachelor thesis, titled “Exploring Ectomycorrhizal Communities: A Comparative Study of Forest Types in Costa Rica,” is my original work and was written by me independently.

Place, Date, Signature ____

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Abstract

This bachelor's thesis explores ectomycorrhizal (EM) fungi in tropical ecosystems, an area that has received far less attention than temperate or boreal regions. To help close this gap, the study investigates how EM exploration types are distributed across three forest stages—Planted, Natural Regenerated, and Old-Growth—within the Cloudbridge Nature Reserve in Costa Rica. The aim was to create a first baseline dataset for EM fungi in this tropical montane cloud forest.

Fieldwork involved sampling soil and fine roots from nine permanent plots, three per forest type, at two depth intervals. The roots were examined under a light microscope, and EM colonization was classified into six exploration types based on the structure of the extramatrical mycelium: Contact, Short-Distance, Medium-Distance (fringe, mat-forming, and smooth), and Long-Distance. Statistical analysis using a non-parametric Friedman test returned a p-value of 0.13, indicating that the overall distribution of exploration types did not differ significantly among the three forest categories.

Although the results were not statistically significant, clear ecological patterns emerged. Contact types dominated across all forest stands (40.9% in Planted, 47.6% in Natural Regenerated, and 49.3% in Old-Growth), reflecting a conservative strategy for nutrient uptake. Planted forests showed a codominance of Contact and Long-Distance types (34.0%), suggesting adaptations to early successional conditions, whereas Old-Growth stands exhibited the highest evenness (0.76) and a more balanced mix of Short- and Medium-Distance types, indicating functional diversification with increasing forest maturity.

Together, these findings provide the first comprehensive baseline for EM fungal communities in the Cloudbridge tropical montane cloud forest. They highlight the role of EM diversity in forest recovery and underline how functional traits shift with ecosystem development. Future studies could expand this work by incorporating molecular identification methods and extending sampling across a broader spatial scale to deepen understanding of EM functional ecology in tropical systems.

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Abbreviations

EM	Ectomycorrhiza
EDM	Endomycorrhiza
ERM	Ericoidmycorrhiza
ORM	Orchidmycorrhiza
AM	Arbuscularmycorrhiza
C	Contact (Type)
SD	Short-Distance (Type)
MD	Medium-Distance (Type)
LD	Long-Distance (Type)
P	Planted (forest stand)
NR	Natural Regeneration (forest stand)
OG	Old Growth (forest stand)

1 Introduction

Gymnosperm and angiosperm trees have co-evolved with symbiotic fungal partners for several million years, enabling them to grow on various soil types and take over terrestrial ecosystems (Khalfallah et al., 2024). These relationships are called mycorrhizae, and are present in most tree species, which allow them to obtain resources below ground (Smith & Read, 2008). These mycorrhizae, meaning "fungus-roots" (Brundrett, 2002), are mutualistic relationships between specialized soil fungi and the roots of plants (Smith & Read, 2008). Because they enhance plant nutrient uptake and influence soil structure, nutrient cycling, and plant community composition (Treseder, 2004; Yang et al., 2022), mycorrhizae are essential to ecosystem stability and productivity (Smith, 2008).

The symbiosis is based on a reciprocal exchange of resources between plants and fungi (Brundrett, 2002; van der Heijden et al., 2015a). Extraradical mycelial networks extend from colonized roots into the soil, allowing fungi to forage efficiently for phosphorus, nitrogen, micronutrients, and water (Agerer, 2007; Khalfallah et al., 2024). In return, plants supply carbon compounds and vitamins, as fungi lack photosynthetic capacity (Martin & van der Heijden, 2024). This carbon transfer can be substantial, with plants allocating 5–30% of their photosynthates to their fungal partners, representing a flux comparable in size to roughly 36% of global fossil fuel emissions (van der Heijden et al., 2015b).

Several types of mycorrhizae are recognized, each differing in evolutionary origin, structure, and ecological role (Brundrett, 2002). Overall, there are two main groups that can be distinguished: Ectomycorrhiza (EM) and Endomycorrhiza (EDM) (Brundrett, 2002). Arbuscular mycorrhizae (AM) are the oldest and most widespread type of EDM (Cordts, 2021), formed by fungi of the order *Glomales* (Voigt, 2025). These fungi penetrate root cortical cells and are dependent on their plant hosts. Ericoid mycorrhiza (ERM) typically occurs in Ericaceae growing in acidic, nutrient-poor soils (Brundrett, 2002), and orchid mycorrhiza (ORM) is critical for orchid germination and development, with many species relying entirely on fungal partners for nutrients and carbon (van der Heijden et al., 2015; Brundrett, 2002).

EM fungi, primarily found in woody plants, create a sheath around root tips. Both symbioses receive carbohydrates in exchange for improved nutrient uptake, drought tolerance, and pathogen resistance (Smith & Read, 2008). Although EDM are widespread, EM fungi are particularly sensitive to abiotic factors such as soil pH, nitrogen deposition, and temperature, making them valuable bioindicators of forest ecosystem health (Rosinger et al., 2018). Furthermore EM associations occur in only about 2% of plant species (Janowski & Leski, 2023), they dominate many forest ecosystems (Rosinger et al., 2018). Tree families such as Pinaceae, Fagaceae, and Betulaceae primarily form EM symbioses, while some predominantly AM families, such as Malvaceae, also contain EM-forming taxa like *Tilia* (Janowski & Leski, 2023). In addition, certain groups, including the Salicaceae, contain species capable of forming multiple mycorrhizal types simultaneously (Brundrett, 2002).

Although the diversity of EM host plants is relatively low compared to AM hosts (Janowski & Leski, 2023), the diversity of EM fungi themselves far exceeds that of other mycorrhizal groups (Tedersoo et al., 2012). Even though they are considered polyphyletic, therefore having acquired or lost mycorrhizal capability in different lineages, they span three major fungal lineages—Ascomycota, Basidiomycota, and Zygomycota (Brundrett, 2002). Together, they comprise more than 250 genera and an estimated 20,000 species (Janowski & Leski, 2023). Host tree species are a major determinant of EM community composition, whose diversity and abundance contrast with the comparatively small number of host tree species. EM communities are spatially patchy at local scales and influenced by disturbances, light conditions, temperature, precipitation, nitrogen inputs, soil characteristics, and land-use history (Shahin et al., 2013). However, large-scale patterns and the relationship between EM functional diversity and environmental variation remain poorly understood, particularly in tropical ecosystems (Tedersoo et al., 2014). The long-lasting effects of previous land use further complicate predictions of EM responses to changing environmental conditions (Khalfallah et al., 2024). As climate change progresses, understanding how EM fungi influence nutrient acquisition and soil carbon dynamics becomes increasingly important (Read & Perez-Moreno, 2003; Defrenne et al., 2019).

The amount of extraradical mycelium in the soil reflects the fungi's ability to explore space in EM symbiosis, effectively functioning as extensions of tree roots (Smith & Read, 2008). EM fungi can be categorized by exploration types based on their extraradical mycelial proliferation (Shahin et al., 2013). Some produce minimal hyphae, while others form large networks capable of long-distance transport (Wasyliw & Karst, 2020). These functional differences influence nutrient cycling and forest-level processes, ultimately affecting tree phenotypes (Cordts, 2021).

This bachelor's thesis investigates the distribution and diversity of EM exploration types across planted, naturally regenerating, and old-growth forest stands in the Cloudbridge Nature Reserve in Costa Rica. The study establishes a baseline dataset for EM fungi in this tropical montane cloud forest. It examines whether functional traits, such as exploration strategies, vary among forest types in relation to forest age, soil conditions, and management history. In addition to presenting original field data, the thesis compares findings from existing literature to situate the results within the broader context of mycorrhizal ecology. The work is structured to provide the necessary background on mycorrhizal symbioses, outline the ecological and conservation context of Cloudbridge, and describe the methods used to assess EM communities. The results and discussion then evaluate observed diversity patterns, test the stated hypotheses, and highlight how combining local field data and global research perspectives can inform conservation and reforestation practices in tropical mountain ecosystems (Voigt, 2025).

1.1 History of Ectomycorrhiza

This section provides an overview of the history of EM research, tracing the development of early morphological studies, including the emergence of exploration type concepts, to the later shift toward molecular methods that now shape our understanding of EM diversity and function.

The first documented initial understanding of mycorrhizal interactions dates to the late 19th century, when Kamieński (1881) observed nutrient exchange in *Monotropa* species (*Monotropa hypopitys* or Yellow Bird's-Nest), (Janowski & Leski, 2023) and Frank (1885) coined the term "mycorrhiza" while studying fungal sheaths on tree roots of *Fagus sylvatica*, *Carpinus betulus*, and *Quercus*. With increasing recognition of its ecological importance, research soon revealed that most plants engage in and depend on mycorrhizal associations.

Early land plants, resembling bryophytes, may have formed endophytic associations similar to AM roughly 400 million years ago, even before the appearance of true roots (Cordts, 2021). EM associations are estimated to be about 100 to 200 million years old, making them severely younger (Brundrett, 2002). Pinaceae was likely among the first plant groups to form EM symbioses around 180 million years ago (Cordts, 2021).

Still, fossil evidence for Paleozoic fungi is scarce, but molecular analyses suggest that fungi diverged early in the Proterozoic. It is probable that the first terrestrial fungi colonized land long before plants, forming early microbial soil communities with algae (Brundrett, 2002). The evolutionary transition to the EM lifestyle has occurred multiple times (van der Heijden et al., 2015) across various fungal lineages, driven by ecological factors such as easy access to soluble carbon compounds available from plant roots, in nutrient-poor environments (Ruytinx et al., 2021). During the late Cretaceous, EM fungi diversified rapidly, likely in parallel with the rise of angiosperms. This co-evolution enabled fungi to recognize different hosts and suppress plant defense responses, allowing them to colonize new ecological niches, particularly in nutrient-limited soils. These processes continue to drive EM diversification today (Martin & van der Heijden, 2024).

A defining genetic hallmark of EM fungi is the reduction of gene families responsible for plant cell wall–degrading enzymes (PCWDEs) (Ruytinx et al., 2021). In contrast to saprotrophic fungi, most EM taxa lack ligninolytic class II peroxidases, invertases, endocellulases, cellobiohydrolases, and cellulose-binding motifs (van der Heijden et al., 2015). This reduction reflects their evolutionary shift from decomposing plant material toward a dependency on plant-derived carbon sources (Martin & van der Heijden, 2024). Nevertheless, EM genomes typically retain a small subset of PCWDE genes, which enables limited scavenging from soil organic matter (Defrenne et al., 2019). Dating analyses further suggest that families such as the Endogonaceae, which also form EM symbioses, originated as early as late Perm or early Trias, potentially playing a role in the early colonization of land by plants (Ruytinx et al., 2021).

Early EM research was mainly grounded in morphological and anatomical identification (Cordts, 2021). Root tips were classified based on mantle color, shape, and surface structure, as well as hyphal and rhizomorphic characteristics. These traits formed the basis of what later became known as "exploration types" (Wasyliw & Karst, 2020). Renowned mycologists like Agerer (2001, 2006) developed comprehensive classification systems and atlases, such as the *ColorAtlas of Ectomycorrhizae*, to categorize these morphological characteristics and their presumed ecological significance. Online resources like the DEEMY platform[1] (Agerer & Rambold, 2004–2024) continue to support morphological identification today.

The introduction of molecular methods, particularly DNA-based phylogenies and sequencing of the Internal Transcribed Spacer (ITS) region marked a turning point in EM research (Janowski & Leski, 2023; Suz et al., 2014; van der Heijden et al., 2015). These techniques revolutionized the field by enabling the identification of fungal species that could not be linked to above-ground fruiting bodies, and by an abundance of undescribed and cryptic taxa (Brundrett, 2002), especially in biodiverse regions like the tropics. Genomics and transcriptomics have deepened understanding by providing insights into mycorrhizal symbioses, their genetic information, molecular mechanisms, and evolutionary patterns (van der Heijden et al., 2015). The ITS region, particularly ITS1, remains the standard universal DNA barcode for fungi due to its generally low intraspecific and high interspecific variability (Janowski & Leski, 2023).

The field advanced further with the publication of the first EM fungal genomes, including *Laccaria bicolor* and *Tuber melanosporum* (Martin & van der Heijden, 2024), as well as the AM fungus *Rhizophagus irregularis* (van der Heijden et al., 2015). Comparative genomics has helped distinguish symbiotic fungi from saprotrophs and elucidated the genetic architecture of symbiosis (Martin & van der Heijden, 2024). The Joint Genome Institute's MycoCosm platform[2] now hosts more than 2,700 fungal genomes, including over 220 mycorrhizal species, providing a foundation for metagenomic and transcriptomic studies. These tools allow researchers to map DNA and RNA from soil and root samples, identify fungal species, and quantify the expression of genes involved in nutrient exchange, signaling pathways, and symbiosis development. Metatranscriptomic approaches now offer insights into whether multiple fungal partners colonizing the same host tree fulfill overlapping or distinct ecological roles (Martin & van der Heijden, 2024).

Current research continues to investigate EM fungal diversity and functional traits, often integrating morphotyping with molecular identification (Rosinger et al., 2018) to assess species richness and community composition (Tedersoo & Bahram, 2019). A central focus is the functional interpretation of exploration types, which are believed to represent distinct strategies for nutrient foraging, water uptake, and carbon allocation, and to reflect adaptations to different environmental contexts (Hobbie & Agerer, 2010). While widely used, the functional validity of

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[1] Link to the website (19.12.2025): http://www.deemy.de

[2] Link to the website (03.10.2025): https://mycocosm.jgi.doe.gov/mycocosm/home

exploration types across diverse forest ecosystems, encompassing different host species, soil conditions, and climatic gradients, remains an active area of research and debate (Rosinger et al., 2018).

Finally, progress in EM research continues to be complicated by ecological complexity, limited visibility of belowground organisms, and methodological challenges (Janowski & Leski, 2023; Tedersoo et al., 2012). As mycorrhizal studies draw from multiple disciplines, including botany, mycology, soil science, and ecology, but still receive little emphasis in most educational curricula, relatively few students pursue careers in this field (Janowski & Leski, 2023). Many EM species remain undescribed, particularly in tropical and Southern Hemisphere regions (Brundrett, 2002), and fungal diversity continues to be underrepresented in conservation assessments compared to plants and animals (Brundrett, 2002; Tedersoo et al., 2014). Growing conservation concerns include documented local declines and extinctions of EM fungi, and several countries have introduced Red Lists for threatened fungal species (Janowski & Leski, 2023; Suz et al., 2014).

1.2 The symbioses between fungi and plants

This section examines the diverse symbiotic relationships between fungi and plants, outlining their structural features, functional roles, and ecological importance.

As already mentioned EM fungi form a characteristic mantle around fine roots, from which hyphae extend into the soil. Internally, they develop the Hartig net between the cortical root cells, creating a highly efficient interface for nutrient exchange and effectively extending the plant's phenotype into the surrounding soil (Khalfallah et al., 2024). The morphology of EM root tips, such as branching patterns, coloration, and the presence of hyphae or rhizomorphs, varies among fungal species. Many EM fungi also produce sporocarps, whose external morphology has historically served as a basis for fungal taxonomy, although the correspondence between above-ground fruiting bodies and below-ground root tips is not always straightforward (Janowski & Leski, 2023).

By binding soil particles through their hyphae and exudates, EM fungi enhance soil structure and stability (Martin & van der Heijden, 2024; van der Heijden et al., 2015). Mixed mycorrhizal systems often show high productivity and species richness, suggesting complementary interactions between different functional types. Experimental studies confirm that mycorrhizal diversity positively correlates with aboveground productivity, and that the relationship between tree richness and productivity often depends on the type of fungal association (Martin & van der Heijden, 2024).

In natural soils, single trees are typically colonized by numerous fungal taxa, sometimes several dozen per adult tree (Simard et al., 2012). Even individual root branches can host multiple fungal taxa (Rosinger et al., 2018). These associations frequently connect plants through common mycorrhizal networks (CMNs), which link different individuals and species (Martin & van der Heijden, 2024). CMNs facilitate the exchange of resources, mediate plant interactions, and influence biodiversity (Simard et al., 2012). Their persistence depends on continuous carbon allocation from the host plants (Martin & van der Heijden, 2024).

At the molecular and genetic level, EM fungi exhibit distinct adaptations. Genes encoding small, secreted proteins play key roles in modulating host immunity, stabilizing the symbiosis, and regulating microbial entry (Ruytinx et al., 2021). Symbiotic interactions involve the expression of specialized gene sets related to nutrient transport, redox processes, and metabolic adjustments. Many of these genes have orthologs in saprotrophic fungi, suggesting that the EM lifestyle emerged through modifications of ancestral metabolic pathways. Resulting functional shifts affect carbohydrate, amino acid, and organic acid metabolism, highlighting the tight metabolic integration between hosts and their fungal partners (Martin & van der Heijden, 2024).

Functional differences among EM fungi are also reflected in their exploration types. Some species form dense hyphal mats or long-distance rhizomorphs, whereas others produce only short-range hyphae (Rosinger et al., 2018). These traits directly shape nutrient foraging strategies and influence community composition (Khalfallah et al., 2024). Although host tree identity is the primary determinant of EM community structure (Morris et al., 2008), environmental factors, including soil chemistry, nitrogen deposition, light availability, temperature, precipitation, and historical land use, also shape EM assemblages (Defrenne et al., 2019; Khalfallah et al., 2024).

EM symbioses are not uniform. Some interactions are balanced and mutually beneficial (Brundrett, 2002), while others are exploitative or mycoheterotrophic, providing disproportionate advantages to either the fungus or the plant (Simard et al., 2012). EM fungi also appear to diversify more rapidly than their plant hosts, producing a remarkable range of taxa and root morphologies (Brundrett, 2002). Genetic individuals, or genets, can sometimes reach considerable sizes and colonize multiple trees simultaneously. Still, most genets remain small and function as autonomous units, as vegetative incompatibility loci (*het loci*) prevent hyphal fusion or anastomosis between genetically distinct individuals by triggering cell death upon incompatible contact (Martin & van der Heijden, 2024). As a result, many EM genets are spatially limited (Cordts, 2021). Still, certain long-distance exploration types form exceptionally large, contiguous genets detectable through microsatellite DNA analyses. These individuals can span up to 20.1 meters and connect trees of different age classes within forest stands (Beiler et al., 2010).

1.3 The symbiosis in ecosystems

Building on the basic understanding of plant–fungal partnerships, this section explains the roles that mycorrhizal symbioses play in shaping the structure, functioning, and resilience of terrestrial ecosystems. These interactions extend beyond individual plant–fungus relationships, influencing nutrient cycling, carbon dynamics, plant community composition, and overall ecosystem stability.

Mycorrhizal fungi are major drivers of ecosystem processes, particularly in temperate and boreal forests (Tedersoo et al., 2020). They enhance the uptake of essential nutrients, including nitrogen, phosphorus, and several micronutrients, thereby directly supporting plant growth and productivity. In nutrient-poor soils, they can access nitrogen bound in soil organic matter, making them essential contributors to sustaining forest productivity (van der Heijden et al., 2015).

At the same time, mycorrhizal mycelium represents a substantial deposit of plant-derived carbon in soils. Their biomass and exudates store carbon over long periods, forming a major global carbon pool (Clemmensen, 2013). Through their influence on soil organic matter formation and stabilization, these fungi further promote long-term carbon storage (Martin & van der Heijden, 2024).

Beyond nutrient and carbon dynamics, mycorrhizal associations contribute significantly to ecosystem resilience. They help plants cope with drought stress, enhance resistance to pathogens (Voigt, 2025), and promote soil aggregation through their hyphae and exudates (van der Heijden et al., 2015). These functions are essential for maintaining soil structure and ecosystem health (Martin & van der Heijden, 2024). At the community scale, mycorrhizal fungi mediate plant–plant interactions, influencing biodiversity, productivity, and forest composition (Simard et al., 2012). Research has shown that forests with mixed mycorrhizal strategies, incorporating both AM and EM fungi, often exhibit higher productivity and species richness than systems dominated by a single mycorrhizal type (Martin & van der Heijden, 2024).

The ecological importance of mycorrhizal symbioses is quite context-dependent and varies across ecosystems, environmental gradients, and plant communities (Martin & van der Heijden, 2024). As mentioned, EM fungi are particularly influential in temperate and boreal regions, where they play a central role in nutrient uptake (Rosinger et al., 2018; Tedersoo et al., 2020). In contrast, EDM fungi are more common in grasslands, though their influence on biomass production appears less pronounced. Agricultural and heavily disturbed systems typically show reduced or absent mycorrhizal associations, with opportunistic species and pathogens becoming more prevalent. Still, some crops and plant species benefit from mycorrhizal interactions, reflecting significant variability in responsiveness. Exceptions occur, such as members of the Proteaceae or certain Australian flora with cluster roots, which thrive without mycorrhizal partners (Martin & van der Heijden, 2024).

Multiple environmental drivers shape EM community composition and function (Defrenne et al., 2019). Host tree species represent the most important factor, strongly influencing community structure and the distribution of exploration types (Morris et al., 2008). Soil properties, including pH, phosphorus, and nitrogen availability, also have major effects and often show unimodal relationships with fungal diversity (Rosinger et al., 2018). Climatic conditions, such as temperature, precipitation, forest age, and land-use history, further influence EM communities at local and regional scales (Voigt, 2025).

A geographical bias in mycorrhizal research is noticeable, with most studies focusing on temperate and boreal ecosystems for example Courty et al., 2008 and Defrenne et al., 2019. This emphasis has likely overlooked the full range of mycorrhizal and non-mycorrhizal strategies occurring in subtropical and tropical ecosystems (Tedersoo et al., 2014). Expanding research into these regions is essential for a more complete understanding of global mycorrhizal functions (Khalfallah et al., 2024).

Mycorrhizal symbioses' ecological significance depends on the context, changing with the type of ecosystem and environmental conditions (Yang et al., 2022). When the community composition of EM fungi shifts, it can lead to changes in functional traits at the ecosystem level (Fernandez & Koide, 2014). Different species exhibit unique strategies for hyphal proliferation, nutrient acquisition, and the turnover of fungal biomass, all of which influence ecosystem functioning. In some forests, EM fungi contribute up to 75% of phosphorus and 80% of nitrogen acquisition, and can account for roughly 40% of microbial carbon biomass in soils, playing a major role in autotrophic CO_2 respiration (Rosinger et al., 2018). Gene expression studies further show that EM species regulate nutrient uptake and allocation, ultimately influencing tree growth and ecosystem nutrient dynamics (Martin & van der Heijden, 2024; Ruytinx et al., 2021). Human-driven nitrogen deposition interferes with these processes by altering EM diversity and metabolic functioning (Lilleskov et al., 2019).

1.4 Ectomycorrhizal Exploration Types

Mirrored in the morphology of their extraradical mycelium, EM fungi exhibit significant functional diversity (Khalfallah et al., 2024). To encompass this diversity, Agerer (2001, 2006) developed the concept of “exploration types” to functionally classify ectomycorrhizal fungi based on the extent of extra-matrical mycelium and rhizomorph development for efficient nutrient acquisition and adaptation to environmental conditions (Fernandez & Koide, 2014; Yang et al., 2022). This categorization is now a commonly used tool to study EM communities because it goes beyond taxonomy and links fungal morphology to ecology. This chapter will look closely at the exploration types according to Agerer, focusing on their structure, ecological significance, abundance, and distribution.

The exploration types are determined by the extension and differentiation of emanating hyphae and the presence of rhizomorphs. At one end of the spectrum is the contact type, which forms dense, hydrophilic mantles with very few emanating hyphae. The mycelial cords are highly carbon-efficient and well-adapted to resource-rich microsites.

Contact Type (CT)

Characterized by having a smooth, dense mantle with few emanating hyphae

They possess only a few short hyphae, but no rhizomorphs

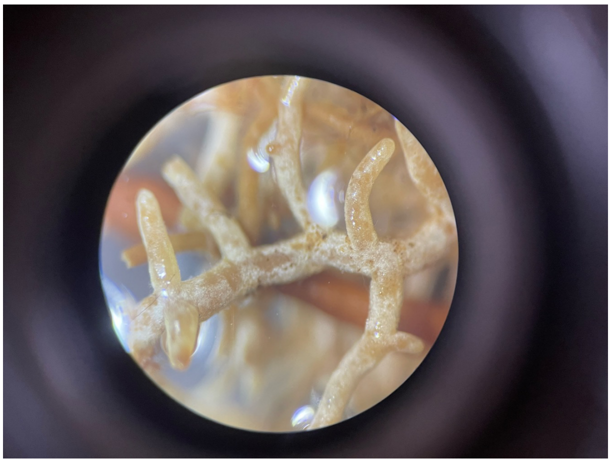

Figure 1. Picture from under the microscope, Contact Type

Short-Distance type (SD)

This type is limited in its extent and lacks rhizomorphs, although hyphal projections extend a short distance into the soil.

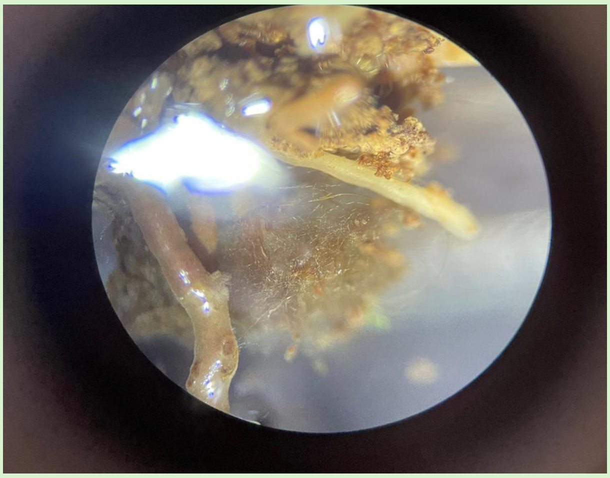

Figure 2. Picture from under the microscope, Short-Distance Type

Medium-Distance (MD)

This type is distinguished in three forms:

1. **1.** Fringe type: interconnected networks of rhizomorphs that constantly divide and fuse.
2. **2.** The mat-forming type: it creates dense hyphal mats, merging into a homogeneous structure
3. **3.** Smooth type: Rhizomorphs with a soft mantle and margin.

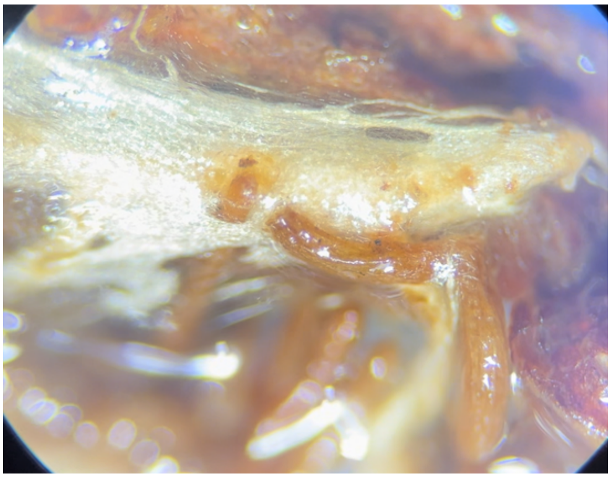

Figure 3. Picture from under the microscope, Medium-Distance Type

Long-Distance (LD)

LD rhizomorphs form thick structures aiming to plant vascular bundles and efficiently translocate water and nutrients over considerable distances. These long-distance explorers can tap organic nitrogen sources and adaptations for nutrient acquisition in spatially restricted environments.

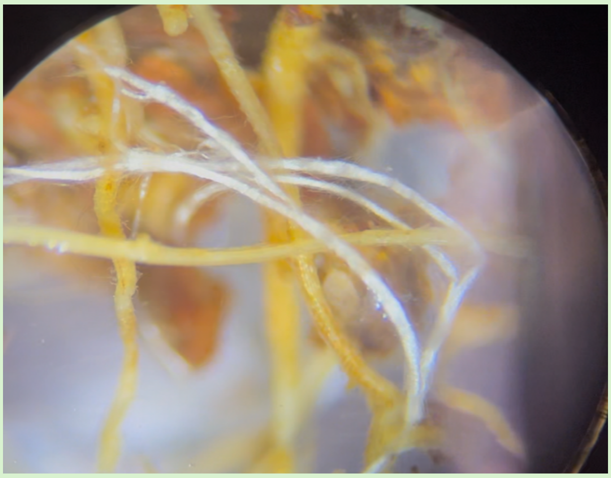

Figure 4. Picture from under the microscope, Long-Distance Type

Table 1. The Exploration Types according to Agerer

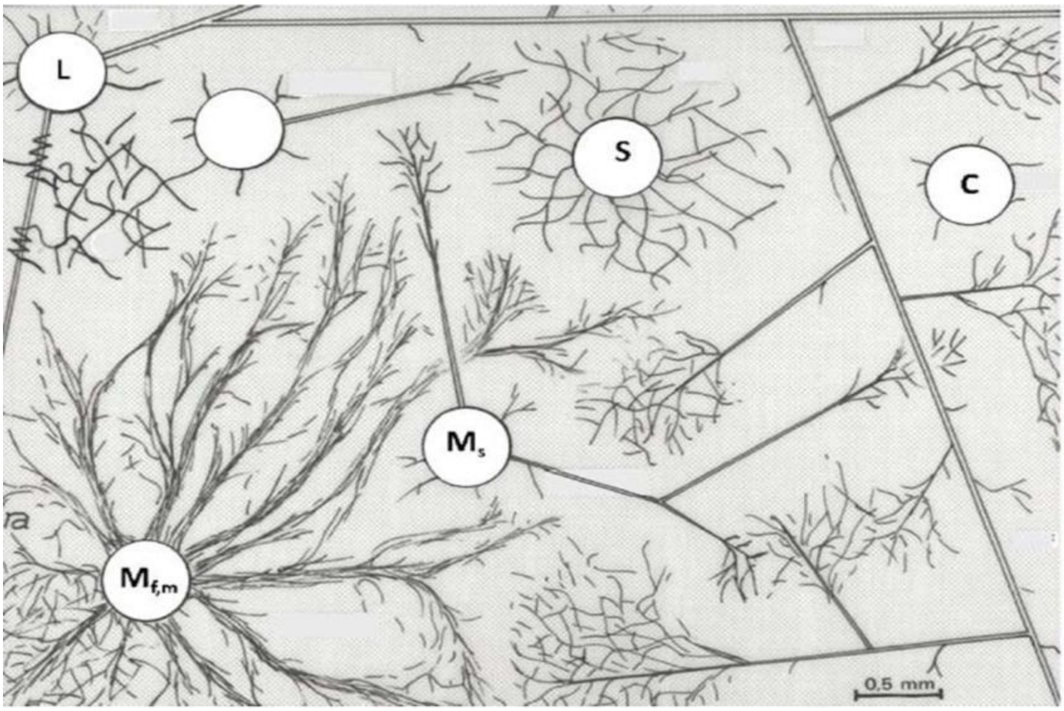

Figure 5. Exploration types of ectomycorrhizal fungi. The schematic extent of hyphae emanating from ectomycorrhizae of different exploration types is shown here for contact (C), short-distance (S), medium-distance smooth (Ms), medium-distance fringe, mat (Mf,m)

This ecologically significant classification offers a practical trait-based approach to EM fungal diversity. While taxonomy reveals which fungal species exist, exploration types demonstrate how fungi interact with their environment and host plants by implementing different strategies of nutrient acquisition, water transport, carbon allocation, and root colonization (Agerer, 2006, as cited in Defrenne et al., 2019). Soil factors such as pH, nutrient availability, and organic matter content also have a strong influence (Voigt, 2025). For instance, SD and CT are often associated with organic-rich upper horizons of the soil layers (Shahin et al., 2013), while LD and MD types may dominate in deeper or nutrient-poor layers. Established isotope studies support this discrepancy, showing that LD types are generally more enriched in nitrogen, indicating a reliance on organic nitrogen (Khalfallah et al., 2024).

These strategies also vary in their carbon requirements. Generally, SD types are less carbon-costly, making them valuable when carbon fixation by the host plant is limited (Suz et al., 2014). In contrast, LD types require greater carbon allocation to sustain their extensive rhizomorphic networks (Rosinger et al., 2018). Still, in return, they provide access to more distant or patchily distributed nutrient and water sources (Khalfallah et al., 2024). This capacity for long-range foraging also makes them particularly important under drought conditions, where continuous mycelial networks can enhance water transport through the soil (Defrenne et al., 2019).

The abundance and distribution of exploration types are shaped by a combination of host identity, soil properties, climate, forest age, and land-use history (Voigt, 2025). Host tree species are consistently identified as the main drivers of EM community composition, with different trees favoring types (Rosinger et al., 2018). Climate adds another layer of complexity, as fungi with long rhizomorphs are often favored in dry and less fertile environments, while SD explorers may thrive under nitrogen-rich conditions (Lilleskov et al., 2019). Forest age can also affect exploration type composition, with competing hypotheses suggesting an increased reliance on long-distance types as fine root density declines, or a decrease in long-distance kinds as carbon availability becomes limiting in older stands (Wasyliw & Karst, 2020).

Despite the ecological appeal of this framework, the link between morphology and function remains an active area of research (Khalfallah et al., 2024). Some studies have correlated the distribution of exploration types with soil physical properties and nitrogen availability (Shahin et al., 2013), while others emphasize the importance of enzymatic traits (Fernandez & Koide, 2014), nitrogen uptake strategies (Lilleskov et al., 2011), and carbohydrate-active enzyme profiles. These findings suggest that exploration types reflect fundamental functional differences among EM fungi. Linking fungal morphology with nutrient foraging strategies, carbon use, and environmental adaptation provides a practical framework for analyzing EM fungi in comparative studies across different forest types, soil conditions, and climatic gradients (Defrenne et al., 2019). Although SD and C types often dominate abundance, MD and LD explorers play critical roles in nutrient and water transport, soil structuring, and ecosystem resilience (Rosinger et al., 2018). However, consistent patterns have not been fully validated across different forest ecosystems and environmental conditions (Khalfallah et al., 2024).

2 Research Context

Costa Rica, a Central American country of about 51,000 km² between Nicaragua and Panama, is internationally renowned for its extraordinary biological diversity. Its geographical location, nestled between the Pacific and Atlantic oceans, contributes to its ecological richness. Nearly 6% of the world's plant and animal species are found within its borders, making it one of the most biodiverse countries on the planet (OECD, 2025). More than 12,000 vascular plants have been recorded, including approximately 1,400 orchid species (Pupulin et al., 2023) and nearly 2,300 tree species (Figueroa-Mata, 2022). The country's fauna is equally remarkable, with around 34,000 species of insects, 1,000 butterflies, 160 amphibians, 220 reptiles, and 850 bird species, six of which are endemic. Mammals such as sloths, four monkey species (spider, howler, capuchin, and squirrel monkeys), jaguars, and white-nosed coatis are also characteristic of Costa Rica (Saunier, 2016).

Costa Rica is home to approximately 3,8 million inhabitants, most of whom are of Spanish, indigenous of the Americas, or African descent. The indigenous population was displaced mainly during the colonial period. Still, cultural diversity remains an essential part of the nation's identity. The capital, San José, is located near the Talamanca Mountain range. Historically, the economy relied heavily on agricultural exports, with bananas, coffee, sugar cane, pineapples, and cocoa as primary products (Morgan, 2023). In recent decades, tourism has become a significant economic driver, primarily supported by the country's commitment to conservation and its reputation as a global biodiversity hotspot (Saunier, 2016).

Within this context, the Cloudbridge Nature Reserve is significant as a conservation and research site (Philips, 2024). Established in 2002 by Ian and Genevieve Giddy (Saunier, 2016), the reserve was founded as a response to deforestation and to protect the endangered cloud forest biome of the Cordillera de Talamanca or Talamanca Mountain range (Hance, 2023). Covering approximately 182 hectares across two mountain slopes, including parts of the Cerro Chirripó (Saunier, 2016). Cloudbridge encompasses both old-growth and early-seral forests (Philips, 2024). Since its founding, the reserve has pursued the dual mission of ecological restoration and environmental education.

Managed by the Cloud Forest Conservation Alliance (CFCA), Cloudbridge is a U.S.-based non-profit organization that supports its operations and promotes conservation, education, and sustainability initiatives. The alliance collaborates with individuals, academic institutions, and organizations that share similar values, with the vision of making Cloudbridge an internationally recognized learning laboratory for cloud forest ecosystems (*Cloudbridge Nature Reserve*, 2024).

Education and research have become integral to the reserve's mission. Through its Welcome Center, art gallery, and field-based training programs, Cloudbridge promotes awareness of conservation challenges and climate change while encouraging sustainable tourism practices. By 'leading through example' and fostering international collaboration, the reserve contributes directly to Costa Rica's broader goals of environmental stewardship and carbon neutrality (Saunier, 2016).

2.1 Ecological Importance of the Study Area

This section outlines the unique geographical setting, biodiversity richness, climatic and edaphic characteristics, and the ongoing threats and conservation efforts that shape the functioning of the tropical montane cloud forest in Costa Rica. Doing so highlights why the area provides an exceptional case study for investigating fungal diversity and ecosystem dynamics.

The Cloudbridge Nature Reserve in Costa Rica represents a site for studying ecological dynamics within a tropical montane cloud forest (Voigt, 2025). Located on the Pacific side of the Talamanca Mountain range, next to Chirripó National Park which is bordering the UNESCO World Heritage Site of La Amistad International Peace Park (UNESCO World Heritage Centre, n.d.), it is centered in one of the world's most significant biodiversity hotspots. The Talamanca Mountains alone are estimated to harbor nearly 4% of all terrestrial species (UNEP-WCMC, n.d.), including around 9000 flowering plants and approximately 800 fern species (Ecology Prime, 2025). This richness also extends to fauna: nearly 300 bird species (Voigt, 2025), all six of Costa Rica's wild felines, and large mammals such as Baird's tapir, puma, and jaguar inhabit the reserve. Many of these species are rare, vulnerable, or endangered, underlining the area's global conservation value (Philips, 2024).

Since its establishment, reforestation has been at the core of the reserve's work. More than 50,000 native trees were planted by 2017, contributing to the restoration of degraded pasture and agricultural lands (Voigt, 2025). Today, the reserve protects existing old-growth forests, continues to expand tree planting efforts, and assists neighboring communities in reforestation initiatives (Saunier, 2016), carried out with the help of a small permanent staff and a fluctuating number of volunteers and interns who contribute both labor and expertise. Volunteers from around the world engage in activities ranging from seed collection and tree planting to biodiversity monitoring, enabling the reserve to gather valuable data on less studied aspects of flora and fauna (Voigt, 2025; Saunier, 2016).

Defined by its high altitude, Cloudbridge tropical rainforest is a cloud forest environment, shaped by orographic cloud formation, where moisture-laden air rises against the mountain slopes, cools, and condenses into a persistent cloud cover (Saunier, 2016). This phenomenon creates a typical microclimate characterized by reduced solar penetration and a stratified forest structure (Philips, 2024). Vegetation is organized into the ground layer, dominated by fungi and shade-tolerant plants, an intermediate shrub layer, the canopy rich in epiphytes such as orchids, bromeliads, and vines, and an emergent layer of towering trees (Backer, n.d.). Such conditions support highly specialized shaded environments, including numerous mycorrhizae essential in nutrient cycling and ecosystem stability (Saunier, 2016).

The cloud forest soils are typically acidic and nutrient-poor, with textures ranging from sandy-silty to silty-clayey. In higher altitude forests with a large percentage of oak, soil fertility is low. External processes partially offset this limitation: long-range atmospheric dust transport from the Sahara and Sahel provides essential inputs of phosphorus and other minerals. However, this mechanism is vulnerable to disruption due to global climate change, which alters wind patterns and dust deposition. Additional pressures arise from pesticide residues transported through atmospheric currents from agricultural areas, further affecting soil health. These environmental constraints create conditions where fungal communities are essential in facilitating plant nutrient acquisition and supporting forest regeneration (Saunier, 2016).

Cloud forests such as the one around Cloudbridge make up barely 1% of the world’s forests (Voigt, 2025) and are among the most threatened ecosystems globally (Womack, n.d.). They are susceptible to deforestation and climate change, particularly through rising temperatures and shifting precipitation patterns, which significantly affect these mountainous areas (Saunier, 2016). As warming forces the cloud belt to move to higher elevations, the total area suitable for cloud forest shrinks, intensifying ecological pressures. In Costa Rica, deforestation reduced national forest cover to just 28% by 1990. Although large-scale reforestation initiatives have since increased cover, many plantations rely on non-native tree species (Saunier, 2016). Cloudbridge pursued a different strategy by planting over 50,000 native trees and protecting more than 200 hectares of forest to foster natural succession and restore native biodiversity (Voigt, 2025). Today, the reserve encompasses planted, naturally regenerated, and remnant old growth forests, creating a unique mosaic for studying forest recovery processes (Womack, n.d.).

The ecological importance of the forest of Cloudbridge lies not only in its exceptional biodiversity and conservation role but also in its environmental functionality (Philips, 2024). The tropical forests around Cloudbridge provide habitat connectivity within Central America's most significant remaining natural forest corridor (UNEP-WCMC, n.d.). At the same time, it serves as a living laboratory for examining how biotic and abiotic factors influence tropical montane forest dynamics (Yang et al., 2022). Understanding these processes is critical for regional conservation strategies and global efforts to mitigate biodiversity loss and climate change.

In summary, the Cloudbridge Nature Reserve represents a sanctuary for biodiversity and a model for active conservation and environmental research (Voigt, 2025). A combination of habitat protection, reforestation, volunteer engagement, and educational outreach demonstrates how local and international efforts can converge to restore and safeguard the tropical montane cloud forest, one of the world’s most threatened ecosystems (Philips, 2024).

2.2 Forest Types

The area of Cloudbridge holds a mix of forests, primary and secondary, with the secondary forests reflecting both natural growth and human planting efforts. The mix creates value for studies and forms a basis for understanding forest growth and planting strategies in a mountain cloud forest. The reserve contains three forest types: Old growth, and Natural Regenerated, and planted forests (Voigt, 2025).

The Old-Growth Forests (OG) are mature and untouched areas. These cover a smaller part, which is vital for studies since these serve as reference points, hold seed sources, and can act as benchmarks (Womack, n.d.). Originally the Natural Regenerated Forests (NR) areas were cleared for farms and cattle but then left to grow back. These forests, ranging from 10 to 30 years old, show natural plant recovery because some have grown since the 1990s and others since 2002. Their plants show a return of native life and create homes for fungi, birds, and mammals (*Cloudbridge Nature Reserve*, 2024). The Planted Forests (P) areas were actively reforested following the establishment of the reserve in 2002. These sites, up to 21 years old today, were initially agricultural lands. Reforestation was implemented to accelerate forest recovery (Womack, n.d.).

Early planting strategies included non-native and native species, such as *Cupressus lusitanica* and *Alnus acuminata*, though sustainable forestry trials with these species ultimately proved unrealistic. Since then, the focus has shifted toward exclusively using native trees or species with high ecological or reforestation potential. Examples include *Quercus costaricensis, Quercus rapurahuensis, Quercus seemannii, Alnus acuminata, Sapium pachystachys*, *Magnolia poasana,* and *Ocotea ira* (Saunier, 2016). Over time, the planting strategy evolved from large-scale hillside plantings to targeted enhancement planting, where pioneer species establish initial cover and shade, followed by the introduction of long-lived climax species such as oaks. Seedlings are raised in the reserve's tree nursery, supplemented by collections from OG stands and neighboring areas maintained through intensive early care, including mulching, invasive grass removal, and composting to overcome degraded soil conditions (Philips, 2024; Saunier, 2016).

The composition of tree species across forest types directly influences EM communities (Rosinger et al., 2018). Oaks dominate certain high-altitude zones and associate with distinct fungal partners. At the same time, *Alnus acuminata*, with its nitrogen-fixing capacity, creates favorable conditions for plant growth and specific fungal taxa such as *Gyrodon monticola (Saunier, 2016).* Studies have shown that fungal diversity is particularly high in OG and NR forests. P areas require more time to develop comparable fungal communities, reflecting the slower establishment of soil symbioses in younger stands (Voigt, 2025; Womack, n.d.).

Cloudbridge's reforestation mission has been central to its conservation strategy since 2002. Annual planting campaigns, carried out by staff, volunteers, and researchers, have reintroduced native tree species and supported the natural spread of pioneer vegetation. Techniques have gradually improved, with higher seedling survival rates achieved through selective planting on ridgelines, careful maintenance, and enhancement planting that mimics natural succession (Saunier, 2016). These efforts have yielded measurable ecological outcomes: oaks planted two decades ago now produce acorns, wildlife such as tapirs and jaguars have returned, bird diversity has expanded to over 300 species, and soil stability and fertility have significantly improved (Voigt, 2025). Still, it is essential to mention that not all the planting actions have been successful. In a P area of the Reserve, next to the trail called *Montaña,* an unknown sort of fungi has affected the oaks. Because only oak species (*Quercus costaricensis* and *Quercus copeyensis* (Saunier, 2016)) have been planted here, this part of the forest is facing a big problem (Voigt, 2025).

In summary, the coexistence of OG, NR, and P forests within Cloudbridge creates a dynamic landscape. This diversity not only strengthens the ecological resilience of the reserve but also provides an exceptional framework for studying fungal diversity, forest recovery, and the long-term impacts of conservation interventions in tropical montane cloud forests.

2.3 Objectives

The primary aim of this project was to investigate community composition of EM fungi in differing forest types within the Cloudbridge Nature Reserve in Costa Rica. For this, we quantified the richness and relative abundance of EM exploration types present in P, NR, and OG forest stands (Voigt, 2025).

The study evaluates the broader ecological effect of EM fungal diversity and functional traits for forest conservation and reforestation strategies using the Cloudbridge Nature Reserve model. By linking scientific findings to practical applications, the research provides recommendations that support the reserve's mission to deepen the understanding of fungal influence on cloud forest development and to promote effective, ecologically informed reforestation practices. The findings contribute to the generic knowledge of the interaction of EM and trees to support forest conservation.

2.4 Hypothesis

Functional traits of EM fungi, particularly their exploration strategies, are known to shift in response to environmental conditions such as soil nutrient availability and forest developmental stage. In planted forests, limited nutrient resources often favor EM species that produce extensive extraradical mycelium and rely on long-distance foraging structures, enabling them to access patchily distributed nutrients more effectively. Recent work has shown that such exploration types occur more frequently in nutrient-poor soils compared to natural regeneration or old-growth stands, where resource conditions are typically more stable (Yang, Zhang & Liu, 2022).

Based on this understanding, it is reasonable to expect that forest age and land-use history at Cloudbridge influence the distribution of EM exploration types. However, factors such as soil nutrient availability, pH, or other abiotic drivers were not measured in this study, meaning that any observed patterns cannot be directly linked to these environmental variables.

To clarify how these dynamics may manifest in this study system, a null hypothesis was formulated specifically for the research conducted in the Cloudbridge tropical montane cloud forests. This hypothesis applies solely to the assessment of EM exploration type distributions among old-growth, naturally regenerating, and planted forest stands within the reserve.

Null Hypothesis:

The proportion and overall abundance of EM exploration types (contact, short-distance, medium-distance, and long-distance) do not differ between old-growth, natural regeneration, and planted forest stands in the Cloudbridge Nature Reserve (K. Beiler, *personal communication,* October 2024).

3 Methodology

3.1 Study area

Study plots were established in the Cloudbridge Nature Reserve, within the Talamanca Mountains of Costa Rica (9°31'42.0" N 83°36'47.0" W). The time period of when the sampling took place started in the beginning of November and ended mid of December 2024. The reserve calculates just under 200 hectares (Greilin Fallas Rodríguez, *personal communication*, October 2025) of recovering tropical montane cloud forest, from 1550m to 2000m above sea level (Womack, 2023). According to the Holdridge life zone classification system, the site lies within the Tropical Premontane Wet Forest to Lower Montane Wet Forest life zones. (Schembre, 2009). Annual precipitation ranges from 3500 mm to 4000 mm per year, with a pronounced wet season between May and November. Temperatures generally vary from 15°C to 22°C, with modest seasonal differences (see Fig. 6). Persistent cloud cover and low-lying mist add to its high humidity and further define the environmental and cloud forest conditions (Phillips, 2024). The three forest types represent distinct successional stages as defined by zonation maps. *Key EM host tree species in the area include Quercus costaricensis and Quercus copeyensis (syn. Q. bumelioides)*, which provide critical symbiotic relationships for sustaining fungal diversity. The soils of the reserve are typically acidic and nutrient-poor, with morphological types such as Dystrudepts and Kandihumults (Saunier, 2016).

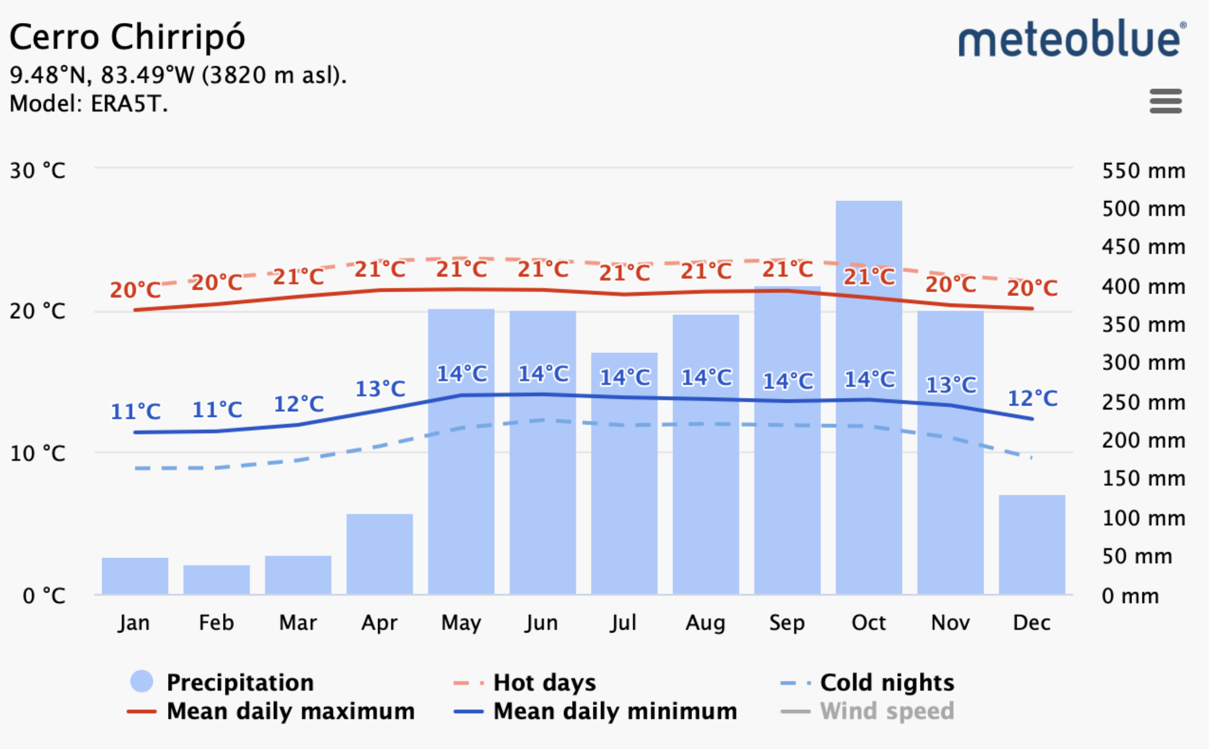

Figure 6. Historical weather data for Cerro Chirripó, hourly measured since 1940 (https://www.meteoblue.com/en/weather/historyclimate/climatemodelled/cerro-chirripó_costa-rica_3624238 (01.10.25))

3.2 Sampling design

This study's sampling design targeted EM fungi's diversity and exploration strategies across forests of different seral stages. The study includes nine permanent plots, with three plots assigned to each of the three forest stands representing different age classes and origin (see Fig. 7).

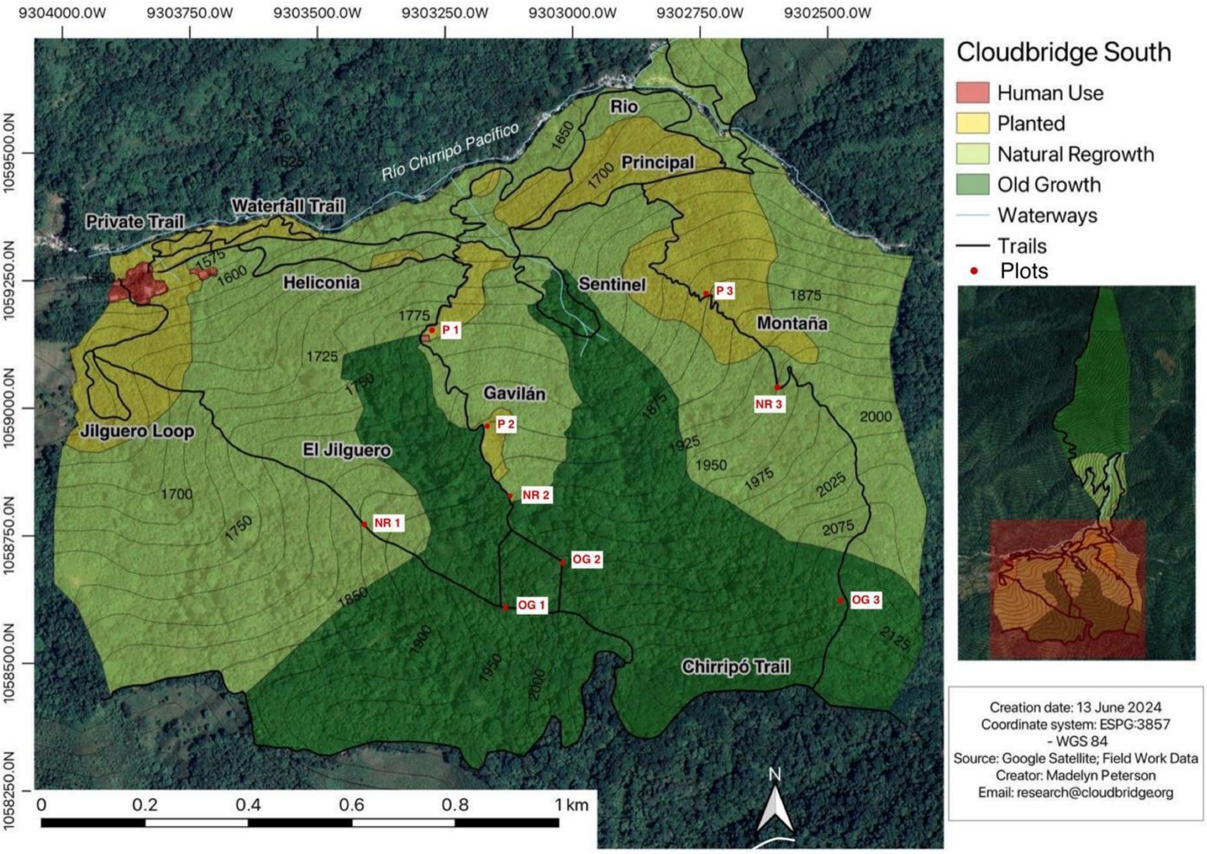

Figure 7. Map of Cloudbridge South with marked plots, Modified from: Madelyn Peterson (Google Satellite; Field Work Data)

Measuring tape and marking tape were used to set up the plots. All the plots were intentionally located on the same mountainside to minimize variability in environmental conditions and microclimate. Uniform conditions within the plots and limitation of ecological variance were the primary intention to decouple the effect of forest age and management method on EM diversity from that of other variables. Locating the plots for sampling at the same worksite/mountainside also simplified the access logistics. The plots should be approximately 10 meters by 10 meters in dimension to provide a sufficient sampling area.

Each plot provided five samples, one from the center and one from each of the four corners. At each of the five sampling points, two soil samples were collected; one from the uppermost 7cm of soil and another from a depth of 20-30cm. Stratified sampling facilitates the study of potential variations in EM communities and provides detailed analysis across different soil depths. This stratified approach enabled the study of potential vertical variation in EM community structure (Defrenne et al., 2019).

A total of ninety samples, collected from nine plots, comprised the study's dataset; each plot yielded ten individual samples (five sampling points at two different depths). A sampling intensity of 90 samples guarantees sufficient catch of the diversity and distribution of EM communities across the forest stand gradients. Its design eases the precise detection of complex patterns and subtle variations in EM diversity and exploration types. This methodology should offer meaningful understanding into how EM communities change with forest age, and sampling across multiple age categories will help with that (Kevin Beiler, 2024).

3.3 Ectomycorrhizal sampling techniques

Because mushrooms comprise 85 to 90% water, temperature and humidity play a significant role in their physiology (Al Qutaibi & Kagne, 2024). Sampling was done only in the wet season to ensure that fungal activity was at its peak and samples were homogeneous. Using a spade, a square of soil was extracted to standardize sample volume; each sample was placed in a labeled plastic bag and transported to the laboratory. In the laboratory, root samples were gently separated from soil over a mesh to eliminate bulk soil particles. The roots were soaked in a water bath for a few minutes to loosen soil and then rinsed very carefully to remove loose soil from the roots to minimize destruction to fungal structures. Repeat the procedures for each set of 10 samples collected at each plot. Samples were kept in a cooling box or refrigerator before the process to prevent deterioration of fungal structures. This technique offers minimum disturbance of fungal structures and efficiently separates roots from soil with minimum loss of EM integrity (K. Beiler, *personal communication*, October 2024). Furthermore, the standardized protocol provided a reliable basis for subsequent identification and classification of EM exploration types (Voigt, 2025).

Figure 8. Labeled bags in the field before taking samples, Source: Carlotta Voigt

3.4 Identification and classification of exploration types

In the laboratory, fine root samples were inspected under a light microscope with a 0.10x lens. The examination of each root tip focused on identifying characteristic EM structures, such as fungal mantles and associated hyphae. Roots were sorted and prepared using scalpels and scissors, ensuring precise handling and minimizing structural damage. At each stage of processing, observations were recorded systematically.

The identification of EM fungi relied on morphological criteria such as the color, texture, and structure of the mantle, as well as the arrangement of emanating hyphae. Root tips colonized by EM fungi were classified into exploration types according to the morphology and differentiation of their extrametrical mycelium. This functional classification helps to distinguish the foraging strategies from C types with little hyphal development to LD types characterized by extensive hyphal networks and differentiated rhizomorphs.

Root tips were grouped into six exploration types representing a continuum of foraging strategies for analysis. Where the number of samples was too high to process thoroughly, a random subset of approximately 100 root tips was selected for classification. After counting the total number of living EM root tips per sample, the abundance of each exploration type was quantified. Relative frequencies were then calculated for each plot, allowing the distribution of exploration types to be assessed across all three forest stands (Voigt, 2025).

3.5 Statistical Analysis

To examine whether the distribution of ectomycorrhizal (EM) exploration types differed among the three forest stands a non-parametric statistical approach was applied. This analytical framework was chosen because the data consisted of counts and relative proportions of EM exploration types, which did not meet the assumptions of normality and homoscedasticity required for parametric tests.

All statistical analyses were conducted using aggregated plot-level data. For each of the nine plots, the absolute abundance and relative frequency of each exploration type were calculated. Medium-Distance types (fringe, mat-forming, smooth) were also evaluated collectively as a functional group, since these categories are similar and encountered in low frequency.

To test for overall differences in exploration type distribution across forest stands, the Friedman test was applied. This test accounts for the paired structure of the data, where plots are treated as blocks and exploration type frequencies as repeated measures within each block. The Friedman test was selected because it is robust against non-normal distributions and suitable for small sample sizes (Hessing, n.d.).

When the Friedman test indicated trends toward significance, post-hoc pairwise comparisons were conducted using the Wilcoxon signed-rank test with Bonferroni correction to control for Type I error inflation. These comparisons were performed to identify potential differences between pairs of forest types (P vs. NR, P vs. OG, NR vs. OG). Effect sizes were also calculated to assess the magnitude of observed differences (Field, 2009).

Exploration type evenness within each forest stand was calculated using Pielou's evenness index to complement abundance-based metrics. This allowed assessment of how evenly exploration types were distributed within each forest category, providing additional insight into functional diversity patterns (Jost, 2010).

All results of the statistical analyses, including p-values, effect sizes, and visual summaries, are reported in the Results section. The significance threshold was set at $\alpha = 0.05$ for all tests.

3.6 Research for comparable Literature

This section of the methods briefly touches on the chosen strategy for the literature research. It is essential to mention that this is an empirical thesis focusing on the study at Cloudbridge Nature Reserve. However, it was decided to conduct this literature research, which was a comparably small effort compared to the total effort to produce this thesis. The study will mention this research part in the Discussion, Methods, and Results sections for a better understanding, even if it is unconventional.

The strategy for this literature research was as follows: my supervisor provided an initial set of relevant publications, which served as the starting point. I employed the Research Rabbit tool to identify related studies to expand this basis. I also used ChatGPT as a supporting instrument to generate preliminary summaries and locate potentially relevant papers. Beyond these tools, I also relied on my academic knowledge, searches conducted during the research process, and databases such as Google Scholar to identify additional literature. The summaries produced by ChatGPT were used exclusively for orientation; all works deemed relevant were subsequently read in full and analyzed in detail. The selection criteria for inclusion were that publications had to focus primarily on EM and its exploration types. This approach was fundamental because comparatively few studies on EM from tropical regions are available. Nevertheless, the review intended to find meaningful comparisons and to situate the empirical findings of this thesis within a broader research context.

4 Results

This section presents the frequency of occurrence and relative proportions of EM fungal exploration types across P, NR, and OG stands in the Cloudbridge Nature Reserve. Exploration types were identified and classified according to their morphological features and then grouped into C, SD, MD (fringe, mat-forming, smooth), and LD categories. The provided data was all self-selected; therefore, the source is Voigt, 2025.

4.1 Diversity patterns across forest types

The absolute number of colonized EM root tips counted for each exploration type in the three forest stands is detailed below:

Exploration Type	P (EM Root Tips)	NR (EM Root Tips)	OG (EM Root Tips)
Contact Type	864	636	523
Short-Distance Type	38	18	98
Medium-Distance Fringe	126	9	161
Medium-Distance Mat-Forming	29	11	11
Medium-Distance Smooth	337	164	41
Long-Distance Type	717	497	230

Table 2. Exploration types and colonized roots

P stands exhibited the highest abundance of EM root tips, with exploration types strongly dominated by C (864) and LD (717) forms. MD smooth types were also widespread in this forest type (337). Fringe types reached 126 tips, while SD forms were comparatively scarce (38). P stands showed the highest number of EM root tips among the three forest categories.

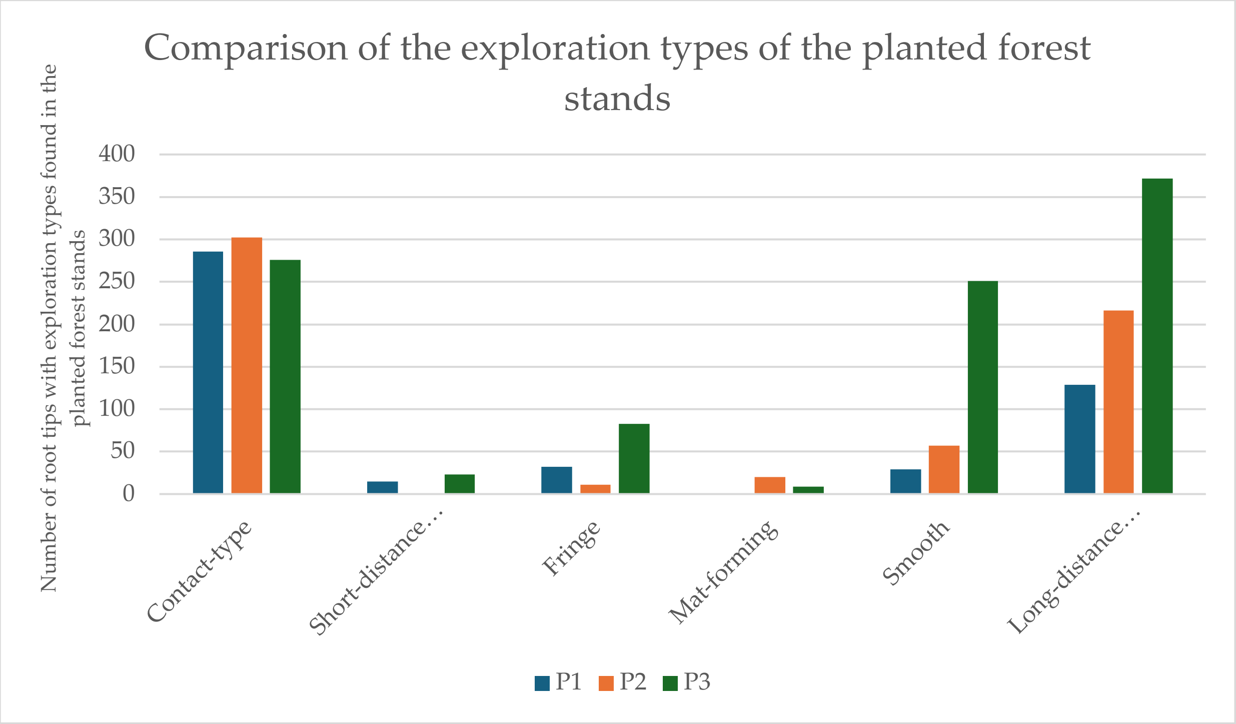

Figure 9. Comparison of the exploration types of the planted forest stands

NR yielded an intermediate total abundance of EM root tips. C types were again dominant (636), followed by LD types (497). MD smooth types (164) were more abundant here than in OG forests but lower than in P stands. In contrast, fringe types were nearly absent in this forest category, with only nine tips observed. SD types remained low, with 18 tips recorded.

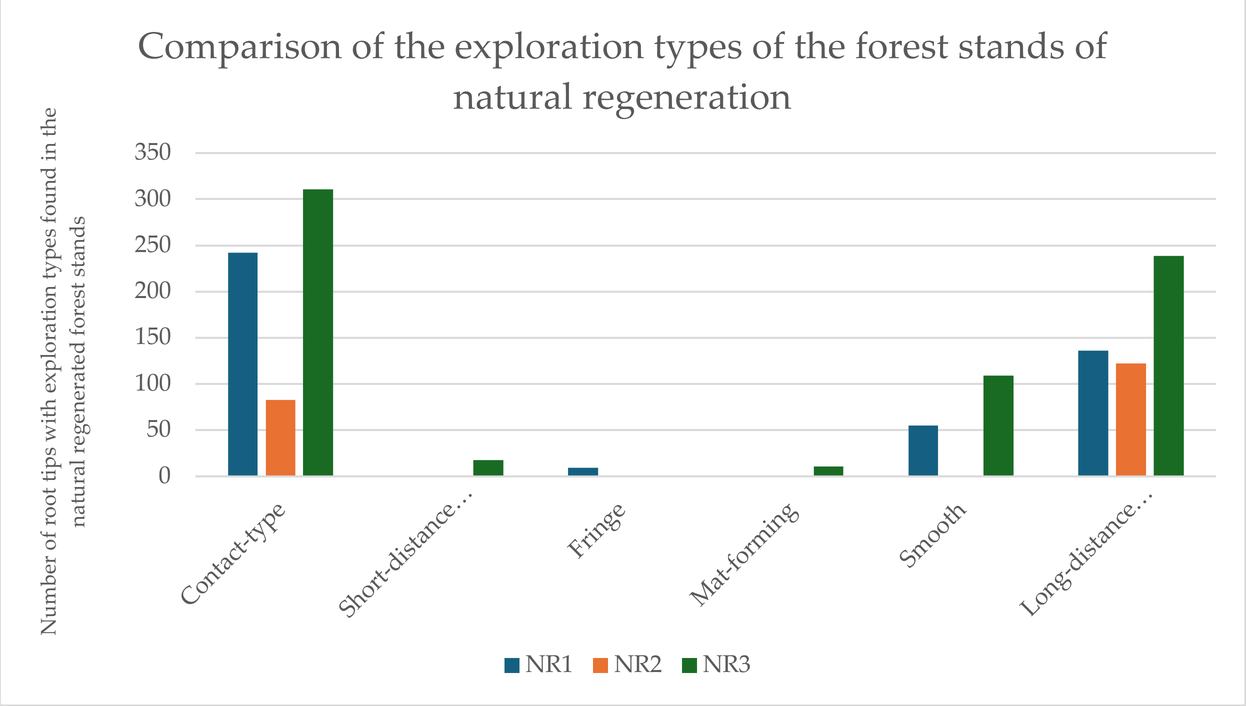

Figure 10. Comparison of the exploration types of the planted forest stands

OG stands displayed the lowest total abundance of EM root tips, yet they showed the highest structural heterogeneity in exploration types. C types remained the most abundant (523), but both SD (98) and MD fringe types (161) increased considerably compared to younger stands. Smooth types were much less frequent here (41), while LD types (230) occurred at markedly lower levels than in P and NR forests.

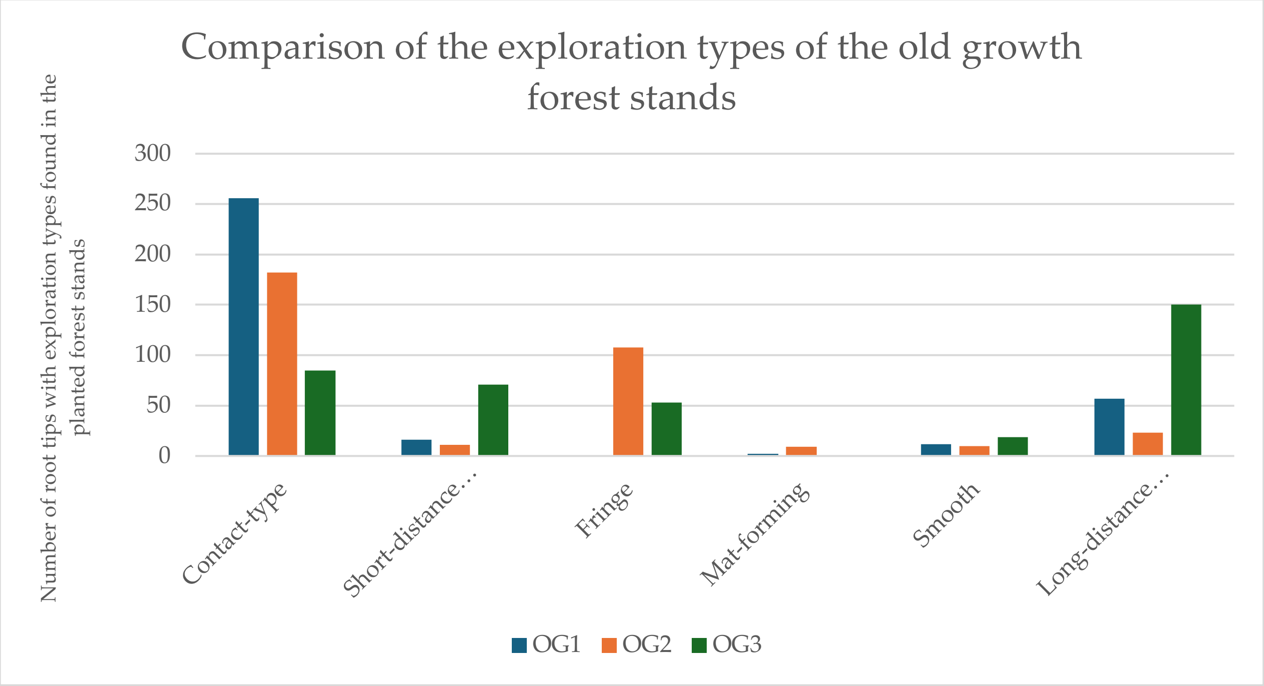

Figure 11. Comparison of the exploration types of the old-growth forest stands

Across all forest categories, C types were the most abundant. However, characterized by the highest overall abundances, P forests are primarily driven by C, LD, and smooth types. In contrast, while supporting fewer root tips overall, OG stands showed a relative increase in SD and fringe types and a reduction in LD exploration types. NR occupied an intermediate position, with a strong dominance of C and LD types but low representation of fringe and SD forms.

4.2 Exploration type distribution patterns

The analysis of exploration type distribution based on relative abundances provides a functional perspective on the EM community structure across the successional forest stages in the Cloudbridge Nature Reserve.

The relative abundances for the most prominent exploration types in each forest category are summarized below:

Exploration Type	P	NR	OG
Contact Type	40.9%	47.6%	49.3%*
Short-Distance Type	1.8%	1.3%	9.2%
Medium-Distance Fringe	6.0%	0.7%	15.1%
Medium-Distance Smooth	16.0%	12.3%	3.8%
Long-Distance Type	34.0%	37.2%	21.6%

Table 3. Relative abundance of exploration types

C and LD exploration types strongly dominated the EM community composition in P and NR forests. In P stands, C types accounted for 40.9% of all root tips, while LD types comprised 34.0%. NR showed a similar pattern, with a slightly stronger dominance of C types (47.6%) alongside 37.2% LD types. In contrast, OG stands displayed greater heterogeneity, increasing SD (9.2%) and fringe (15.1%) exploration types, while LD types decreased to 21.6%.

The Pielou's evenness indices further highlight the structural contrasts among forest categories. OG forests exhibited the highest evenness (J'= 0.76), indicating a more balanced distribution of exploration types. P stands showed a slightly lower value (J'= 0.74), while NR had the lowest evenness (J'= 0.61), reflecting a strong dominance of only a few strategies.

These patterns suggest functional shifts in EM strategies along the successional gradient. The communities were skewed towards simpler, dominance-driven structures in P and NR forests, while OG forests showed higher diversity and functional balance. The increase in SD and fringe types in OG stands highlights the emergence of more specialized foraging strategies, contrasting with the firm reliance on C and LD types in younger forests.

The observed distribution corresponds to morphological traits of EM mantles and emanating hyphae. C types dominate in P and NR stands and are characterized by dense, smooth mantles with few emanating hyphae, maintaining close contact with the substrate. In contrast, the higher evenness in OG stands is associated with more complex mantle morphologies, including SD types with localized hyphae and fringe types with differentiated hyphal cords. The reduction of LD exploration in OG forests corresponds to a decline in rhizomorph-forming structures, reflecting a shift towards more structurally diverse and functionally balanced EM communities.

4.3 Statistical results

This section presents the descriptive statistics and outcomes of the statistical analyses used to compare EM exploration type distribution patterns across the three forest successional categories investigated in the Cloudbridge Nature Reserve: P, NR, and OG stands.

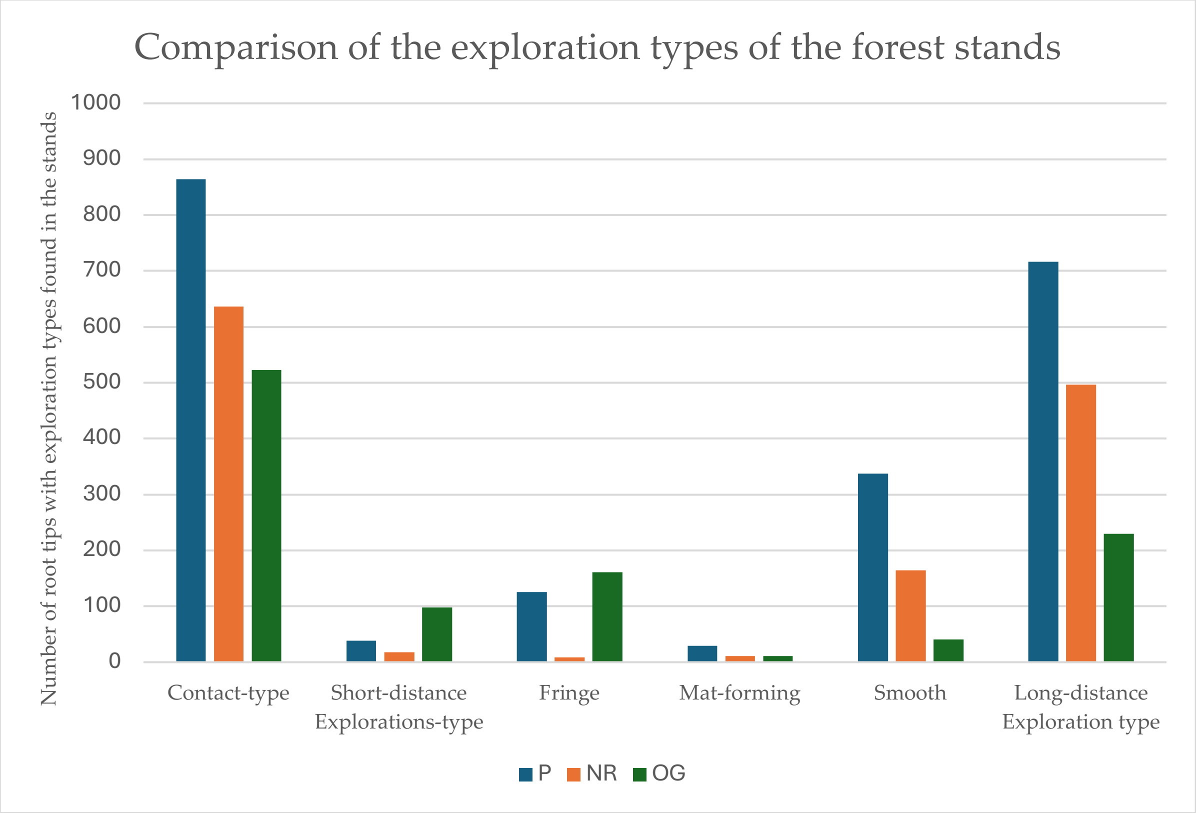

Figure 12. Comparison of the descriptive statistics of the forest stands

Preliminary trends are assessed by calculating descriptive statistics for each forest type. P forests recorded the highest mean abundance of EM exploration types (351.83), but also showed the widest variability, as indicated by the most significant standard deviation (360.44). NR areas presented a lower mean abundance (222.5), while OG forests exhibited the lowest mean value (177.33). Median, minimum, and maximum values further illustrate this variation, ranging from as low as 9 EM root tips in NR to a maximum of 864 tips in P stands (Tab. 3, Fig. 12).

Friedman Test (Omnibus Test)

$$Q = (12 / [N * k * (k + 1)]) * \Sigma(R\_j^2) - 3 * N * (k + 1)$$

A non-parametric Friedman test was applied to determine whether these differences were statistically significant. This approach was chosen because the data is not normally distributed and due to the repeated-measures nature of the study design. The test yielded a chi-square value of 4.08 with 2 degrees of freedom and a p-value of 0.13. Since the p-value exceeded the significance threshold ($\alpha = 0.05$), the Friedman test indicated no significant difference in the overall distribution of EM exploration types among the three forest categories.

Despite the lack of significance in the omnibus test, post-hoc analyses were conducted to examine potential differences between specific pairs of stands. The results were as follows:

Comparison	**Test Statistic**	**Std. Error**	**Std. Test Statistic**	**p-value**	**Adjusted p-value**
Total P - Total NR	1.08	0.58	1.88	0.061	**0.182**
Total P - Total OG	0.92	0.58	1.59	0.112	**0.337**
Total NR - Total OG	-0.17	0.58	-0.29	0.773	**1.000**

Table 4. Results of the post-hoc analyses

All adjusted p-values exceeded the significance level of 0.05, confirming that none of the pairwise differences were statistically significant.

5 Discussion

This chapter interprets and places the study's results into a broader ecological context. The section begins with evaluating the main findings, focusing on the diversity and distribution of EM exploration types across the forest categories at Cloudbridge, as already done in the research report. These patterns are then compared with results from other studies to assess how far the observed trends align with or diverge from established knowledge of EM functional ecology. The discussion further considers the ecological and practical implications of the findings, particularly about forest development, soil conditions, and conservation strategies. Finally, attention is given to the potential outlook for future research and forest management at Cloudbridge, highlighting the role of EM fungi in supporting restoration and long-term ecosystem stability.

5.1 Interpretation of results

The results from this study offer insight into the distribution and diversity of EM exploration types across different categories of forests within the Cloudbridge Nature Reserve. Although descriptive statistics suggested clear trends, particularly the higher mean abundance in P stands and the greater variability across categories, the statistical analyses did not support significant differences in EM exploration type distribution among the forest types. The large standard deviations within categories likely contributed to the absence of statistically significant results, emphasizing the high variability inherent in EM community structures across successional stages.

The Friedman test with a p-value of 0,13, higher than the decided significance level 0.05; therefore, the variation observed in the proportion of exploration type among the three forest categories may not be considered statistically significant. While there was no significant evidence of statistical differences in forest types, a few trends deserve more elaborate discussion. The differences among forest types point to ecophysiological adaptations of EM. The dominance of hydrophilic C types in plantations versus hydrophobic long-distance types in NR matches the spectrum of exploration types described by Suz et al. (2008). Increasing mantle complexity with age in OG stands (from plectenchymatous to pseudoparenchymatous) reflects the maturation of EM communities and confirms that forest age and soil development are crucial factors for functional diversity (Wu, 2013).

This diversity gives an interesting pattern in the distribution of EM exploration types across forest categories, supplemented with evenness values: 0,74 for P forests, 0,61 for NR areas, and 0,76 for OG forests. Together with the observed distribution of exploration types, these values provide a good insight into the ecology of EM fungi across different forest stages in this tropical mountain cloud forest ecosystem. In plantations, the codominance of C types (40,9%) and long-distance exploration types (34,0%) suggests a double adaptation to the environment of these younger, managed ecosystems. The dominance of C types, with their smooth mantle structures, can be an opportunistic response to readily available nutrients, perhaps induced by management practices like fertilization. Meanwhile, the large proportion of long-distance exploration types with well-differentiated rhizomorphs indicates a strategy for nutrient capture in more distant or depleted soil patches, which can occur even in fertilized, young forest ecosystems. These rhizomorphs are effective at transporting water and nutrients along extensive distances.

The evenness for planted forest is high at 0,74, showing that other exploration types are relatively evenly represented, perhaps due to management practices or the developing nature of these forests. Note that these planted areas are no older than 21 years since reforestation at Cloudbridge began in 2002. It is essential to mention that the numbers seen in plot P3 (see Fig. 9) were markedly different from the rest, probably because this plot is situated within a stand of diseased oak trees. Most likely, the trees have been infected with a fungus or fungus-like spores, which probably influence the EM community in this area. On top of that, oaks are known to be trees that are more likely to be in symbiosis with many fungi. These could be possible explanations for the noticeably high numbers in plot P3.

NR areas showed a similar pattern, but with a slight increase in C types (47,6%) and maintained presence of long-distance types (37,2%). These could reflect transitional stages in mantle development typical for regenerating ecosystems, for example, the pseudoparenchymatous mantle types. The low evenness value observed (here 0.61) suggests a transient stage in the development of the forest and its EM community. These represent the mix of active planting and natural regeneration approaches adopted at Cloudbridge. It is important to note that NR2 had a much lighter canopy in the first and second tree layers and showed many signs of peccary activity. Peccaries (*Tayassuidae*), which forage for roots, fungi, and other plant material, may influence the EM fungal community through their rooting activity and alteration of soil structure. This plot, compared to the rest when considering the diagrams, has a lower number of mycorrhizal associations, most likely due to increased light penetration and soil disturbance by wildlife, compared to the exploration types of the forest stands of NR.

While OG forests had the most even distribution of exploration types, SD and fringe types increased to 9,2% and 15,1%, respectively. Reduced LD exploration (21,6%) might indicate more efficient nutrient cycles in the OG forests, while an increase in hydrophilic SD types hints at well-developed organic soil layers (Schembre, 2009). The highest evenness value of 0.76 supports this diversity. It would thus suggest a more balanced distribution of the EM strategies in mature, stable ecosystems with complex soil structure and diverse microhabitats. No pattern could be observed when comparing the three plots in the OG forest stand.

One influencing factor in these observed patterns could be the fast plant growth, as some of the planted trees here at Cloudbridge have reached a height of 20 meters after only 20 years. These rapid growth patterns may affect the development of the communities of EM fungal communities and possibly explain some variation in exploration type between younger and older forested stands. The results follow the general research of EM diversity in different forest ecosystems. Estimates are that between 8,000 and 25,000 EM fungal species exist worldwide, with approximately a third of about 6,000 macrofungal species being EM in Switzerland. Generally, temperate and boreal forests have higher diversities of EM fungi than tropical forests, with up to 60% of trees in these regions forming EM associations. With an increase in the proportion of younger Douglas firs (< 50 years), the number of tubercles from *Rhizopogon vesiculosus* grew while the amount of its related genotypes, *R. vinicolor,* declined. More generally, this implies a differential impact on forest changes in a stand structure and regeneration among EM species.

5.2 Comparison with other studies

To contextualize the patterns observed at Cloudbridge and to evaluate whether the detected trends reflect broader ecological mechanisms, it is necessary to compare the findings of this study with existing research on EM exploration types. Previous work conducted in temperate, boreal, and Mediterranean forests provides a valuable framework for interpreting the Cloudbridge results, as these studies examine how exploration strategies respond to factors such as stand age, nutrient availability, soil structure, and climate. By integrating insights across these different systems, it becomes possible to determine whether the dominance of specific exploration types and the absence of strong statistical differences at Cloudbridge are consistent with global patterns or reflect ecological and methodological uniquenee of tropical cloud forests.

Across the reviewed literature, forest stand age frequently emerges as a major driver of EM exploration type distributions. Many temperate and boreal studies report clear successional patterns, with shifts in dominance from C and SD types in younger stands to more balanced communities in older forests. For example, findings from Rosinger et al. (2018), Wasyliw et al. (2020), and Defrenne et al. (2019) indicate that stand development can strongly influence EM functional traits, often increasing evenness and shaping the relative importance of LD types.

In contrast, the results from Cloudbridge showed no statistically significant differences in exploration type distribution across forest categories, although trends were visible. P stands displayed higher proportions of LD types, while OG stands showed increased evenness and slightly elevated SD and fringe types. These patterns resemble expectations from successional theory but lack strong statistical support. The difference between Cloudbridge and temperate studies likely reflects the more limited spatial scale, narrower environmental gradients, and reliance on purely morphological classification. So, while many temperate studies identify strong age-related structuring, the Cloudbridge dataset suggests that such patterns may be weaker or harder to detect in tropical cloud forests.

Nutrient dynamics are frequently linked to EM exploration strategies. Several studies, particularly from temperate and Mediterranean systems, associate the abundance of LD types with nutrient-poor conditions, while C and SD types tend to dominate in more balanced or nutrient-richer microsites. Defrenne et al. (2019) noted that soil fertility, precipitation, and temperature interact to filter EM traits across broad gradients. Similarly, Khalfallah et al. (2024) described depth-related nutrient partitioning that influenced the distribution of exploration types.

At Cloudbridge, soils are shallow, nutrient-poor, and highly leached, concentrating fungal activity in the upper humus layers. This may explain the strong dominance of C types and the modest increases in LD types in early-successional stands. However, because soils across the reserve are relatively uniform, the expected nutrient-driven differentiation may not be easily detectable, resulting in minimal statistical differences. The Cloudbridge patterns nonetheless align with the broader literature in showing that C and SD types represent effective strategies in nutrient-limited environments.

Many studies emphasize vertical gradients in EM communities. Courty et al. (2008) documented substantial differences between upper and deeper soil horizons, with strong C-type dominance in surface layers and functional differentiation at depth. Khalfallah et al. (2024) reported clearer vertical partitioning in Mediterranean soils, likely due to more pronounced soil horizons and nutrient differences.

In contrast, Cloudbridge data showed minimal vertical stratification, with almost no root tips recorded in the lower soil layer. This divergence likely reflects differences in soil profiles: tropical cloud forests typically have shallower organic layers and weaker horizon differentiation, reducing the potential for vertical niche partitioning. Methodological factors may also contribute, as studies combining morphological and molecular approaches tend to detect finer-scale patterns that purely morphological analyses cannot resolve.

Climate emerges as a key factor influencing exploration strategies. Temperate systems exhibit strong seasonality, which affects nutrient pulses, root activity, and fungal specialization. Studies such as Courty et al. (2008) and Rosinger et al. (2018) show that seasonal shifts can reinforce trait differentiation and niche partitioning.

Cloudbridge, by contrast, is characterized by year-round moisture and comparatively stable temperatures. These conditions likely favor more generalist strategies and may explain the reduced functional divergence among exploration types. The consistent dominance of C types across all forest categories fits with this interpretation and aligns with findings from tropical montane forests (e.g. Soethe et al. 2006), where continuous resource availability supports strategies with lower carbon costs and high efficiency.

Methodological differences among studies play a substantial role in shaping detectable patterns. Several studies, such as Defrenne et al. (2019), Suz et al. (2014), and Khalfallah et al. (2024), combine morphological and molecular techniques, enabling higher taxonomic resolution and more accurate detection of functional diversity. These approaches often reveal environmental filtering and vertical structuring that remain undetected when using morphology alone.

The Cloudbridge study relied solely on morphological identification, which may conceal subtler patterns in community composition and exploration strategies. The small spatial scale—nine plots within a single reserve—further limits the detection of environmental effects compared to studies spanning broader gradients.

Across ecosystems, the dominance of C types is a consistently reported pattern, and Cloudbridge aligns strongly with this global trend. However, many temperate and Mediterranean studies detect clear structuring by forest age, nutrient gradients, soil depth, and climatic seasonality—patterns that were weaker or not statistically significant in Cloudbridge. These differences likely reflect both ecological contrasts between tropical and temperate systems and methodological constraints.

Taken together, the comparisons support the null hypothesis of no significant differences among forest categories. Yet the observed trends—higher LD proportions in P stands and greater evenness in OG stands, suggest that underlying ecological mechanisms may still be operating but are less pronounced or more difficult to detect with morphological methods alone. The Cloudbridge dataset therefore provides an important tropical baseline that complements the predominantly temperate literature and underscores the value of integrative approaches for testing functional trait hypotheses in EM communities.

5.3 Possible future outlook for CB

Although the study provides a baseline for understanding EM exploration types in the Cloudbridge Nature Reserve, no significant statistical difference exists across forest categories. Still, the observed ecological trends point to important directions for future work. Building on these foundations requires long-term monitoring, methodological advancement, and practical applications in conservation and reforestation.

Continuation of Research and Long-Term Monitoring

This study reflects only a short temporal window, yet EM fungal communities and forest structure develop for decades. Long-term datasets are therefore crucial to link fungal dynamics with forest succession and tree development (Martin & van der Heijden, 2024). Monthly surveys at Cloudbridge, like in Courty et al. (2008), would allow for baseline refinement and may reveal temporal fluctuations not visible in a single-season study. Expanding the scope to additional zones within the reserve and cross-referencing results with other ecological studies would strengthen the understanding of the factors shaping EM community composition. Moreover, further investigation of the environmental trends observed here—such as the relative dominance of C versus LD types in P and NR stands—would help clarify the functional role of EM fungi in forest development (Defrenne et al., 2019).

Methodological and Technological Advancements

The current reliance on morphological identification provided essential insights and limited taxonomic resolution. For more comprehensive research in the future, studies at Cloudbridge should partly integrate morphological classification with molecular approaches such as DNA metabarcoding to resolve fungal diversity more precisely. This integration would allow researchers to confirm whether functional differences inferred from exploration types correspond to underlying species-level variation. Beyond taxonomy, trait-based and functional analyses—including enzymatic activity or isotopic studies—could test whether exploration types are reliable indicators of nutrient acquisition strategies in tropical soils. Still, those kinds of methodical research would not be possible at the research station at Cloudbridge (Cordts, 2021). Samples would have to be sent to laboratories, which would be a lot of effort and bring a lot of costs; therefore, it is unrealistic at this moment. Still, standardized methods and protocols could be introduced, enabling comparisons across sites and helping establish baselines for undisturbed tropical cloud forest soils, contributing to a better understanding of ecosystem resilience.

Conservation and Reforestation Applications

One of the goals of this line of research is to inform Cloudbridge’s reforestation strategies. Insights into EM diversity and function can guide the selection of tree species and their fungal partners to improve survival, growth, and ecosystem recovery. Results suggest that reforestation campaigns should prioritize diversity rather than monospecific stands, promoting stable EM communities. In addition, practical applications could involve testing which exploration types best support indigenous trees under nutrient-poor conditions, for example, *Quercus* species commonly planted in the reserve (Saunier, 2016). Linking EM communities directly to tree growth success will provide actionable knowledge for forest managers. Protection of the soil microbiome must also remain a priority, as it underpins both carbon cycling and forest resilience under changing climatic conditions (van der Heijden et al., 2015).

Integrating Climate and Educational Dimensions

Future work should also consider how EM communities influence forest resilience under pressures such as altered rainfall and temperature regimes, which are expected to impact montane cloud forests severely (Womack, n.d.)—understanding whether certain exploration types of buffer trees against drought or nutrient stress may prove crucial for adaptation strategies. Beyond science, Cloudbridge’s role as a conservation and education center offers the opportunity to translate findings into practical guidelines for volunteers, practitioners, and local communities, fostering greater awareness of the role of fungi in forest health.

Cloudbridge in the Global Context

Finally, the reserve’s unique environment is an essential reference point within global mycorrhizal research. Most existing exploration type studies originate from temperate regions, and tropical ecosystems remain underrepresented (Rosinger et al., 2018). By continuing and expanding this work, Cloudbridge can establish itself as a key site for filling these knowledge gaps, providing data directly relevant to tropical montane forests and comparing temperate systems. Filling this gap would support local conservation goals and contribute to the broader scientific effort to understand how EM fungi shape forest ecosystems worldwide.

Future outlooks for Cloudbridge center on consolidating the current baseline into a long-term dataset, applying modern molecular, and translating scientific insights into conservation practice (Cordts, 2021). By doing so, the reserve can strengthen its reforestation strategies, safeguard soil biodiversity, and position itself as a critical node in global research on EM ecology.

6 Conclusion

This study sought to investigate the distribution and diversity of EM exploration types across three forest categories, within the Cloudbridge Nature Reserve in Costa Rica. The primary aim was to address a critical knowledge gap in tropical mycorrhizal ecology, as most research on EM functional traits originates from temperate and boreal ecosystems. By establishing a baseline for tropical montane cloud forests, this thesis provides regional insight and a valuable reference point for comparative research.

The statistical analysis did not reveal significant differences in the proportion of EM exploration types across forest categories. The Friedman test ($p = 0.13$) supported the null hypothesis, indicating that any apparent variation in mean abundance among forest types cannot be considered statistically significant. High variability within categories, reflected by large standard deviations, likely contributed to these results.

Despite the lack of statistical significance, several notable trends were observed. C exploration types were consistently dominant across all stands, representing a conservative and efficient strategy for nutrient acquisition. P forests showed a codominance of C and LD types, suggesting dual strategies under early successional conditions. NR forests also exhibited high proportions of C types but lower evenness, reflecting transitional dynamics influenced by disturbance and canopy structure. OG forests demonstrated the highest evenness (0.76) and a more balanced representation of strategies, with increased SD and Fringe types and reduced LD representation, consistent with mature soil systems and stable ecological conditions. While some trends suggested adaptive shifts related to forest type and nutrient conditions, the data did not provide sufficient statistical evidence to confirm these expectations conclusively.

The results confirm that Cloudbridge's EM communities exhibit functional traits broadly consistent with global literature, particularly the dominance of C types across forest stages. However, the absence of clear stratification and the limited statistical support highlight differences from findings in temperate or Mediterranean systems. These divergences may reflect both the unique ecological conditions of tropical montane cloud forests and the methodological limitations of the present study, which relied on morphological classification within a relatively small sample size.

This thesis contributes an essential baseline dataset for tropical EM research. The distribution patterns and evenness values observed across forest stages underscore the importance of continued monitoring and more refined methodologies. Future studies at Cloudbridge should integrate morphological and molecular approaches, expand spatial and temporal coverage, and directly measure functional traits to capture the ecological dynamics of EM communities more accurately.

For conservation and reforestation, these findings reinforce the importance of fostering fungal diversity as part of forest recovery strategies. The dominance of generalist C types, combined with trends toward higher representation of LD forms in P stands, suggests that soil nutrient conditions and stand history influence fungal strategies. Such insights can inform reforestation campaigns, guiding the selection of tree species and their fungal partners to improve resilience and ecosystem function in this biodiversity hotspot.

In conclusion, although this study did not detect significant statistical differences among forest categories, it provides the groundwork for future research and management at Cloudbridge. By combining long-term monitoring, methodological advancements, and practical applications, the reserve can further develop its role as both a conservation site and a research platform for understanding the ecological significance of EM fungi in tropical montane forests

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Appendix

Glossary

Anastomosis	the process of fusion between branches of the same or different hyphae to produce a mycelial network (Milne, R. I., 2004)
Genets	the entire genetic individual, a group of physically separate but genetically identical offspring, called ramets, that all originated from one original ancestor via asexual reproduction (ScienceDirect, 2017)
Mycoheterotrophic	parasitic plant relationship where plants get all or part of their carbon from fungi, therefore stealing nutrients from trees connected to a mutualistic fungal network, rather than making their own food via photosynthesis (Kiers, E. T., & Hager, M. C. 2020)
Plectenchymatous	a tissue made of loosely interwoven, tangled hyphae where their thread-like nature is still somewhat visible, distinct from pseudoparenchyma, where hyphae are so tightly packed they look like true plant cells (DiMarino, E., 2008)
Polyphyletic	a group of organisms that includes species with different immediate common ancestors. These groups are formed based on similarities that are not inherited from a common ancestor (Fiveable, 2025)
Proliferation	rapid growth and increase in number or size of a fungus, which happens when the fungus extends its hyphae, produces spores, or forms dense networks of cells (mycelium). This process is important for the fungus to spread, break down nutrients, cause disease, or decompose organic matter (Sustainability Directory, 2024)
Pseudoparenchymatous	a tissue formed from a compact, interwoven mass of hyphae that looks like plant parenchyma (DiMarino, E., 2008)

Saprotrophic	Fungi that produce enzymes that help them break down tough plant materials like cellulose, hemicellulose, and pectin. Some can also break down lignin, a tough substance in wood. This helps recycle important nutrients, especially carbon and nitrogen, from dead plants and organic matter (RHS, 2025)

Figure a. Light microscope used for looking at the samples

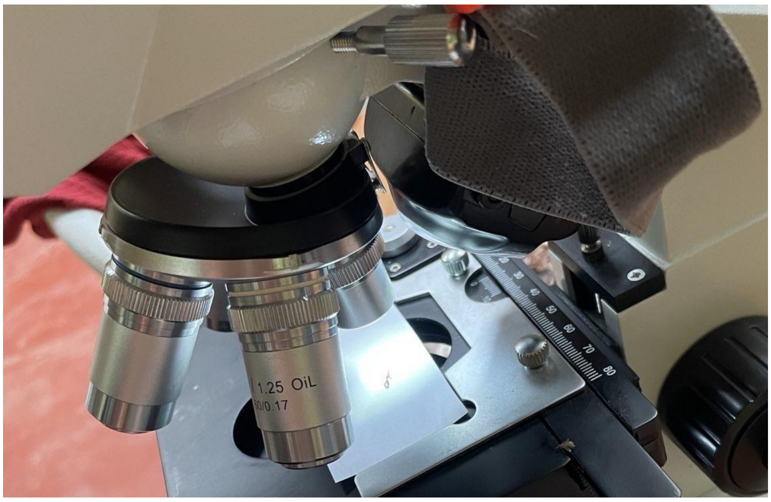

Figure b. Light microscope, adjusted with light from above

Figure c. Plot: Natural regrown 2

Figure d. Mushroom found at Cloudbridge

Figure e. Injury of a tree at Planted 3

Figure g. Tree on the Plot: Planted 3

Figure f. Fruiting body on Plot: Planted 3

Figure h. Soil layers on Plot: Old growth2

Figure i. Fruiting body of a mushroom on a tree at Cloudbridge

Figure j. Plot: Old Growth 2

Figure k. Plot: Natural Regrowth 3

Figure l. Middle point of Plot: Old Growth 1

Figure m. Fine root samples, distributed for counting

Figure n. Soil sample before washing

Figure o. Labeled bag of soil sample

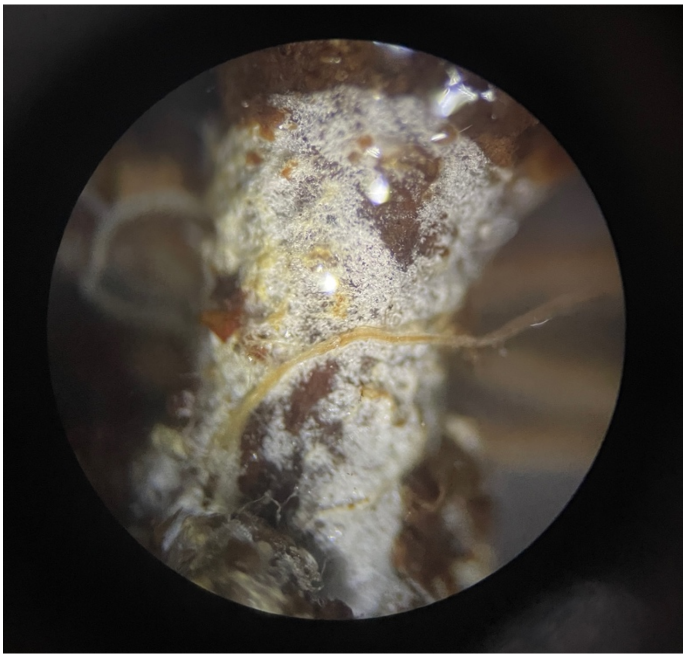

Figure p. Exploration-Type: Medium-Distance Smooth

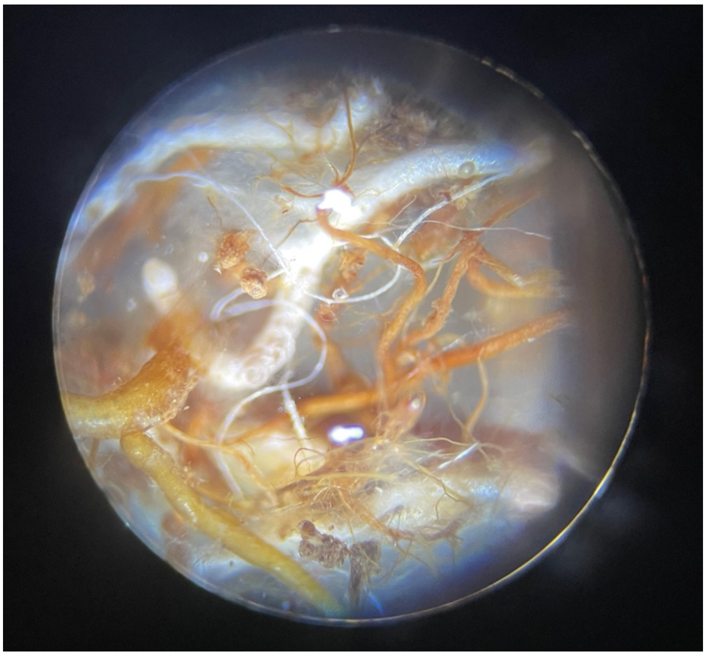

Figure q. Exploration-Type: Medium-Distance Fringe

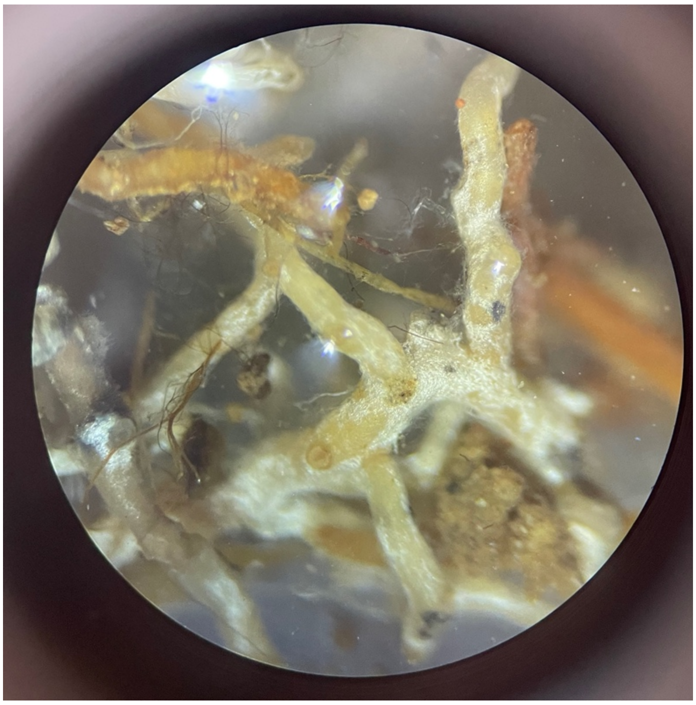

Figure r. Exploration-Type: Medium-Distance Smooth

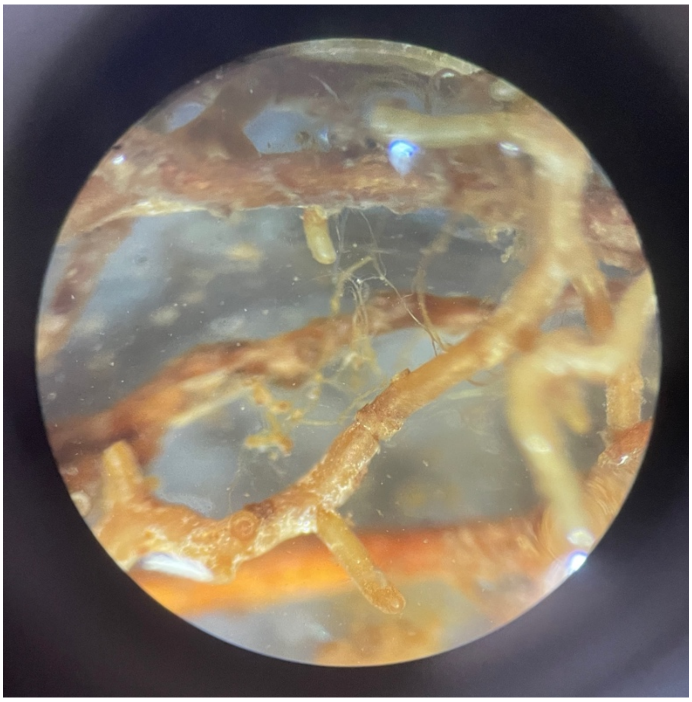

Figure s. Exploration-Type: Contact-Type

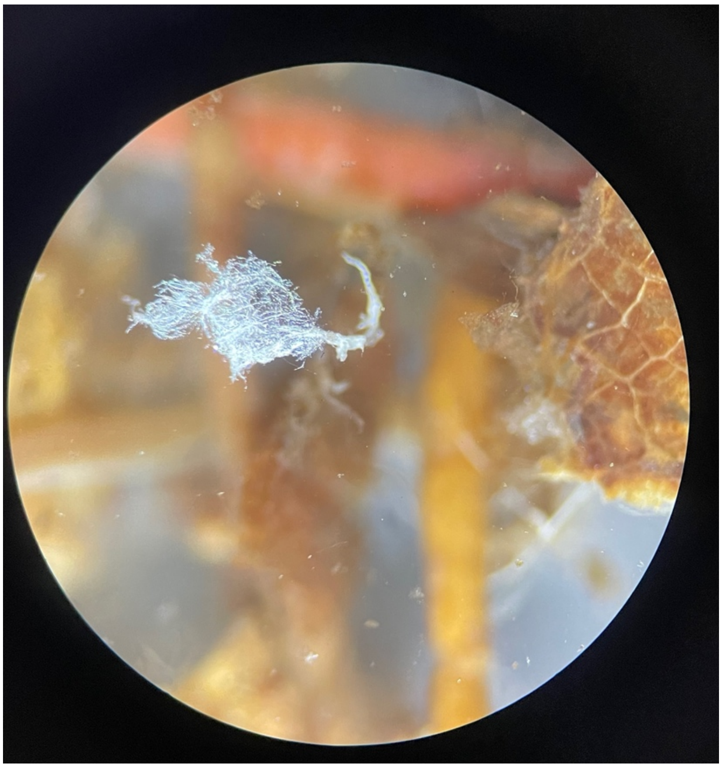

Figure t. Exploration-Type: Medium-Distance Mat-forming

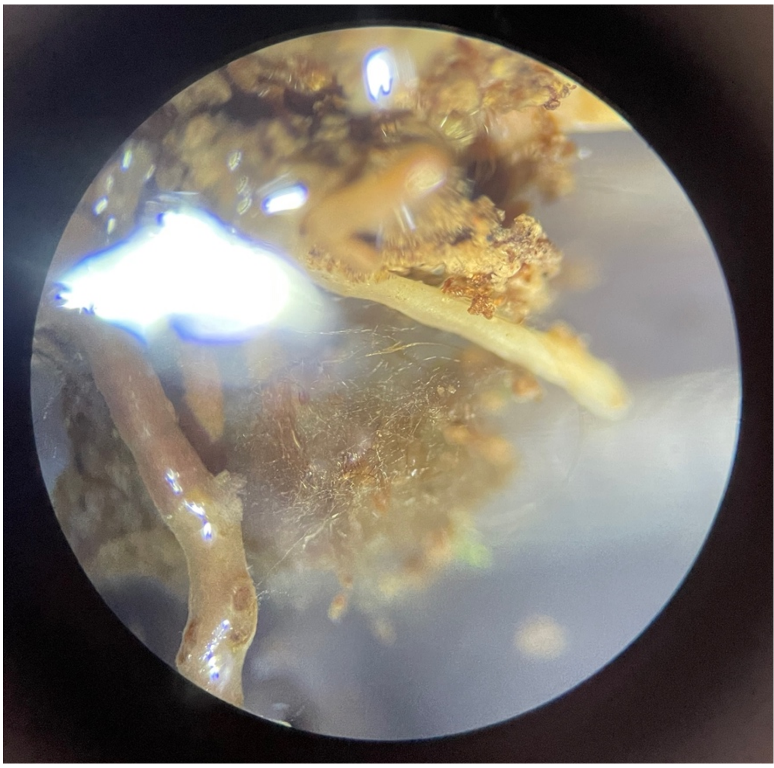

Figure u. Exploration-Type: Short-Distance

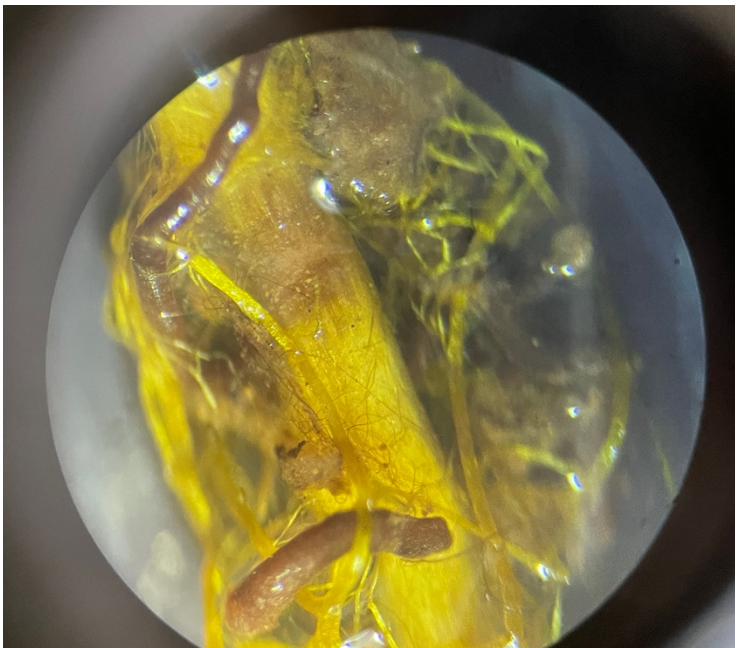

Figure v. Exploration-Type: Long-Distance Rhizomorphs

Figure w. Exploration-Type: Contact-Type

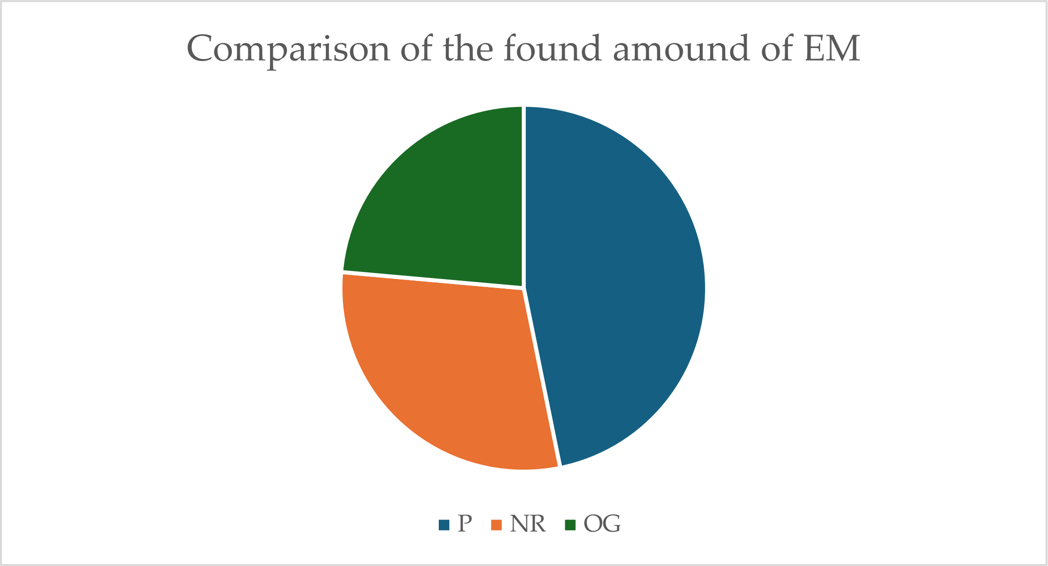

Figure x. Comparison of the found amount of EM

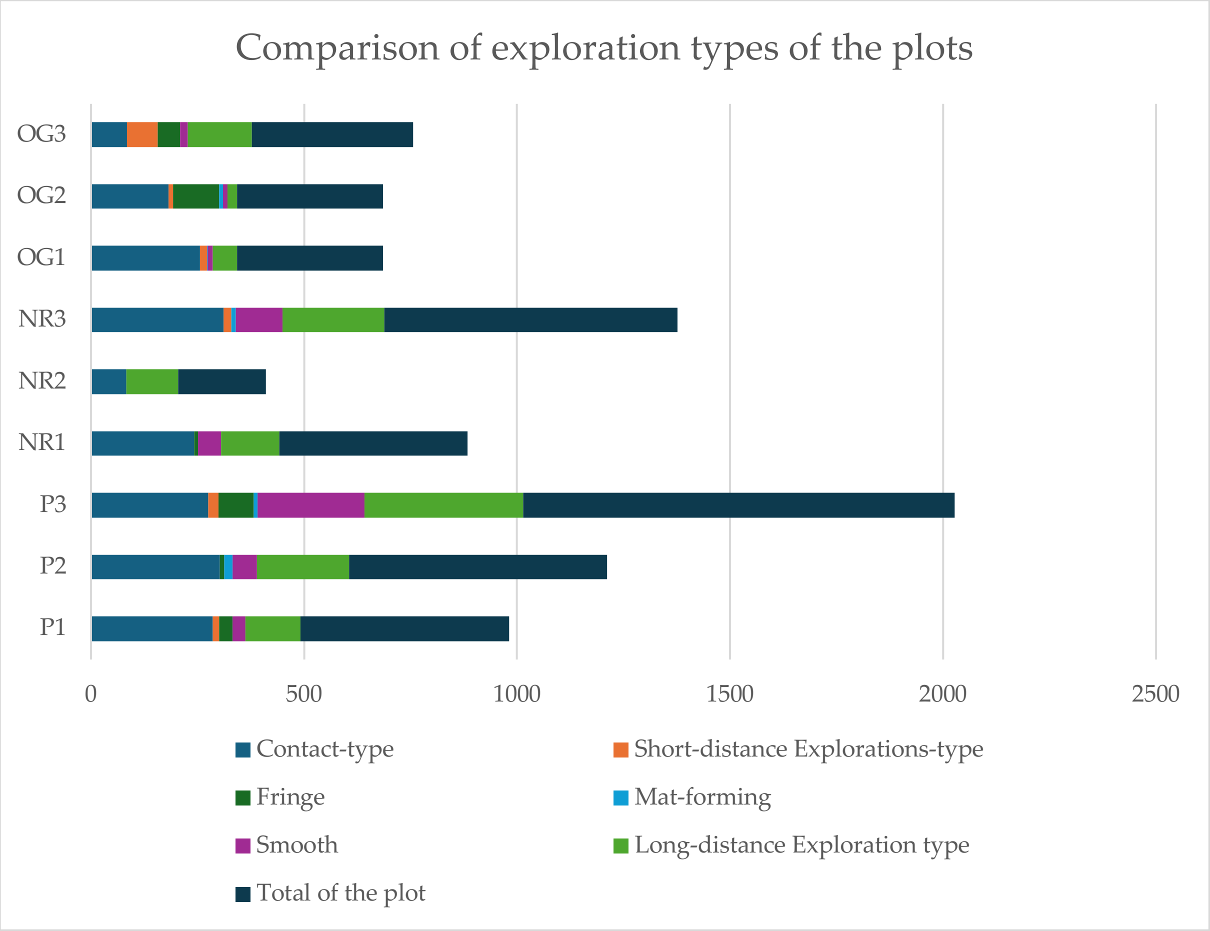

Figure y. Comparison of the exploration types of the plot

Plots		**Corner 1 Upper Layer**	**Corner 1 Loweer Layer**	**Corner 2 Upper Layer**	**Corner 2 Lower Layer**	**Corner 3 Upper Layer**	**Corner 3 Lower Layer**	**Corner 4 Upper Layer**	**Corner 4 Lower Layer**	**Middle-point Upper Layer**	**Middle-point Lower Layer**
Planted 1	Kontakt-Typ	56	2	30		45		89		63	1
	Short-distance Explorations-Typ		1			5		9			
	Fringe			7		7		11		7	
	Mat-bildende										
	Smooth			7				22			
	Long-distance Exploration Typ	14		35		6		55		19	
Planted 2	Kontakt-Typ	17		83		88		51		62	1
	Short-distance Explorations-Typ										
	Fringe	8								3	
	Mat-bildende					20					
	Smooth					57					
	Long-distance Exploration Typ	39		8		91		38		40	
Planted 3	Kontakt-Typ	23		55		84		69		45	

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	Short-distance Explorations-Typ	5		9		9					
	Fringe			36		18		11		18	
	Mat-bildende					9					
	Smooth	4		73		73		37		64	
	Long-distance Exploration Typ	14		85	2	92	2	100		77	
Natural Regenerated 1	Kontakt-Typ	56	3	93	19	9		36	4	22	
	Short-distance Explorations-Typ										
	Fringe							9			
	Mat-bildende										
	Smooth	23		27				5			
	Long-distance Exploration Typ	34		56				23		23	
Natural Regenerated 2	Kontakt-Typ	34			1	31		5		12	
	Short-distance Explorations-Typ										
	Fringe										

	Mat-bildende										
	Smooth							6			
	Long-distance Exploration Typ	26	1	22		28		9		35	1
Natural Regenerated 3	Kontakt-Typ	83	3	63		9		75	14	64	
	Short-distance Explorations-Typ	5		13							
	Fringe										
	Mat-bildende							11			
	Smooth	10		38		17				44	
	Long-distance Exploration Typ	43	2			20		83	15	76	
Old Groth 1	Kontakt-Typ	27		66	2	5		73		79	4
	Short-distance Explorations-Typ					16					
	Fringe										
	Mat-bildende	2									
	Smooth	3		2						7	
	Long-distance Exploration Typ	5		9		21		6		13	3

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Old Growth 2	Kontakt-Typ	66	6			43	17	24		24	2
	Short-distance Explorations-Typ		3	5	3						
	Fringe	23		42	2	12	13			16	
	Mat-bildende		3		6						
	Smooth	2						8			
	Long-distance Exploration Typ	7						11	5		
Old Growth 3	Kontakt-Typ	3				5		76			1
	Short-distance Explorations-Typ	5		13	12	15			14	12	
	Fringe			35		18					
	Mat-bildende					2					
	Smooth									19	
	Long-distance Exploration Typ	32	1	9	8	24	2	32	8	33	1

Table a. Data of the collected EM and exploration types from all the plots

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