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A comprehensive review on the concept and dynamics of earth's mycobiome: AMF networks and processes

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Abstract
The plant mycobiome, which associates with plants, plays a crucial role in enhancing ecological management and environmental conditions. Its effectiveness in maintaining ecological fitness is evident in several ways, including improving plant growth, exhibiting parasitism against harmful microorganisms, providing cross-protection, producing microbial compounds, competing with disease-causing microbes for nutrients, space, and colonization, and inducing systemic resistance against abiotic and biotic stresses. The plant-associated mycobiome, particularly plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), significantly contributes to plant growth and development by facilitating nutrient acquisition, promoting plant growth, producing siderophores, aiding in rhizoremediation, generating phytohormones, and enhancing abiotic and biotic stress tolerance. Recent advancements in genetic sequencing techniques have enabled the study of complex plant-AMF interaction networks in natural environments. Various molecular techniques, such as metagenomics, metatranscriptomics, metabolomics, and metaproteomics, provide a holistic understanding of microbial community structure, function, and dynamics. Additionally, imaging technologies, including dyeing methods, epifluorescence microscopy, vital and non-vital destructive staining techniques, and phosphatase assays, facilitate the visualization of AMF networks. However, research on plant-associated mycobiomes faces several challenges, such as sample complexity, limited cultivation, DNA extraction and sequencing bias, database and annotation limitations, as well as difficulties in understanding interactions, dynamics, and functional profiling.

Keywords: AMF networks, mycobiome, metagenomics, metatranscriptomics, metabolomics, metaproteomics

Introduction
The plant mycobiome, which interacts with plants, plays a crucial role in ecological management and adapting to environmental conditions (Finzi *et al*, 2011, Panago *et al*, 2017) [20, 41]. Mycobiome communities inhabit diverse natural environments, including soil, plant material, decaying wood, and water. Their composition is influenced by various factors such as the plant host, nutrient availability, environmental conditions, plant density, and interactions with external mycobiomes, including fungi and bacteria (Bahram *et al*, 2015, Nilsson *et al*, 2019) [6, 39]. The plant mycobiome comprises five main fungal groups: epiphytic, endophytic, saprotrophic, pathogenic, and mycorrhizal fungi (Porras-Alfaro and Bayman 2011, Beckers *et al*, 2016) [40, 7]. The fungal mycobiome associated with plants supports various functions, including carbon distribution, water acquisition, mineral conduction, interplant competition, and enhancing tolerance to both biotic and abiotic stresses (Bucher *et al*, 2009, Cai *et al*, 2013) [4, 10]. The application of diverse mycobiomes to plants enhances ecological fitness by promoting plant growth, acting as parasites against harmful microorganisms, providing cross-protection, producing microbial compounds, competing with disease-causing microbes for nutrients, space, and colonization, and inducing systemic resistance to both abiotic and biotic stresses (Hardoim *et al*, 2015, Cai *et al*, 2015) [27, 13]. Recent studies on rhizospheric mycobiomes suggest their potential as biofertilizers to enhance soil fertility. These mycobiomes contribute to plant growth, protect against stress by inducing systemic resistance, act as parasites against harmful microorganisms, exhibit antibiosis, and aid in rhizoremediation for plants (Hu *et al*, 2018) [28].

Arbuscular mycorrhizal fungi (AMF) form associations with approximately 75% of vascular plant species (Garces-Ruiz *et al*, 2019) [24]. They establish symbiotic relationships within terrestrial ecosystems (Brundrett and Tedersoo 2018) [8]. AMF form a symbiotic relationship with plants, where plants supply them with carbon derived from photosynthesis, while AMF, in return, provide essential nutrients like phosphorus and nitrogen for plant growth and development. Additionally, AMF enhance plant defense against pathogenic microbes and offer protection against abiotic stress (Delavaux *et al*, 2017) [16].

Agricultural Practices and Mycobiome Shifts: Technological Advances in Studying Mycobiome and AMF Networks

Agricultural practices play a crucial role in shaping the mycobiome, particularly the network of arbuscular mycorrhizal fungi (AMF) associated with plants. Advances in technology have enabled the study of these shifts through various approaches. Understanding the influence of agricultural practices on AMF networks is essential for sustainable farming. These technological advancements provide valuable insights for optimizing agricultural methods to improve plant health, enhance soil fertility, and boost crop productivity by leveraging the beneficial interactions between plants and AMF.

Multilayer Network Interactions Between Plants and Arbuscular Mycorrhizal Fungi

In two Mediterranean pine-oak forest regions of the southern Iberian Peninsula, researchers conducted a study on multilayer network interactions between plants and arbuscular mycorrhizal fungi (AMF), which share a symbiotic relationship. By analyzing these networks, they gained insights into plant community structure, dynamics, and the influence of AMF on plant interactions. To assess this impact, they developed a novel metric called Interlayer Node Neighborhood Integration (INNI) to explore the role of each node in shaping plant structure across different layers. The findings revealed variations in plant-AMF relationships between the two forest regions. Researchers also identified a specific AMF that facilitates plant-to-plant recruitment interactions, significantly influencing both functional and structural plant interactions. This has a profound effect on the recruitment network structure and overall plant dynamics in these forest ecosystems (Garrido *et al*, 2023) [25].

The Role of Mycorrhizal Fungi in Resource Allocation Across Multilayer Plant Networks

Mycorrhizal fungi play a crucial role in resource allocation by supplying host plants with essential nutrients that are often unevenly distributed in the soil. Arbuscular mycorrhizal fungi (AMF) facilitate the transfer of these scarce resources across multilayer networks, enabling plant-to-plant nutrient exchange. This interaction is mutually beneficial—AMF provide plants with vital nutrients, while plants offer AMF nutrition, space, and colonization opportunities. However, the exact mechanisms by which AMF distribute resources across plant networks remain largely unknown. To explore this, researchers developed a quantum dot tracking technique to observe phosphorus distribution through AMF networks. This method uses fluorescent nanoparticles of different colours to trace phosphorus movement, revealing where it is collected, transported, and allocated to host plants. AMF regulate resource distribution by identifying rich and poor nutrient patches within plant networks. They gather nutrients from resource-abundant areas and transport them to deficient regions where they are most needed. Additionally, researchers have employed single particle tracking and high-resolution video imaging to study how different AMF species allocate resources between plants, effectively counteracting resource inequality in the soil (Whiteside *et al*, 2019) [52].

Plant-AMF Interactions in the Rhizosphere

The interactions between host plant roots and arbuscular mycorrhizal fungi (AMF) in the rhizosphere significantly influence plant growth, soil health, and carbon dynamics. Within this zone, metabolite exchange and molecular mechanisms facilitate communication between plants and AMF. However, the precise process of this exchange remains a challenge to fully understand. To address this, researchers have developed a 3D root cartography platform to accurately observe the molecular and microbial components exchanged between host plant roots and AMF during their interactions in the rhizosphere. This innovative approach provides deeper insights into plant-AMF relationships, paving the way for the development of sustainable agricultural practices (Handakumbura *et al*, 2021) [29].

Reduction of AMF network complexity due to intensification of Agriculture

Researchers conducted studies on various farming systems, including organic farming (low agricultural intensification), conventional farming (moderate agricultural intensification), and no-tillage farming (high agricultural intensification), to determine whether increased agricultural intensification reduces AMF network complexity. They analyzed wheat-associated AMF samples from 60 farmlands using PacBio SMRT sequencing to assess the impact of different farming practices on AMF networks. The results revealed that organic farming supported the highest AMF network complexity with increased AMF colonization. In contrast, both conventional farming and no-tillage farming exhibited lower AMF network complexity and reduced AMF colonization, likely due to their higher levels of agricultural intensification. These findings suggest that agricultural intensification negatively affects AMF network complexity, potentially impacting soil health and plant-microbe interactions (Banerjee *et al*, 2019) [9].

Mycobiome analysis by using molecular techniques

Various molecular techniques are utilized to analyze mycobiomes, offering valuable insights into the diversity, composition, and functional potential of fungal communities. The integration of multiple molecular approaches with bioinformatics analyses enhances our understanding of mycobiomes, their dynamics, and their roles across different ecosystems, including agriculture and environmental processes. These advanced techniques help in studying the intricate interactions between fungal communities and their surroundings. The major molecular techniques used for mycobiome analysis are detailed in Table 1, which highlights the efficacy of various methods in examining plant-associated mycobiomes.

Metagenomics

Plant-associated mycobiome communities exhibit a highly complex diversity, which often results in lower genome coverage and poor assembly of specific fungal genomes (Sczyrba *et al*, 2017) [46]. This metagenomic strategy estimates the required sequencing depth needed to retrieve essential information for a target genome, in contrast to the data obtained from 16S rDNA amplicon sequencing (Peterson *et al*, 2021) [42]. The selection of a metagenomic dataset depends on the type of analysis being conducted, with the required sequences generated

accordingly. There are four major computational techniques used for metagenomic data analysis taxonomic binning, taxonomic profiling, target-gene reassembly, and genome binning. These methods help in classifying and reconstructing microbial genomes from complex environmental samples. Several publicly available plant-associated mycobiome metagenomic datasets, including ENA-SRA metagenomes, MG-RAST metagenomes, and IMG metagenomes, provide valuable resources for researchers to study fungal communities and their interactions with plants (Lucaciu *et al*, 2019) [33]. Metagenomic analysis is used to detect and analyze various plant-associated mycobiome communities in rhizospheric soil. In soybean cultivation, taproot decline disease, caused by *Xylaria necrophora*, leads to significant yield losses. To better understand the microbial dynamics associated with this disease, metagenomic techniques are employed to examine both fungal and bacterial mycobiomes in healthy and diseased soybean samples collected from the Mississippi Delta. This approach provides valuable insights into the microbial composition, aiding in disease management and sustainable agricultural practices (Popescu *et al*, 2022) [43].

Metatranscriptomics

Analyzing transcript sequences within a microbiome under specific conditions offers valuable insights into microbial activity and function. RNA-Seq is a powerful and widely used tool for transcriptome analysis due to its high sensitivity and ability to provide a comprehensive view of gene expression. The application of metatranscriptomics to study whole plant-associated mycobiome communities in the rhizosphere of *Arabidopsis thaliana* across various developmental stages presents a promising approach. Investigating how these mycobiome communities evolve and function throughout different growth stages can provide critical insights into their roles in plant health, nutrient cycling, and overall ecosystem functioning (Chaparro *et al*, 2014) [12]. Proper RNA-Seq data preprocessing is essential to ensure the reliability and accuracy of downstream analyses. Standard preprocessing steps, such as rRNA removal, trimming low-quality bases, and eliminating sequence tails, enhance sequencing data quality by focusing on mRNA transcripts while reducing biases from non-coding or contaminant RNA. In plant microbiome analysis, distinguishing between host RNA and microbial RNA is particularly important. A common approach involves mapping reads to a closely related reference plant genome or transcriptome, allowing researchers to filter out host-related sequences and focus specifically on mycobiome RNA. A read-based approach helps further classify these sequences by aligning reads to reference databases, such as the NCBI nonredundant protein database for mRNA and SILVA for rRNA. This method helps differentiate mRNA reads (representing protein-coding genes) from non-rRNA reads (potentially including mycobiome-derived mRNA) and rRNA reads (originating from both the host and mycobiome). Utilizing reference databases like NCBI and SILVA provides a valuable framework for annotating and characterizing sequences based on known genetic information. This enables researchers to assign functional roles to mycobiome taxa within the plant's ecosystem, leading to a deeper understanding of their contributions to plant health, nutrient cycling, and overall ecosystem function (Quast *et al*, 2012, Agarwala *et al*, 2014) [44, 3]. It is utilized in plant-associated mycobiome studies to analyze various rhizospheric mycobiomes associated with host plants such as wheat, oat, and pea (Turner *et al*, 2013 [48]. This analysis also aids in evaluating bacterial gene expression in relation to *Arabidopsis* development (Lambais *et al*, 2013) [32].

Metabolomics

Metabolomics is a comprehensive approach to studying small-molecule metabolites within a mycobiome system, providing a snapshot of these compounds under specific conditions. The metabolome consists of a diverse range of compounds, broadly classified into primary and secondary metabolites (Wink, 2003) [50]. Several advanced technologies are commonly used in metabolomics research, including nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS). Each of these techniques offers unique advantages, making them suitable for different analytical needs. NMR spectroscopy provides detailed structural information about metabolites by analyzing their nuclear magnetic properties. It is a non-destructive technique that enables the identification and quantification of metabolites in complex mixtures while also helping to determine molecular structures, chemical bonding, and molecular dynamics. GC-MS is particularly effective for analyzing volatile and thermally stable compounds by separating metabolites through gas chromatography and subsequently identifying them using mass spectrometry. This method is widely used for studying smaller, volatile molecules. In contrast, LC-MS is well-suited for a broader range of metabolites, including non-volatile and polar compounds. It utilizes liquid chromatography for separation, followed by mass spectrometry for precise identification and quantification. Together, these metabolomic techniques play a crucial role in understanding the chemical composition and functional dynamics of mycobiome systems (Emwas 2015) [19]. It is utilized for the detection and analysis of the soil mycobiome associated with the *Arabidopsis* plant, as this mycobiome influences root exudate composition throughout plant growth and development (Chaparro *et al*, 2013) [11]. Research has shown that plant-associated mycobiomes play a crucial role in enhancing the phyllosphere of plants, contributing to overall plant health and resilience (Ryffel *et al*, 2016) [45].

Metaproteomics

Metaproteomics is an emerging field within microbial ecology that focuses on characterizing the collective protein complement of a mycobiome community derived from environmental samples. This approach provides a direct insight into the functional dynamics of microbial communities and complements other omics strategies such as metagenomics and metatranscriptomics. Metaproteomics offers several advantages over other omics techniques by analyzing proteins, providing direct evidence of the functional capabilities of the mycobiome community. In addition to identifying proteins, it reveals post-translational modifications, protein-protein interactions, and protein turnover rates, offering valuable insights into metabolic pathways, cellular processes, and adaptive mechanisms employed by microbial communities in specific environments. The data obtained through metaproteomics helps uncover the metabolic activities of microbial taxa, their responses to environmental changes, and their interactions within the community. It is particularly useful in studying uncultured or poorly characterized mycobiomes, shedding light on their functional roles. The integration of metaproteomics with other omics approaches, such as metagenomics and metatranscriptomics, enables a more comprehensive understanding of mycobiome community structure, function, and dynamics (Hettich *et al*, 2013) [26]. In plant-associated mycobiome studies, metaproteomics plays a crucial role in

analyzing changes in plant root exudates influenced by bacterial mycobiomes, such as plant growth-promoting bacterium (*PGPB*) *Bacillus amyloliquefaciens* (Kierul *et al*, 2015) [31].

Table: Indicating the efficacy of various molecular techniques in plant associated mycobiome analysis.

S. No	Molecular techniques used for mycobiome analysis	Effectiveness of these techniques in mycobiome analysis	References
1	Metagenomics	It aids in estimating the sequencing required to retrieve the necessary information for the target genome of a plant-associated mycobiome.	Tamames *et al*., (2012) [47]. Ni *et al*., (2013) [38].
2	Metatranscriptomics	It allows researchers to assign functions and identify potential mycobiome taxa contributing to the transcriptome, providing deeper insights into the functional roles of the microbial community within the plant's ecosystem.	Agarwala, (2014) [3].
3	Metabolomics	It offers insights into the metabolic activities of plant-associated mycobiome communities across different environmental conditions.	Emwas, (2015) [19].
4	Metaproteomics	It provides insights into the functional and metabolic activities, including protein expression, of plant-associated mycobiome communities across diverse environmental conditions.	Hettich *et al*., (2013) [26].
5	Polymerase chain reaction (PCR)	For the purposes of sequencing and quantifying the targeted genome in plant-associated mycobiomes, it amplifies mycobiome DNA sections (such as ITS, LSU, and SSU).	Yang *et al*., (2018).
6	Next-generation sequencing (NGS)	It is an amplicon sequencing technique for high-throughput targeted DNA sequencing of plant-associated mycobiomes (e.g., Illumina MiSeq, Ion Torrent).	McCoy *et al*., (2019) [35].
7	Quantitative PCR (qPCR)	It aids in measuring the quantity of particular mycobiome taxa or active genes in the mycobiome linked to plants.	Bellemain *et al*., (2017) [5].
8	Fluorescence in situ hybridization (FISH)	It employs fluorescent probes to visualize and locate various mycobiome DNA within samples collected from their natural environment and inside plant tissues. This technique enables researchers to study the spatial distribution and organization of mycobiome communities, providing valuable insights into their interactions with plant hosts and surrounding ecosystems.	Wassermann *et al*., (2019) [51].
9	Single cell genomics (SCG)	It facilitates the genomic analysis of individual plant-associated mycobiomes, aiding in the understanding of genetic diversity within targeted unculturable mycobiomes.	Mauger *et al*., (2022) [37].
10	Stable isotope probing (SIP)	It assists in tracking the intake of substrates, enabling the identification of potential plant-associated mycobiomes. By analyzing substrate utilization patterns, researchers can gain insights into the metabolic activities and functional roles of these mycobiomes within plant ecosystems.	Cahanovitc *et al*., (2022) [14].

Imaging technologies are utilized for visualizing arbuscular mycorrhizal fungi (AMF) networks

Non-destructive methods are available for visualizing arbuscular mycorrhizal fungi (AMF) associated with the roots of higher plants without the need for staining. These methods include laser scanning and microscopy, which enable the observation of dynamic fungal processes and structures. One significant advantage of these techniques is that they allow the study of whole plants, and after the experiment, the plants can be re-sown to continue their growth. The simplest approach for identifying AMF is through light microscopy; however, detecting AMF structures clearly under a light microscope is challenging. In this method, only thin roots are examined without any additional processing, making it inadequate for detailed morphological studies and AMF quantification (Vierheilig *et al*, 2001) [49]. Table 2 provides an overview of various imaging technologies and their effectiveness in visualizing AMF networks.

Dyeing techniques for AMF networks

Neutral red is commonly used as a dye for visualizing arbuscular mycorrhizal fungi (AMF) associated with plant roots (Guttenberger 2000a, 2000b) [22, 23]. This staining process is based on the ion-trap mechanism. However, in some cases, higher plant roots and other fungi associated with the roots may also absorb the stain. Despite this, AMF structures can be easily distinguished from other stained fungi and plant roots. The duration required for staining depends on factors such as the complete penetration of the dye and the root diameter. Additionally, certain mechanically stressed roots may not absorb the dye effectively. It is also important to note that there is no conclusive evidence confirming that all arbuscules are stained using this method.

Epifluorescence microscopy for AMF network identification:

In 1982, Ames *et al* [1]. detected autofluorescent spots on roots colonized with arbuscules and proposed a technique to quantify AMF based on these autofluorescent spots. A thorough investigation comparing the number of autofluorescent arbuscules with the degree of arbuscular colonization in various plants after staining with acid fuchsin, chlorazol black E, and Trypan blue concluded that a higher number of arbuscules corresponded to stronger staining. The AMF structures observed under epifluorescence were identified as collapsed arbuscules. Fluorochromes are commonly used to observe fungal structures in contact with plant roots. Recent research has shown that treating tobacco leaves with fluorochromes such as 5(6)-carboxyfluorescein (CF) or 5(6)-carboxy-seminaphthorhodafluor enhances the visibility of all AM fungal forms, from intraradical hyphae to arbuscules. Notably, these findings were obtained from live roots, making this method a valuable tool for studying the *in vivo* dynamics of AM symbiosis. It allows researchers to track the establishment of the symbiosis, beginning with intraradical hyphal development and ending with the degradation of arbuscules. Although this method is relatively quick and effective for observing AMF in roots, it requires a laser-scanning confocal microscope and expensive fluorochromes, limiting its application to specialized research requiring *in vivo* observations.

Vital and non-vital destructive staining techniques

Staining techniques used in destructive approaches can be categorized as either vital or non-vital. Vital stains highlight metabolically active fungal tissues by targeting specific

enzymatic activities unique to fungi, while non-vital stains mark both viable and non-viable fungal structures. This method stains all active AMF structures, including hyphae, spores, vesicles, and arbuscules, except for compressed and clumped arbuscules, which do not retain the stain (Vierheilig *et al*, 2001) [49]. One commonly used enzymatic staining technique involves the tricarboxylic acid cycle enzyme succinate dehydrogenase (SDH), which reacts with nitro blue tetrazolium chloride (NBT) to form insoluble formazan, making it easily detectable in root tissues. To assess SDH activity, viable mycorrhizal roots are incubated overnight in an NBT-succinate solution at room temperature. The NBT solution consists of 2.5 ml of NBT (4 mg/ml), 5 ml of Tris–HCl buffer (0.2 M; pH 7.4), 1 ml of sodium succinate (2.5 M), 1 ml of $MgCl_2$ (5 mM), and 3 ml of distilled water. However, before staining, root tissues need to be cleared. MacDonald and Lewis (1978) [34] recommended boiling roots in a 75% (w/v) chloral hydrate solution for approximately 15 minutes to remove excess material, though this process can leave roots opaque. My findings suggest that roots stained with SDH and cleared solely with KOH become dark and nearly opaque. The best clearing results are achieved using a combination of chloral hydrate and KOH. The process involves boiling the roots for 15 minutes in 20% (w/v) chloral hydrate, followed by three washes with deionized water. The roots are then incubated overnight at 55°C in 5% (w/v) KOH, followed by three additional rinses. After clearing, the stained roots become visible under a standard optical microscope while submerged in water. This entire procedure takes approximately two days, including clearing and staining. A comprehensive study was conducted to compare various clearing techniques following SDH staining, providing additional insights into optimizing these methods.

Phosphatase assays for AMF networks

Short segments of roots or longitudinally sectioned roots are stained at sites of alkaline phosphatase (ALP) activity in AM fungal tissues, including hyphae and arbuscules, using either a fluorescent (Aarle *et al*, 2001) [2] or a non-fluorescent approach. The vacuolar compartments of arbuscules and intraradical hyphae are the primary locations of AM fungal ALP activity (Gianinazzi *et al*., 1979) [21]. In the fluorescence technique, freshly blotted roots are immediately placed into the reaction solution. The "ELF-97 Endogenous Phosphatase Detection Kit" (Molecular Probes, Eugene, OR) provides all necessary components. The staining solution is prepared by diluting an alkaline detection buffer (Solution B) with an enzyme-labeled fluorescent substrate (Solution A) at an optimal dilution of 20–30 times. Roots are stained for 30 minutes in the dark at room temperature, then washed with a Tris solution (30 mM Tris, 1.5 M NaCl, and 0.05% TritonX-100, pH 8.0). The stained root pieces are then mounted using the provided mounting medium. Samples are examined under an epifluorescence microscope with a 425-nm long-pass fluorescence emission filter and an excitation wavelength of 340–380 nm (UV light), revealing bright green fluorescent precipitates against a dark background. In the non-fluorescent method, roots must first be cleared using a solution containing 15 units/ml of cellulase and 15 units/ml of pectinase (both derived from *Aspergillus niger*), 0.05 M Tris/citric acid buffer (pH 9.2), and 0.05% sorbitol. Clearing occurs at room temperature for two hours, after which roots are rinsed in deionized water before being immersed in the staining solution. The staining solution consists of Fast Blue RR salt (1.0 mg/ml), α-naphthyl acid phosphate (1.0 mg/ml), $MgCl_2 \cdot 6H_2O$ (1.07 mg/ml), and $MnCl_2 \cdot 4H_2O$ (0.8 mg/ml), prepared in 0.05 M Tris buffer (pH 9.2). Roots are incubated in this solution overnight at room temperature, followed by thorough rinsing with water and a five-minute incubation in a 1% sodium hypochlorite solution. After a final rinse, roots are mounted and examined under a light microscope, where a deep purple precipitate marks sites of phosphatase activity. Both staining techniques effectively differentiate AM fungal structures from root tissues, though (Aarle *et al*, 2001) [2] found the ELF method to be more sensitive than the Fast Blue RR method. To optimize chemical use, staining can be performed directly on microscope slides. However, adjusting the ELF solution's pH to 4.8 is not recommended, as it leads to widespread precipitate formation throughout the roots, making it difficult to distinguish individual fungal structures.

Table 2: indicating Various Imaging technologies and their efficacy in visualization of AMF networks

Imaging Technology	Efficacy of Various imaging technologies in visualization of AMF networks	References
Confocal laser scanning microscopes (CLSM)	It allows high-resolution imaging of individual AMF structures and their networks from samples collected from plant roots and surrounding soil.	De Novais *et al*., (2020) [17].
Microcomputed Tomography (Micro-CT)	It generates 3D visual representations of individual AMF colonization and their networks from samples collected from plant roots.	Ivanov *et al*., (2019) [30].
Scanning Electron Microscopy (SEM)	It provides high-resolution surface imaging of diverse AMF structures and hyphal growth from samples collected in the rhizospheric zone.	De Jaeger *et al*., (2010) [15]
Fluorescence Microscopy	It facilitates the visualization of various individual AMF structures and their networks through the use of multiple fluorescent probes.	Morris *et al*., (2019) [36].
X-ray Microscopy	It provides non-destructive visual imaging of individual AMF networks from samples collected within plant roots.	Duncan *et al*., (2023) [18].

Conclusion

In this review we concluded on plant-associated mycobiomes and their networks faces several limitations, requiring methodological advancements and interdisciplinary collaboration across plant biology, microbiology, and ecology. Addressing these challenges necessitates improved databases, refined analytical techniques, and the integration of cutting-edge technologies. High-throughput sequencing, omics approaches, and advanced bioinformatics are essential in overcoming these barriers, enabling a deeper understanding of the structure, function, and ecological significance of plant-associated mycobiomes. These advancements will enhance our ability to unravel the complexities of plant-mycobiome interactions and their broader implications for plant health and ecosystem dynamics.

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