Establishing a Standardized Approach for Elucidating Glomeromycota Life-History Traits: Advancing Consistency in Mycorrhizal Fungi Research

Pedro Madeira Antunes[1], Sidney L. Stürmer[2], James D. Bever[3], Pierre-Luc Chagnon[4], V. Bala Chaudhary[5], Coline Deveautour[6], Catherine Fahey[1], Vasilis Kokkoris[7], Ylva Lekberg[8], Jeff R. Powell[9], Carlos A. Aguilar-Trigueros[10], and Haiyang ZHANG[9]

[1]Biology Department, Algoma University, Sault Ste. Marie, Ontario, Canada, P6A 2G4
[2]Departamento de Ciências Naturais, Universidade Regional de Blumenau, Blumenau, SC, 89030-903, Brazil
[3]Kansas Biological Survey and Center for Ecological Research and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, Kansas, USA
[4]Institut de Recherche en Biologie Vegetale, Universite de Montreal, 4101 Sherbrooke Est, Montreal, QC, H1X2B2, Canada
[5]Department of Environmental Studies, Dartmouth College, Hanover, New Hampshire, USA
[6]UniLaSalle, Rouen, France
[7]Amsterdam Institute for Life and Environment (A-LIFE), Faculty of Science, Section Systems Ecology, Vrije Universiteit Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, the Netherlands
[8]MPG Ranch & Department of Ecosystem and Conservation Sciences, W.A. Franke College of Forestry and Conservation, University of Montana, Missoula, Montana, USA
[9]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, New South Wales, Australia
[10]Institute of Biology, Freie Universität Berlin, Altensteinstrasse 6, 14195 Berlin, Germany.

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[1]Pedro M Antunes*, [2]Sidney L. Stürmer*, [3]James D. Bever,[4]Pierre-Luc Chagnon, [5]V. Bala Chaudhary, [10]Coline Deveautour,[1]Catherine Fahey, [6]Vasilis Kokkoris,[7]Ylva Lekberg, [8]Jeff R. Powell,[9]Carlos A. Aguilar-Trigueros,[8]Haiyang Zhang

[1]Biology Department, Algoma University, Sault Ste. Marie, Ontario, Canada, P6A 2G4

[2]Departamento de Ciências Naturais, Universidade Regional de Blumenau, Blumenau, SC, 89030-903, Brazil

[3]Kansas Biological Survey and Center for Ecological Research and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, Kansas, USA

[4]Institut de Recherche en Biologie Vegetale, Universite de Montreal, 4101 Sherbrooke Est, Montreal, QC, H1X2B2, Canada

[5]Department of Environmental Studies, Dartmouth College, Hanover, New Hampshire, USA

[6]Amsterdam Institute for Life and Environment (A-LIFE), Faculty of Science, Section Systems Ecology, Vrije Universiteit Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, the Netherlands

[7]MPG Ranch & Department of Ecosystem and Conservation Sciences, W.A. Franke College of Forestry and Conservation, University of Montana, Missoula, Montana, USA

[8]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, New South Wales, Australia

[9]Institute of Biology, Freie Universität Berlin, Altensteinstrasse 6, 14195 Berlin, Germany.

[10]UniLaSalle, Rouen, France

*Equal contribution

The problem

A trait is an attribute influencing an organism's performance within its environment, encompassing morphological, genetic and physiological characteristics measured at the individual or population levels (Salguero-Gómez *et al.* , 2018; Zhang *et al.* , 2023a). Understanding the ecology of species using a trait-based approach can contribute to a mechanistic explanation of processes mediated by microbes, including those that affect ecosystem functioning (Romero-Olivares *et al.* , 2021). This approach holds particular significance for arbuscular mycorrhizal (AM) fungi - Phylum Glomeromycota. As obligate symbionts of plants, where multiple species colonize both roots and soils in a network, predicting the functional outcomes (e.g., host growth, plant community diversity, soil characteristics) of individual AM fungal genotypes and communities within ecosystems remains challenging, despite major developments in molecular methods in the last two decades (Tisserant *et al.* , 2013; Montoliu-Nerin *et al.* , 2021). Indeed, establishing connections between AM fungal taxa and/or genotypes (e.g., within species) and their functional roles is a laborious process, which is expected to continue in the foreseeable future (Serghi *et al.* , 2021; Manley *et al.* , 2023). This is needed due to the complex links between AM fungi and functional outcomes for both hosts (*e.g.* , plant growth and fitness, nutrient uptake and stress tolerance) and soil functions/properties (*e.g.* , carbon storage, aggregate stability, biotic diversity), which appear to be highly context dependent and relatively poorly predicted by taxonomy alone (Munkvold *et al.* , 2004; Koch *et al.* , 2017; Yang *et al.* , 2017; Qiu *et al.* , 2021). However, this effort is also required because AM fungal traits have not been systematically assessed alongside with hypotheses of adaptation or with specific mechanisms in mind. For example, small-spored AM fungi may be dispersed longer distances by wind than large-spored AM fungi. It is then a reasonable hypothesis that small spore size is an adaptation for wind dispersal. One could empirically observe that small-spored AM fungi are geographically more widespread than large-spored fungi and this potential result could be viewed as evidence in support of this hypothesis. However, this finding would not necessarily prove that such dispersal difference has "functional" or "adaptive" value. Alternatively, producing small spores is a correlated response to producing many spores quickly, which itself could be an adaptive response to the likelihood of unpredictable soil disturbance such as from tillage. In this scenario, the adaptation and/or function is the production of many spores quickly to confer resistance to disturbance and then, after soil disturbance with wind erosion, small spores may also be blown farther (which may or may not improve fitness). Another example, variation in rooting depth among plants in a community may contribute to resource partitioning. But the mechanism (differential resource depletion with depth) still needs to be demonstrated separately from the trait evidence. AM fungi could contribute to equalize resource partitioning if plants with short roots associate with AM fungi that form more extensive extra-radical mycelium and vice-versa. Given these complexities, we consider the development of a robust, universally applicable trait-based framework for predicting key AM fungal functional outcomes a priority. To achieve this objective, first we must identify AM fungal traits that can be measured at morphological, physiological, and genetic levels. Second, considering the important roles of AM fungi in ecosystems, affecting host plants, soil, and the AM fungi themselves, we need to discern/hypothesize how measuring AM fungal traits impacts each of these components. For the host plant, it is crucial to consider nutrition, biomass, fitness, and survival in face of pathogens, heavy metals, salinity, drought, etc. (Delavaux *et al.* , 2017; Wehner*et al.*). Within the soil environment, AM fun-

gal effects on soil structure (Rillig & Mummey, 2006), nutrient cycling, carbon storage, and other members of the soil food-web are paramount (Antunes & Koyama, 2016; Frew *et al.* , 2021; Horsch *et al.* , 2023a). Regarding the fungal organism, we should focus on key aspects of their life-history strategies; reproduction and fitness, survival, dispersal, competitive ability, infectivity and abundance both within the host and soil environments (Aguilar-Trigueros *et al.* , 2019; Chaudhary*et al.* , 2020; Deveautour *et al.* , 2020). This requires identifying relevant proxies (sometimes termed "soft traits" in the plant ecophysiology literature) to provide easy-to-measure quantitative metrics for such complex facets of fungal life-history that can be measured across several species. Third, we need to evaluate existing standardized methods and experimental designs, or develop new ones, to measure such relevant (soft) traits, as has been done in plant ecophysiology (Pérez-Harguindeguy *et al.* , 2013). Measurement standardization and relevant metadata for hypothesis-driven analysis and interpretation is essential if we are to aggregate trait information from different studies into a public database, facilitating their incorporation into earth system models (e.g., (Fry *et al.* , 2019) and enhancing the predictability of functional processes and/or adaptations associated with AM fungi. Analogous libraries on plant traits (Kattge *et al.* , 2020) have proved useful to better understand trait variation along global climatic gradients (Butler*et al.* , 2017). Here, we aim to:

1. To comprehensively catalog and define AM fungal functional traits (morphological, physiological/phenological, and genetic) while avoiding redundancy.
2. To elucidate the relationships between these traits and their functional outcomes for host plants, soil environments, and the AM fungi themselves.
3. To critically review the historical methods and experimental designs employed in measuring AM fungal traits, highlighting their strengths and limitations.
4. To propose standardized methodologies and protocols for measuring AM fungal traits.
5. To explore the integration of AM fungal trait information into ecological models to enhance ecosystem processes' predictability.

Contextual basis

The scientific literature on the life-history traits of AM fungi (i.e., the biological characteristics and features that influence their growth, reproduction, and survival) has predominantly centered on aspects related to plant growth and nutrition, largely through an agronomic lens. Although not explicitly reported as such, early studies employing experimental approaches to assess, for example, AM fungal root colonization, abundance of external hyphae, and spore counts for specific species under certain experimental conditions have yielded insights into AM fungal trait variation (Abbott, 1982; Reich, 1988; Jakobsen *et al.* , 1992a; Gazey *et al.* , 1992; Bever *et al.* , 1996). Given the wide variation observed, these and other seminal studies provided a foundation for further inquiry into the complex dynamics of AM fungal life-history traits and their broader implications to the AM symbiosis.

Studies of distinct traits within a taxonomic framework started with the comparison of mycelium form and function, and root colonization strategies among major families of the Glomeromycota. For example, (Dodd*et al.* , 2000) compared the morphology and mycelial architecture of different AM fungal genera, discussing form and function, (Hart & Reader, 2002b) showed in a comparative study of 21 AM fungal isolates (i.e., an AM fungus isolated in the laboratory into pure culture but without genetic characterization, at which point it becomes a certified strain with a collection number) spanning 16 species from North America that the isolates of the Glomeraceae family, on average, colonized roots before those of Acaulosporaceae and Gigasporaceae families. Additionally, the proportion of fungal biomass in roots versus soil also diverged, on average, among isolates of those families. Those in the Glomeraceae exhibited high root colonization but low soil colonization, Gigasporaceae tended to have low root colonization but high soil colonization, while Acaulosporaceae displayed low colonization in both roots and soil. These findings revealed a strong association between AM fungal function and taxonomy for these fungi, as isolates from the main families could be differentiated based on colonization rate, biomass allocation, and the onset of sporulation. These observations were corroborated by subsequent studies, albeit using AM fungi from the same community and, possibly, the same isolations (Hart & Reader, 2002b, 2005; Maherali & Klironomos, 2007; Powell *et al.* ,

2009; Sikes*et al.* , 2009). In fact, using the same data, Aguilar-Trigueros et al. 2019, showed that large-spore species produced, on average, fewer spores than small-spore species, suggesting that AM fungi experience similar resource allocation constraints during reproduction as plants seeds (Moles *et al.* , 2005). Results from these studies suggest differences between Glomeraceae and Gigasporaceae concerning life-history traits and their relationship with host benefits. However, new comparative studies that include more fungal species isolated from other ecological contexts are necessary to confirm these differences.

The patterns described above underscore the utility of employing a comparative framework to test hypotheses concerning AM fungal function by examining trait expression. For instance, based on soil mycelium production, Gigasporaceae would be expected to outperform Glomeraceae in nutrient uptake (Maherali and Klironomos, 2007). Alternatively, if early or extensive root colonization (with abundant coils/arbuscules) is more important for nutrient delivery to the host, then Glomeraceae could be more beneficial partners under nutrient limiting conditions (e.g., (Horsch *et al.* , 2023b). Despite inconsistencies among studies, which may to some extent be explained by variability in mycorrhizal dependency among hosts (Pringle & Bever, 2008; Sikes *et al.* , 2009), a meta-analysis (Yang *et al.* , 2017) suggested that, on average, Glomeraceae were better at acquiring P and reducing pathogen growth compared to other AM fungal families. Is it also of interest, that this family also appears to be the most abundant in many locations (Öpik *et al.* , 2010).

The preceding studies lacked a broader environmental perspective. For instance, considering diverse environmental conditions, such as varying soil types or climatic factors, could unveil how AM fungal traits respond and adapt. Currently, most data reporting the impact of different AM fungi on their host originate from short-term experiments, using fungal taxa that readily sporulate and are easily amenable to pure cultures (Ohsowski *et al.* , 2014) which may not reflect the reality in natural environments. The study by Sikes et al. (2009) investigating differences in plant pathogen protection between AM fungal taxa, as well as that of (Lerat *et al.* , 2003) on C-sink strength among different AM fungal families suggests that certain functional outcomes resulting from the symbiosis depend on the combination of plant and fungal traits (Johnson *et al.* , 1997). As such, considering fungal traits alone (i.e., in absence of plant and soil characteristics) may limit predictions of functional outcomes of the symbiosis. This brings an additional layer of complexity to the study of AM fungal ecophysiology, or trait-based ecology, as intricate relationships between fungal and plant traits are to be expected (Chagnon *et al.* , 2013).

Proposed trait-based frameworks for AM fungi

(Van Der Heijden & Scheublin, 2007) conducted the first comprehensive review on AM fungal traits to predict plant growth and ecosystem functioning. The authors provided a list of 13 AM fungal functional traits, which they categorized into morphological traits (*e.g.,*hyphal length, rate and extent of root colonization, spore production) and physiological traits (*e.g.* , fungal carbon acquisition, host preference, nutrient uptake efficiency). Subsequently, (Behm & Kiers, 2014) noted substantial intraspecific trait variation among AM fungal species (also see (Koch *et al.* , 2017)), complicating the characterization of traits and their incorporation into functional trait models. To address this issue, they proposed a five-part framework for characterizing intraspecific trait variation of AM fungal species within the context of nutrient cycling, based on experimental design and trait measurement considerations. According to Behm and Kiers (2014), AM fungal genetic units should be subjected to diverse environmental conditions (e.g., host plants, soil nutrient concentrations). Subsequent measurements would encompass the degree of variation, trait reversibility, relationships among traits, the adaptive nature of variation, and the potential for variation to evolve. Through these five dimensions, researchers could map traits onto an evolutionary tree and incorporate them into functional models for predicting nutrient cycling dynamics.

Chaudhary et al., (2022) highlighted the challenges in defining traits for organismal networks such as the mycorrhizal symbiosis. They proposed a unified trait framework, complemented by a standardized vocabulary, with the objective of establishing a clear connection between trait-based mycorrhizal ecology, AM fungal niches and community assembly rules. The authors categorized traits into three main groups: morphological, physiological, and phenological. Within each of these categories, they pinpointed distinctive mycorrhizal traits specific to both the host plant (such as root:shoot ratio, growth form, photosynthetic pathways) and

the fungal partner (e.g., spore size, hyphal length, and melanin content). Beyond these bifurcated traits by plant or fungal traits, (Chaudhary *et al.* , 2022) introduced the concept of mycorrhizal traits. These are unique attributes that emerge during symbiosis and are co-dependent on both partners. They encompass aspects such as root colonization-induced structures, plant mycorrhizal response, and resource exchange rates. This novel framework provides an enriched understanding of mycorrhizal ecology and serves as a basis for the empirical framework proposed here.

(Chagnon *et al.* , 2013) put forth an AM fungal trait-based framework building on Grime's CSR (competitive, stress-tolerant, ruderal) framework - which identifies stress, disturbance and competition as the major filters driving trait selection and evolution in natural communities. By allowing speculative connections to be made regarding potential linkages between fungal traits (e.g., hyphal fusion, sporulation phenology, carbon sink strength, growth rates, etc.) and environmental filters (e.g., soil disturbances, scarce C transfer from host, low soil pH), this framework could tentatively identify priority traits for measurement, and combinations of host and fungal traits that may lead to the highest mutual benefits. Building on the apparent family-level conservatism of many traits or responses to environmental filters, parallels were drawn between AM fungal major families and C, S and R strategies. However, as stressed by Chagnon et al. (2013), this family-to-strategy association is simplistic and unlikely to stand the test of time. In addition, it several AM fungal families (e.g., Pacisporaceae, Entrophosporaceae, Diversisporaceae, or more basal lineages in the Paraglomerales or the Archaeosporales). It also fails to consider the relative distribution of different AM fungal families in certain biomes or at certain latitudes. For example, *Acaulospora*is a common genus in the tropics, where it can be dominant both in natural forests and under intensive land-use where ruderal traits are crucial (e.g., (González-Cortés *et al.* , 2012). The primary significance of the CSR framework in AM fungal trait-based ecology should not be considered as merely a framework for associating families with strategies. Instead, it should be recognized as a tool for leveraging well-established life-history trade-offs in plant ecology to pinpoint pertinent fungal traits that should be incorporated into our research agenda.

We build upon prior frameworks, emphasizing that the discrepancies observed among studies, stemming from non-standardized experimental approaches and the absence of a comprehensive database on AM fungal traits, represent significant barriers to achieving a more predictive understanding of AM fungal ecology. Moreover, the validity and relevance of the isolates and species employed in these studies are reliant on the taxa available in culture collections or from a few natural communities. A deliberate inclusion of numerous uncultured taxa, or other taxa hitherto overlooked fungal mutualisms in conjunction with AM fungi—such as Mucoromycotina, as suggested by (Hoysted *et al.* , 2023) —remains an important task. Given the existing data showing large variability in plant and soil responses to the AM symbiosis both among and within AM fungal species, we must address these issues to assess if, and to what extent, AM fungal traits determine growth responses or effects on ecosystems.

Traits and Function

Morphological and physiological traits

Arbuscular mycorrhizal fungal traits, including for example hyphal length, arbuscule morphology, or the robustness of spore walls, can modulate key functions/processes with ramifications not only to the health of the fungus itself but also the associated plant and the soil environment (see Table 1 for detailed descriptions of key traits, their hypothesized function, and methods for trait measurement). Here we define AM fungal traits primarily as "functional markers", which are indicators of mycorrhizal function and are dependent on the morphological, physiological, or phenological characteristics of the fungal partner (Chaudhary *et al.* , 2022). In this context, AM fungal traits are most likely instrumental in defining ecosystem resilience and adaptability to environmental fluctuations, as certain fungal isolates with specific traits may demonstrate superior robustness or flexibility under changing conditions.

Conceptualizing the form and function of AM fungal traits becomes clearer when contextualized within the lifecycle of the fungal organism. We can broadly categorize the lifecycle of an AM fungus into two phases: (1) the asymbiotic phase, in which the dispersed spores are activated, germinate and explore the soil for a

compatible host, and (2) the symbiotic phase, which includes the four following stages: a) the initiation of root colonization; b) the formation of structures within the root cortex; 3) the extension of mycelium into the soil matrix and possibly other hosts; and (4) spore production and dispersal. Briefly, spores, hyphal networks, and colonized root fragments, identified as the three principal types of propagules, remain dormant until the proper abiotic/biotic conditions emerge (Lanfranco *et al.* , 2018). Hyphae emerging from these propagules identify a host root, adhere to its surface, and commence root colonization. A swollen hyphopodium forms subsequently, from which a single hypha penetrates the root epidermis to access the root cortex. A series of morphogenetic and molecular processes come into play at these initial stages, enabling the plant to identify the presence of the fungus (as reviewed by (Bonfante & Perotto, 1995; Gianinazzi-Pearson *et al.* , 2007; Bonfante & Genre, 2010; Luginbuehl & Oldroyd, 2017). Upon reaching the root cortex, the fungus colonizes intercellular spaces, forming the intraradical mycelium (IRM). This mycelium then differentiates into structures such as arbuscules or coils, and, in some taxa, vesicles/ spores and the extraradical mycelium (ERM) which consists of runner hyphae, branched absorbing structures (BAS), spore associated BAS, and "asexual" spores. Upon attaining a certain threshold of root colonization, hyphae extend beyond the root system into the soil matrix, forming the extraradical mycelium (ERM) and generating asexual spores. The expansive hyphal network, comprising IRM and ERM and spores, embodies the traits that underpin several ecosystem-level processes attributed to AM fungi. These traits impact not just the host plants and soil environment, but also the fungal organism itself.

AM fungal spores

Arbuscular mycorrhizal fungal spores are among the largest (Aguilar-Trigueros *et al.* , 2023) and most multinucleated spores (Kokkoris et al., 2020) known in the kingdom Fungi and exhibit the phenotypic characteristics that enable species identification. Three types of spore formation are recognized (Walker *et al.* , 2018). Glomoid spores are formed blastically at the tip of a hyphae or by intercalary inflation of a hypha. Acaulosporoid spores involve the blastic formation of a sporiferous saccule with a neck, followed by the differentiation of spores laterally or inside the neck, or inside the sporiferous saccule. Gigasporoid spores are differentiated at the tip of a small bulb or suspensor cell.

Spores range widely in their traits including size, shape, color, and wall thickness (see (Morton, 1988) for a review) across and within species. In fact, single isolates of some species are known to produce more than one type of spores (even the model fungus, *Rhizophagus irregularis* (Kokkoris *et al.* , 2023)). It has been reported that spores can be produced singly in the soil, in loose aggregates and in small or large sporocarps. The cytoplasm of the spores contains not only the nuclei (ranging from hundreds to thousands) but also lipid reserves that assist the germination and early colonization. Based on spore ontogeny, three main phenotypic characters are observed in AM fungal spores: spore wall, germinal walls, and germination structure - with the latter two not present in many species. In addition, the spore walls of many species show appendages and indentations. Some AM fungi produce sporocarps (i.e., aggregations of spores), which are called "glomerocarps" (Jobim *et al.* , 2019). Sporocarps have functions in reproduction (Yamato *et al.* , 2022) and dispersal, including dispersal by mammals (Mangan & Adler, 2002). Overall, variation in spore traits across species are hypothesized to reflect differences in reproduction (investing in fewer larger spores or many small spores), dispersal (long or short, different dispersal vector), survival in the absence of the host (e.g., resistance to desiccation and pathogenesis) and early colonization strategies (Chaudhary *et al.* , 2018).

Intraradical mycelium (IRM)

The AM fungal mycelial system colonizes two distinct environments: the IRM grows inside the roots in a very constant environment while the ERM grows in the soil under highly variable environmental conditions (Smith & Read, 2008). Two broad anatomical groups of IRM can be recognized in mycorrhizal roots , the *Arum* -type, dominated by arbuscules, and the *Paris*- type, dominated by coils, although evidence suggests a continuum between these types, depending on the host plant and the fungus (Dickson, 2004). Presence of 'H' branches in the IRM is more common in Glomeraceae taxa compared to Acaulosporaceae, while looping hyphae and hyphae with small bumped projections are prevalent in species forming gigasporoid spores (see (Dodd *et al.* , 2000)for a review). Arbuscules are highly branched structures with a turnover rate ranging

from 7 to 16 days (Alexander *et al.* , 1989) or longer in woody plants (Brundrett & Kendrick, 1990), and they serve as the primary site of nutrient exchange between the fungus and the host. The main differences observed in arbuscule architecture relate to branching patterns. In Gigasporaceae, the trunk is wide and branching is abrupt, whereas the trunk is narrow and branching is gradual in Acaulosporaceae and Glomeraceae. Vesicles are thick-walled, globose to lobed structures that store lipids and contain many nuclei (Smith & Read, 2008). They are not formed by members of Gigasporaceae, and there is some evidence the same is true for basal families such as Ambisporaceae, Archaeosporaceae, and Paraglomeraceae.

Extraradical mycelium (ERM)

The extraradical mycelium (ERM) is composed of two types of hyphae: unbranched runner hyphae, which run parallel to the root length to initiate secondary colonization, and highly branched absorptive hyphae responsible for nutrient uptake from the soil and subsequent translocation to the host (Friese & Allen, 1991). (Bago *et al.* , 1998a) and Dodd et al. (2000) observed the formation of 'branched absorbing structures' - small groups of dichotomous hyphae - within the ERM in species of Glomeraceae. The ERM is also accountable for the formation of spores and auxiliary cells in the soil. Phenotypic variables associated with the ERM, such as hyphal length and density, interconnectedness, and hyphal diameter, have been studied in some AM fungal species (Dodd *et al.* , 2000; Avio *et al.* , 2006). The extraradical mycelium can form Common Mycorrhizal Networks (CMNs), where a single AM fungus associates with multiple plant hosts, interconnecting them within a shared mycelial network. These networks may serve as biological bridges, facilitating resource exchange and, possibly, communication among different plant hosts (Barto *et al.* , 2011; Babikova *et al.* , 2013). However, more research is needed to determine the role of CMNs in natural environments and to what extent the capacity to form CMNs can be considered a fungal trait (Karst*et al.* , 2023).

Genetic traits

In this section we define genetic measurements that have been proven or have the potential to reflect differences in life history strategies as "genetic traits" and we discuss how they can fit in a trait-based framework. Among these traits are the genetic organization of AM fungal strains, the spore nuclear content, the genome size, and the GC content of the genome. Recent findings demonstrated that AM fungal strains belonging to one species carry thousands of nuclei in their coenocytic mycelia that either belong to one (i.e., homokaryotic strains) or two nuclear genotypes (i.e., dikaryotic strains (Ropars *et al.* , 2016) with each of these genotypes having unique structure, genetic content and epigenetics (Sperschneider *et al.* , 2023). Interestingly, the relative abundance of the coexisting genotypes in the dikaryotic strains appears to be deterministic and their regulation to be responsive to biotic (e.g., plant host identity) (Kokkoris *et al.* , 2021) and abiotic factors (e.g., pH, temperature, nutrient content) (Cornell*et al.* , 2022). Carrying two genomes instead of one may reflect differences in life histories strategies if the same is shown in multiple species (Serghi *et al.* , 2021). Particularly, the homokaryotic strains have higher germination rates and faster germination compared to the low germination rate of the dikaryotic strains. In contrast, dikaryotic strains grow faster and produce larger and more interconnected ERM compared to the homokaryotic ones. The nuclear organization can affect the mycorrhizal response of their plant hosts. Specifically, and in contrast to expectations that two genomes might result in more mutualistic interactions, dikaryotic strains were inferior mutualists compared to the homokaryons when interacting with multiple potato cultivars (a highly mycorrhizal dependent crop) in greenhouse conditions (Terry *et al.* , 2023). While we recognize that nuclear organization may be an important function trait, until mono vs dikaryons are found in more AM fungal species it might be premature to suggest this trait should be included in a program for standardization of trait measurement across all AM fungal taxa.

The nuclear content of the spores also seems to be associated with particular life history traits although further experimental evidence is needed. The range of nuclei present in spores correlates with spore size, ranging from 35000 nuclei for spores of *Gigaspora decipiens*which have an average diameter of 400um, to 130 nuclei for the spores of*Glomus cerebriforme* with an average diameter of 80um (Kokkoris*et al.* , 2020). These huge differences in nuclear content could be associated to spore viability and germination, and overall colonization ability after dispersal. For example, multiple re-germination events have been observed for

Gigasporaceae spores when no host is encountered initially (Sward, 1981), a trait that does not appear in *Glomus*sp. spores which usually demonstrate a more ruderal behavior. It has been hypothesized that the numerous nuclei, could serve as resource reserve via nucleophagy when facing starvation, a phenomenon previously observed in fungi (Shoji *et al.* , 2010; Kokkoris *et al.* , 2020). Despite the number of genotypes and the number of nuclei present in the AM fungal networks and spores, the overall genome size might influence the reproductive rate, the environmental adaptability and in the overall resource economy of a species/strain. Although not very common for fungi, linkage of genome size to life history affiliations is not a novel concept. (Grime & Mowforth, 1982), linked plant genome size to climate growth conditions. (Veselý *et al.* , 2012) linked larger plant genome sizes to early flowering events and preference for humid conditions, and (Bhadra *et al.* , 2023) linked genome size to multiple functional traits related to plant morphology, physiology, performance and survival. Our knowledge on the variation of genome size in AM fungi is limited due to the low number of sequenced genomes. Regardless, we know that the variation is extreme, with larger species (Gigasporaceae) having genomes that reach 740 Mb and smaller species (e.g., *Rhizophagus clarus*) 116Mb (Kokkoris *et al.* , 2020). It is important to keep expanding our datasets with such information to identify links between genome size and the morphological, physiological, and phenological traits of AM fungi. Finally, a particular genetic trait, the guanine plus cytosine (G+C) content of genomic DNA which have the potential to reflect ecological niche or pathogenicity in fungi (Yoder & Turgeon, 2001). Once again with limited data due only few complete genomes available substantial variation in G+C content exists in AM fungi (range from 25 to 36 (mol%) (Malar C *et al.* , 2022). These differences could potentially reflect differences observed in mycorrhizal response and host preference.

The way forward on AM fungal traits research

Considering the above discussion and to overcome challenges related to measurement of AM fungal traits, and to obtain a more accurate understanding of AM fungal-plant interactions, we suggest the following points for future research.

1) *Create and maintain a centralized database of AM fungal traits* . This is a keystone task to integrate data and analyses to use appropriate AM fungal traits to predict plant responses and ecosystem processes. Databases are available on AM fungal traits but they are limited to mycorrhizal type and intensity of root colonization primarily in the early stages of plant development (Soudzilovskaia *et al.* , 2020). We propose the development of a generic structure for the AM fungal database for individual taxa stemming from the traits described in Table 1 and that is consistent with the principles of the Observation and Measurement Ontogeny (Madin *et al.* , 2007). The central tabulation in this database is the taxonomy at the species and strain level, and accession codes if available. Ancillary data are related to a) the site of origin such as latitude and longitude, along with the date and the observer b) its source either from field observations, a culture collection or literature data, and c) metadata and information about experimental treatments used to measure traits including, but not limited to, the levels of replication and variation associated with each estimate. A trait and measurement component integrates all information related to a specific trait (spore, mycelium, arbuscules, vesicles) and its measurement values and units. A trait database could facilitate the synthesis of research findings and improve our understanding of the functional diversity and ecological roles of AMF taxa.

2) *Expand the scope of research to include a broader range of AM fungi, with a particular focus on uncultured and underrepresented taxa* . Traits have been studied focusing on Glomeraceae, Acaulosporaceae, and Gigasporaceae, all common components of AM fungal communities and harboring 76% of the total number of species in the Glomeromycota. Other families such as Diversisporaceae, Claroideoglomeraceae, and basal families such as Paraglomeraceae and Archaeosporaceae are rarely included in experiments and there is very little information on their traits, despite being common components of AM fungal communities. Glomeromycota comprise 13 families and 337 species but likely contain many more (Öpik *et al.* , 2010). However, we estimate that only*ca.* 88 species are represented in culture collections worldwide. To ensure a more comprehensive understanding of AM fungal ecological roles and interactions, it is essential to record traits from taxa in these poorly studied families and attempt to increase taxonomic diversity in culture collections.

3) *Determine traits at fine levels of taxonomic resolution* . Results from distinct experimental approaches indicate that AM fungal traits related with internal and external mycelium and sporulation time exhibit some conservation at the family level, although variation within these clades was also observed (Hart & Reader, 2002a; Maherali & Klironomos, 2012). Seemingly inconsistent with this finding, high intraspecific variability in root colonization, external mycelium length and plant responses has been demonstrated for several species (Mensah*et al.* , 2015; Schoen *et al.* , 2021; Stahlhut *et al.* , 2023). As argued above, there is a need to conduct additional comparative studies using different species within the same genus to investigate trait conservatism.

4) *Measure and report AM fungal traits using standardized experimental approaches* . AM fungal traits reported in the literature have been measured using different experimental approaches, which makes comparisons difficult. We propose herein (Table 2) a set of minimum parameters that can be used when studying AM fungal traits that can minimize biases and account for the complex interplay between fungal and plant traits. We expect that by adopting these standard approaches database data supplied by different laboratories are more directly comparable. An obvious research imperative will be to validate the reproducibility of AM fungal trait measurements by different teams/infrastructures but using the same starting inoculum material. This reinforces the value of culture collections.

5) *Determine the variability (plasticity) of AM fungal trait expression* . A gap in our understanding of AM fungal traits, partially responsible for our inability to predict symbiotic function under field conditions, lies in our knowledge of how consistent traits are under varying environmental conditions. Experiments designed to test how specific environmental factors impact AM fungal traits are needed. Additionally, the scope of trait variations across different traits and taxa could be studied to determine if some traits are more conserved than others and/or vary more significantly in one taxon compared to others.

6) *Embrace AM fungal community diversity* : A basic premise of ecophysiology is that environmental filters will select for specific traits/adaptations (Lambers *et al.* , 2008). Given that some traits can be measured at the community level (e.g., hyphal nutrient stoichiometry (Zhang *et al.* , 2023b)), it can be envisaged to conduct experiments (physical disturbance, nutrient additions, drought etc.) on whole natural AM fungal communities and look at correlations between environmental filters and traits (Chagnon, 2023). Coupled with rotating and static cores (Johnson et al. 2001), these experiments could also assess AM fungal growth and mycorrhizal function. With synthesis studies identifying major drivers of AM fungal community structure at global scales (Davison et al., 2021), the next frontier is to move beyond taxonomy and assess the functional biogeography (e.g., (Violle*et al.* , 2014) of AM fungi.

7) *Utilize AM fungal isolates deposited in culture collections.*Culture collections worldwide uphold a considerable variety of AM fungal isolates in terms of physiology, genetics, taxonomy, and geographic origin. These isolates, which are cultivated either in a mineral substrate or in conjunction with root organ cultures, are well characterized taxonomically, thereby representing important resources in comparative studies of traits. These centers are instrumental in training personnel, and specific workshops can be developed to provide hands-on experience and theoretical knowledge about measurement of AM fungal traits.

8) The use of microphotography and machine learning: The integration of microphotography and machine learning algorithms could not only help standardize and accelerate trait quantification but also eliminate the subjectivity of the observer, a common issue entangled with our current quantification approaches. Successful integration of the two can create automations that will allow for large dataset acquisition, no longer limited by space and time (e.g., continuous growth measurements of ERM and its traits, or continuous progression of root colonization with the help of fluorescent markers). These approaches can help reveal behavioral patterns that have so far remained undetected due to technical limitations.

As mentioned earlier, some ecologically significant functions of the AM fungal symbiosis, such as the ability to promote host plant growth, depend not only on the properties of an individual AM fungal isolate but also on its interaction with the host. While we recognize that this may limit the scope of some trait measurements, we consider that even though a hypothesis-driven approach as outlined in this study for effectively assessing

AM fungal traits may not address all questions about the role and impact of AM fungi in ecology, it represents a significant step towards that goal.

Experimental approaches BOX 1

Various experimental approaches can be employed to investigate morphological and physiological AM fungal traits in semi-realistic conditions including soil or substrate and plant host(s). We identified five main approaches in the literature. Gazey et al. (1992) utilized a**sterile mesh bag** approach to examine the association between the initiation of sporulation and external hyphae production in two*Acaulospora* species. This technique involves the use of 25 µm mesh bags (2 cm wide, 10 cm across, and 10 cm long) containing 200 g of uninoculated, steamed soil. The mesh bags can be placed in pots anywhere along the soil profile. Data are gathered at weekly intervals from 3 to 8 weeks post-plant host germination within the "sterile mesh bag," encompassing external hyphae length, spore numbers. Outside the "sterile mesh bag" host biomass and root colonization, among other variables, can be measured. This approach allows measuring mycorrhizal traits within a controlled sterile soil environment without the interference of propagules present in the original inoculum. The sterile soil should be free from AM fungal propagules and researchers should have controls with non-inoculated mesh bags. Furthermore, as samples are collected from a small soil volume inside the mesh bag, correlations between root colonization, spores, and external hyphae are more likely to represent realistic relationships among these traits. The temporal approach (weekly sampling) permits close monitoring of the entire AM fungal and plant life-cycles. Nonetheless, there are potential limitations to this approach, such as the requirement for propagules to infiltrate the mesh bag, which may be limiting for some taxa (e.g., (Thonar *et al.* , 2011)). Additionally, if many isolates or species are being analyzed concurrently, the entire experiment may be time-consuming and labor-intensive.

A second approach similar to the sterile mesh bag was used by (Jakobsen*et al.* , 1992a) to study the abundance of AM fungal hyphae in soil and P uptake into roots. **Root compartment bags** consisted of cylindrical (60 mm diameter) bags constructed using 25 µm nylon mesh and filled with AM fungal inoculum. These bags are placed in the center of 1.5 L pots, surrounded by steamed sterilized soil and pre-germinated seeds are transplanted into each bag. Thus, the host generated roots are confined within the compartment bag, while AM fungal hyphae are unrestricted to extend into the surrounding soil. After 25 days, root compartment bags can be transplanted into rectangular PVC boxes (L x W x H = 300 x 185 x 130 mm) surrounded by 7 kg of steamed dry soil. To measure hyphal growth, 10 mm diameter soil cores are obtained on five sampling dates at different distances from the root compartment. Similar to the sterile mesh bag method, the root compartment approach enables the measurement of hyphal length in an environment devoid of pre-existing mycorrhizal inoculum. By transplanting the root compartment into large rectangular boxes, this approach is very suitable to study distance and rate of spread of AM fungal external hyphae, allowing a direct comparison of these traits among fungal taxa. However, the experimental design requires a substantial quantity of soil for the setup. A similar approach uses a trap plant on the other end, which allows measurements of resource movement between hosts in a community (Mikkelsen *et al.* , 2008).

A third approach, named **inoculated containers,** was used by (Hart & Reader, 2002a) to establish the taxonomic basis for the observed variation in root colonization strategies among AMF families. Fungal biomass is initially measured based on ergosterol concentration to equalize the amount of inoculum at the onset of the experiment. However, we recommend using a different approach (e.g., fatty acids) as has been shown that AM fungi do not produce ergosterol (Olsson *et al.* , 2003; Olsson & Lekberg, 2022). Containers (4 cm diameter x 20.5 cm deep) are 2/3 filled with soil, then inoculated with soil inoculum containing spores, hyphae and colonized root fragments and sown with leek as a surrogate host. Following a 30 d period, the shoots are harvested, and the soil subjected to experimental treatments, including different hosts. Containers are harvested six times over a 12 w period to measure the extent of root and soil colonization. The inoculated container approach, using small-volume containers, allows the concurrent study of many isolates over time. The standardization of fungal biomass among species allows a direct final comparison among taxa. Given that the abundance of AM fungal external hyphae is assessed in the same container in which the inoculum was introduced, differentiating between newly produced hyphae and those present in the original inoculum

is not feasible.

Direct measurements of physiological/chemical traits can require efficient separation of fungal hyphae from the growth substrate. (Zhang*et al.* , 2023b) employed **hyphal in-growth bags** , consisting of glass beads and fine silt and clay particles (as a nutrient source) and surrounded by fine nylon mesh (pore size = 38 um) based on the original design by (Neumann & George, 2005), to harvest enough mycelium so that carbon and nitrogen concentrations could be assessed following combustion, and phosphorus concentration following wet digestion, after eight weeks of incubation. In-growth bags were 2 cm wide and 10 cm long and filled with 40 g of the soil-glass bead mixture and buried lengthwise in the top 10 cm of each pot. The mesh size was sufficient to prevent in-growth of *Medicago sativa* roots but not root hairs of *Festuca arundinacea* , which requires a mesh size of 10 um to exclude (Zhang *et al.* , 2021). Soil particles in the bag can still stick to hyphae and need to be carefully removed before analysis. This method, while similar to the sterile mesh bag, is more suitable to study physiology.

Another approach to study traits associated with common mycorrhizal networks (CMNs) was proposed by (Johnson *et al.* , 2001) using**rotative cores** . In this method, a slot (2 cm wide and 5 cm long) is made on the side of a conical container (270 ml) and covered with a 40 µm nylon mesh or hydrophobic membrane. Containers are fitted into polystyrene foam and assembled in pots or microcosms, depending on the study's objective. The containers are then filled with soil and sand mixtures, inoculated with AM fungi and seeded with a host plant to establish the CMNs for 2-3 months. Following this period, CMN treatments can be established by either maintaining the containers' positions throughout the experiment (undisturbed CMNs) or rotating them (one full rotation) periodically to physically sever the AM fungal hyphal network. This method is highly suitable for understanding the role of external mycelium and the impact of AM fungal mycelium network disruption on various soil (e.g., bacterial community structure, soil aggregation) and plant (e.g., biomass production, nutrient allocation) parameters (Babikova *et al.* , 2013). Although the preparation of modified containers is somewhat laborious, this approach enables the investigation of the role of CMNs in plant interactions across multiple species simultaneously.

The methods described above are appropriate for assessing fungal traits and their interrelationships; however, these are not the sole approaches available. The study of genetic traits typically requires the use of isolation techniques into *in vitro* culture see (Declerck *et al.* , 2010). For example, *in vitro* root-organ cultures methods have enabled major breakthroughs in the understanding of genetic and physiological traits such as nutrient exchange ratios (Cranenbrouck*et al.* , 2005; Kiers *et al.* , 2011) and patterns of hyphal anastomosis between isolates in the same species (Giovannetti *et al.* , 1999). In addition, *in vitro* systems may be instrumental in investigating trait interactions between AM fungi and other microorganisms (Faghihinia *et al.* , 2023). Considering the vast amount of literature on mycorrhizal research and the diverse factors involved in experiments (e.g., plant hosts, soil type, fertilization regime, environmental conditions), a comprehensive understanding of traits for different AM fungal taxa has not been achieved. Given that AM fungi are influenced by soil parameters, plant hosts, and environmental conditions, we propose a set of standard items that should be considered in experiments aiming to measure AM fungal traits. We acknowledge that certain experimental items could be standardized, while others that are more challenging to standardize (e.g., soil type, lighting conditions) could be collected as metadata. By adopting this approach, experiments could be conducted in different laboratories using the same AM fungal taxa while modulating different environmental conditions (*e.g.,*soil disturbance, salinity, drought, CO_2 concentration, temperature, light intensity) to examine the degree of conservation of AM fungal traits across systems and the validity of predictions based on taxonomic identity.

Toward a standardized approach to measure AM fungal traits BOX2

Standardizing the quantification of mycorrhizal fungal traits can be useful to optimize data quality, reduce bias, facilitate comparability, and reproducibility among studies. This methodological uniformity can enhance the robustness of meta-analyses and promote collaboration among researchers, thereby advancing our ecological understanding of AM fungi. What follows is a proposal, based on the authors' expert opinions, for the standardization of trait-measurement experiments. Researchers may have very good reasons to not

comply with some or all of these recommendations in their work, and we want to make clear that this would not mean that the data lack value in the context of understanding AM fungal life histories. As long as deviations from proposed methods are documented along with the data, they would certainly still hold value.

a) Pot Size and Type: Measuring AM fungal traits might result in experiments with many experimental units. Therefore, pot size and substrate (see below) are two items that should be considered to make the experiment feasible. We propose the use of pots with volumes greater than 2 kg , when mesh bags are used. If inoculated containers or rotative cores are chosen, then containers (4 cm diameter x 20.5 cm deep) with conical open bottoms are recommended (Weremijewicz & Janos, 2019).

b) Soil texture: Production of AM fungal external mycelium and sporulation are certainly affected by soil texture, as it determines the number of pores and available free space for fungi to grow. Inert media like sand: expanded clay could be used to standardize the substrate. These media have the advantage of not containing AM fungal propagules. However, they hardly represent the common habitat of AM fungi. Therefore, extrapolating AM fungal trait measurements obtained using these media to field soil conditions might be challenging. We propose using a sterilized loam soil in standard experiments to measure AM fungal traits. If a loam soil is not available, adding quartzite sand or coarse river sand to the soil is suggested to bring the texture closer to a loam soil.

c) Soil sterilization: Autoclave 121°C (for as long as needed; adjust by placing a rod with autoclave tape to the center) repeated twice with a 24h interval. However, if budget and access to infrastructures allow, gamma radiation is a useful alternative that limits chemical alteration of organic matter and downstream consequences on dissolved organic carbon and aggregate stability (Berns *et al.* , 2008).

d) Nutrient solution: Plant nutrition is an important aspect to be considered as it impacts the establishment of the mycorrhizal symbiosis. We suggest the use of a modified Hoagland's solution for monocots to provide the minimum amount of macro and micronutrients for the host plant (*SupplementaryMaterial*).

e) Typically a microbial wash to introduce and standardize non-mycorrhizal soil organisms is prepared by mixing all the tested AM fungal inocula with water and passing the resulting slurry through a 20 µm sieve to be added to the test pots (Ames *et al.* , 1987). This procedure is arguably the hardest to standardize. We suggest including a control treatment, without a microbial wash to ascertain the role of microorganisms on AM fungal traits.

f) Host plant: Mycorrhizal host plants vary widely in their growth habits (e.g., grasses, trees, forbs), growth rates, and root architecture, which affect the amount of root colonization and spread of hyphae in the soil. Host preference is also another factor to be considered, as it impacts AMF sporulation (Bever *et al.* , 1996). We suggest the use of *Sorghum* × *drummondii* (Sudan grass) as a standard host because a) it has been widely used to grow and maintain a vast array of AMF germplasm in culture collections (Morton *et al.* , 1993), b) it has a fasciculated root system that provides space for root colonization, and c) it is mycorrhizal dependent.

In addition to these recommendations, metadata should include temperature, soil/substrate type, pH, soil moisture content, soil fertility, light intensity and experiment duration.

Table 1: Key traits, their hypothesized function, and methods for trait measurement

Fungal and fungal mycorrhizal traits	**Hypothesized symbiotic effects**	AM fungi	Plant	Soil*	Qualitative/quantitative (unit)	References - methods on how to measure the traits	References**
Spores							

Number	- fitness/competitive ability -dispersal -carbon storage	x x x	x	Quantitative: number of spores/g soil Spores/meter of mycelia	Spores extracted by wet-sieving ((Gerdemann & Nicolson, 1963)) and sucrose gradient centrifugation (Ba, 1982) and counted under dissecting microscope	(Bever *et al.*, 1996), (Chaudhary *et al.*, 2020)
size - diameter	- fitness/competitive ability -dispersal -energy to support hyphal growth in absence of host -carbon storage -resistance to abiotic and biotic stress	x x x x	x x	Quantitative: size measured in µm	Spore diameter measured intact in water using a dissecting or optical microscope ((Morton, 1995, 1996))	(Chaudhary *et al.*, 2020; Deveautour *et al.*, 2020)
germination rate % of total	- fitness/competitive ability -carbon storage	x	x	Quantitative: % of germination	(Douds & Schenck, 1991), Spores over filter paper in a soil-filled Petri plate (Koske, 1981)	(Tommerup, 1983), (Maia & Yano-Melo, 2001)

germination timing	- fitness/competitive ability -resistance to abiotic and biotic stress	x x	x x	Quantitative - % germination per unit of time Qualitative - stratification needed	(Koske, 1981)	(Tommerup, 1984, 1985), (Koske *et al.*, 1996) (Douds & Schenck, 1991) (Juge *et al.*, 2002)
color	- dispersal - palatability - UV protection - germination duration	x x		Qualitative: color based on CMYK color chart Quantitative: RGB color channels extracted from digitized images (JPG, TIF), calculation of luminance and saturation	Spores observed under a dissecting microscope and compared with color chart (Morton, 1996) or imaged and analyzed using computer software (Deveautour et al. 2020)	Deveautour et al. (2020),(Zanne *et al.*, 2020)
ornamentation	-dispersal -resistance to abiotic and biotic stress	x x	x	Qualitative: type of ornamentation. Quantitative: size in µm	Spores mounted on slides and observed under microscope Koske and Walker, 1985	Chaudhary et al. (2020)
wall thickness	- dispersal -palatability - carbon storage - resistance to abiotic and biotic stress	x x x	x	Quantitative: thickness in µm	Spores mounted on slides and thickness measured under microscope Morton (1995, 1996)	(Pawlowska *et al.*, 1999) (Moore *et al.*, 1985)

wall number	-dispersal -resistance to abiotic and biotic stress	x x			x	Quantitative: number of walls	Spores mounted on slides and observed under microscope Morton (1995, 1996)	(Walker, 1983)
sporocarps	-dispersal	x			x	Size and existence of fungal peridium on the sporocarp surface.	Sporocarps are measured under a microscope for size (Redecker *et al.*, 2007) and existence of peridium (Schüßler *et al.*, 2011)	(Mangan & Adler, 2002)
wall chemical receptors	- perception of host/soil environmental cues affecting germination	x	x				Knowledge gap (develop a method to identify specific receptors)	Knowledge gap (are there specific receptors on the spore wall that trigger germination?)
Spore nuclear content	-spore viability and germination - colonization ability after dispersal	x x	x x	x		Number of nuclei per spore	Confocal microscopy, Flow cytometry	(Kokkoris *et al.*, 2020, 2021) (Bianciotto *et al.*, 1995; Marleau *et al.*, 2011)
Elemental composition	-Energy support for hyphal growth	x			x	Elemental composition	Proton-induced X-ray emission	(Hammer *et al.*, 2011)
Extraradical Hyphae								

length	– nutrient/water	x	x	x	Quantitative: hypha	(Miller *et al.*, 1995)	(Wilson *et al.*, 2009)
	acquisition	x	x	x	length in		(Johnson *et*
	-carbon	x	x	x	m/g dry soil		*al.*, 2015)
	storage		x	x			
	-soil	x	x	x			
	aggregation						
	-plant						
	productivity						
	-resistance						
	to abiotic						
	and biotic						
	stress						
architecture	– nutrient/water	x	x	x	Qualitative	(Friese &	Knowledge
(branching	acquisition	x	x	x		Allen, 1991)	gap (e.g., is
rate,	-carbon	x	x	x		or (Bago *et*	hyphal
anastomoses	storage	x	x	x	Quantitative:	*al.*, 1998b)	architecture
rate,	-soil	x	x		Using image	in	akin to root
absorptive/runner	aggregation				analysis in	monoxenic	architecture
hypha)	-plant				*in vitro*	conditions	for nutrient
	productivity				systems		acquisition?
	-resistance					(Hammer *et*	how does
	to abiotic					*al.*, 2023)	hyphal
	and biotic						architecture
	stress						influence soil
							aggregate
							stability?)
inter-host	- transfer of	x	x	x	Quantitative:	(Weremijewicz	Knowledge
connection	nutrient/water/signals				amount of	& Janos,	gap (e.g.,
	among hosts	x	x		nutrient/signal	2019)	are there
	-resistance				transferred	(Frey &	fungi that
	to abiotic				Number of	Schüepp,	interconnect
	and biotic				hosts	1993)	more hosts
	stress				connected		than others?
					by the same		can common
					fungus		mycorrhizal
							networks
							provide
							additional
							pathogen
							protection?)
hyphal	–carbon	x		x	Quantitative:	(Friese &	(Klironomos
diameter	storage	x	x		in μm	Allen, 1991)	& Kendrick,
	- resistance						1996)
	to abiotic	x					
	and biotic						
	stress						
	- palatability						

growth rate	– nutrient/water acquisition - carbon storage - resistance to abiotic and biotic stress	x x x	x x x	x x x	Quantitative: hyphal growth in mm/day	(Schütz *et al.*, 2022)	(Jakobsen *et al.*, 1992b)
hyphal lifespan/turnover	- carbon storage - nutrient/water acquisition	x x	x x	x x	Qualitative	(Pepe *et al.*, 2018)	(Pepe *et al.*, 2018)
Genetic organization/hyphal fusion (homokaryon/dikaryon) (note: also applies to spores)	-fitness – nutrient/water acquisition -carbon storage -soil aggregation -plant productivity -resistance to abiotic and biotic stress - hyphal network interconnectedness	x x x x x x x x	x x x x x	x x	Quantitative ddPCR: number of nuclei?	(Cornell *et al.*, 2022)	(Cornell *et al.*, 2022) (Serghi *et al.*, 2021)
Exudation rate/leakiness	-carbon storage -influence soil pH and fertility -soil aggregation -resistance to abiotic and biotic stress	x x x x x	x x x x	x x x	Quantitative: measure release of a molecule in µM	(Tawaraya *et al.*, 2006)	(Tawaraya *et al.*, 2006)
absorptive capacity	– nutrient/water acquisition	x	x	x	Quantitative: mol m^{-1} s^{-1} or % of nutrient taken up	(Frey & Schüepp, 1993), (Jakobsen *et al.*, 1992a)	(Frey & Schüepp, 1993)

color	-resistance to abiotic and biotic stress	x	x		Qualitative: color described by CMYK model	(de la Providencia *et al.*, 2005) using transformed roots and (Koske, 1981) using spores over filter paper on soil-filled Petri plate	Knowledge gap (are darker hyphae, more melanized, more resistant to fungivores?)
wall/membrane chemical composition	-resistance to abiotic and biotic stress	x		x	Quantitative: in µg	(Bethlenfalvay *et al.*, 1981) for chitin. (Frey *et al.*, 1992, 1994) for chitin and ergosterol (Butler & Lachance, 1986) for melanin; (Harrison & Vanbuuren, 1995) for P transporters	(Deveautour *et al.*, 2020)
	- nutrient/water acquisition	x	x	x			
	-fungal recognition (anastomosis)	x					
	-palatability						
pattern of anastomosis	- fungal recognition	x			Quantitative: number of anastomosis per hyphal length (cm) or percentage of anastomosis (%)	(de la Providencia *et al.*, 2005)	(de la Providencia *et al.*, 2005)
	- fitness	x					
Intraradical Hyphae							

-hyphal thickness	-Resource flux/exchange	x	x	Quantitative: in µm	(Abbott, 1982)	Knowledge gap (e.g., are thicker hyphae more resistant to pathogens? Is there a tradeoff between nutrient transfer and biotic resistance in terms of hyphal thickness?)
	-resistance to abiotic and biotic stress	x	x			
-pattern of colonization (localized / widespread)	-Resource flux/exchange	x	x	Qualitative	(Dickson, 2004) (McGONIGLE *et al.*, 1990) (Abbott, 1982)	Knowledge gap (e.g., is resource exchange more/less efficient when colonization is localized or widespread?)
	-resistance to abiotic and biotic stress	x	x			
-rate of root colonization	-Resource flux/exchange -resistance to abiotic and biotic stress	x	x	Quantitative: % of root colonization over time	(Dickson, 2004)	(Campo *et al.*, 2020)
Arbuscules						
- architecture (Paris/Arum type)	- resource flux/exchange	x	x	Qualitative	(Dickson, 2004)	(van Aarle *et al.*, 2005)
	-resistance to abiotic and biotic stress	x	x			

- turnover rate	-resource flux/ exchange	x	x	Quantitative: number of days	(Alexander *et al.*, 1989)), (Toth & Miller, 1984)	Knowledge gap (e.g., are some arbuscules more short lived than others? How does arbuscule turnover affect resource exchange?)
-number	-resource flux/ exchange	x	x	Quantitative: number of arbuscules	Quantification of Arbuscules Using Morphometric Cytology - (Toth, 1992) Magnified intersections method - (McGONIGLE *et al.*, 1990) Image analysis - (Smith & Dickson, 1991) Direct count - (Menge *et al.*, 1978)	(Koch *et al.*, 2017)
Vesicles						
-size and form (globose/lobbed)	- carbon storage - resistance to abiotic and biotic stress	x x	x x	Quantitative: in μm (for size). Qualitative (for form)	(Abbott, 1982)	Knowledge gap (e.g., are fungi with larger vesicles more resistant to stress?)
-number	- carbon storage	x	x	Quantitative: number per root length	(Abbott, 1982), (Menge *et al.*, 1978)	(Kobae *et al.*, 2016)

-chemical composition (C/lipid storage)	- carbon storage	x	x		Quantitative: % total lipids/ fatty acids	(Jabaji-Hare *et al.*, 1984)	(Jabaji-Hare *et al.*, 1984)
Turnover rate	- carbon storage	x	x	x	Quantitative: number of days	Knowledge gap (adapt the method used for arbuscules)	Knowledge gap (e.g., are some vesicles more short lived than others? How does vesicle turnover affect C storage?)
Other genetic traits							
Genome size	- reproductive rate - survival					Whole genome sequencing Flow cytometry	Sperschneider et al., 2023 (Hosny *et al.*, 1998)
GC content of the genome	- mycorrhizal host response -host preference	x	x x	x		Whole genome sequencing Knowledge gap (missing genomes across phylogeny)	(Malar C *et al.*, 2022)

*abiotic and/or biotic

**Examples/papers showing the relationship between measuring the trait its function. References might not necessarily represent best practice.

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