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REVIEW

Enhancing consistency in arbuscular mycorrhizal trait-based research to improve predictions of function

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Abstract

Arbuscular mycorrhizal (AM) fungi (phylum Glomeromycota) are obligate symbionts with plants influencing plant health, soil a(biotic) processes, and ecosystem functioning. Despite advancements in molecular techniques, understanding the role of AM fungal communities on a(biotic) processes based on AM fungal taxonomy remains challenging. This review advocates for a standardized trait-based framework to elucidate the life-history traits of AM fungi, focusing on their roles in three dimensions: host plants, soil, and AM fungal ecology. We define morphological, physiological, and genetic key traits, explore their functional roles and propose methodologies for their consistent measurement, enabling cross-study comparisons towards improved predictability of ecological function. We aim for this review to lay the groundwork for establishing a baseline of AM fungal trait responses under varying environmental conditions. Furthermore, we emphasize the need to include underrepresented taxa in research and utilize advances in machine learning and microphotography for data standardization.

Keywords Symbiosis · Trait-based ecology · Ecosystem processes · Standardization · Functional diversity · Environmental adaptation

A trait is an attribute influencing an organism's performance within its environment, encompassing morphological, physiological and genetic characteristics measured at the individual or population levels (Salguero-Gómez et al. 2018; Zhang et al. 2023b). 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

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(Romero-Olivares et al. 2021). This approach holds particular significance for arbuscular mycorrhizal (AM) fungi – Phylum Glomeromycota. As obligate symbionts of plants, where multiple fungi colonize both roots and soils in a network, predicting the functional outcomes (*e.g.*, host growth, plant community diversity, changes in soil processes) 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 relationships between AM fungal taxa and/or genotypes (*i.e.*, accounting for within species variability) and their functional roles is a laborious process, which is expected to continue (Serghi et al. 2021; Manley et al. 2023; Corradi et al. 2024). However, it is important to go beyond taxonomy and morphological characteristics, incorporating physiological and genetic traits. This work is needed due to the complex associations that exist between AM fungi and various functional outcomes for hosts (*e.g.*, plant growth and fitness, nutrient uptake and stress tolerance) and soil processes (*e.g.*, carbon (C) storage, aggregate stability, biotic diversity), all of which currently appear 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 over longer distances by wind than large-spored AM fungi, which leads to the 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 difference in dispersal ability has "functional" or "adaptive" value. Alternatively, producing small spores is a correlated response to producing many spores (arguably more quickly if they are structurally simpler), which itself could be an adaptive response to the likelihood of unpredictable soil disturbance (*e.g.*, caused by soil animals or 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). Measuring traits like spore size, spore production rates, and their ability to resist or respond to environmental disturbances can help to disentangle different hypotheses about how these traits contribute to AM fungal success and distribution. Another example to illustrate the complexity of associating traits with function is the variation in rooting depth among plants in a community, which 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. As such, plants and fungal traits cannot be considered in isolation. Given these complexities, we consider the development of a robust, universally applicable trait-based framework towards predicting key AM fungal functional outcomes a priority. To achieve this objective, first we must identify AM fungal traits that can be measured not only at morphological levels of organization but also at physiological, and genetic levels. Second, considering the important roles of AM fungi in ecosystems, affecting host plants, soil processes, and the AM fungi themselves, we need to discern 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 nutrient limitations, pathogens, heavy metals, salinity, drought, etc. (Delavaux et al. 2017; Wehner et al. 2010; Begum et al. 2019). Within the soil environment, AM fungal effects on soil structure (Rillig and Mummey 2006), nutrient cycling, C storage, and other members of the soil food-web are relevant (Antunes and Koyama 2016; Frew et al. 2021; Horsch et al. 2023a). Regarding the fungal organism itself, 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). Connecting traits to functional outcomes 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. For example, small spore size and high spore production rates can serve as proxies for functional traits such as effective dispersal in face of disturbance. Hyphal growth rate and branching pattern can serve as proxies for resource foraging efficiency. Third, we need to evaluate existing standardized methods and experimental designs, or develop new ones, to measure such relevant (soft) traits, as it has been done in plant ecophysiology (Pérez-Harguindeguy et al. 2013). Measurement standardization and relevant metadata for hypothesis-driven analysis and interpretation are essential if we are to eventually aggregate trait information from different studies, 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 of 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:

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 methods and experimental designs used to study AM fungal traits, highlighting their strengths and limitations.
4. To propose standardized approaches for measuring AM fungal traits.
5. To explore the integration of AM fungal trait data into ecological models to potentially enhance ecosystem processes' predictability.

Historical perspective of trait-based approaches

The scientific literature on AM fungal life-history traits (*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. In a comparative study of 21 AM fungal isolates (*i.e.*, defined as 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, Hart and Reader (2002a) showed 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 the isolates of those families. Glomeraceae fungi exhibited high root colonization but low soil colonization, and vice-versa for Gigasporaceae. Acaulosporaceae fungi displayed low colonization in both roots and soil. These findings revealed a strong association between AM fungal morphological characteristics and taxonomy, as isolates of the main families in the phylum could be differentiated based on root colonization rate and biomass allocation patterns. These observations were corroborated by subsequent studies, albeit using AM fungi from the same community and, possibly, the same isolates (Hart and Reader 2002a, 2005; Maherali and 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 plant seeds (Moles et al. 2005). However, to what extent plant trait-frameworks may be applicable to AM fungi is unknown. At present, evidence suggests 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. More recently, a distinction between 'rhizophilic' and 'edaphophilic' life-history strategies has been introduced to categorize AM fungi that allocate more biomass to growth within roots versus soil (Weber et al. 2019), and data show that long-term phosphorus (P) enrichment in subtropical forests shifts AM fungal communities toward edaphophilic guilds (Wang et al. 2023). Since there was more P directly available to the host plant in the soil, the observed shift towards edaphophilic guilds (i.e., Gigasporaceae vs Glomeraceae) suggests that these fungi may offer alternative benefits to P uptake (e.g., water uptake, nitrogen acquisition, pathogen resistance) or are better adapted to the new soil conditions (Rúa et al. 2016).

The patterns described above demonstrate 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). However, evidence suggests that the relationship between mycelium production and nutrient acquisition is not straightforward. If early or extensive root colonization (with abundant coils/arbuscules) rather than growing an extensive soil mycelium is more important for nutrient delivery to the host, then Glomeraceae could be more beneficial partners than Gigasporaceae 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 and Bever 2008; Sikes et al. 2009), a meta-analysis (Yang et al. 2017) suggested that, on average, fungi of the family Glomeraceae were better at acquiring P and reducing pathogen growth compared to other AM fungal families. It is also of interest, that this family appears to be the most abundant in many locations

(Öpik et al. 2010). Perhaps this potential ability of Glomeraceae to outperform Gigasporaceae in P acquisition despite producing less extensive mycelium, can be more accurately understood by considering 'response traits' (how organisms adapt to environmental changes) versus 'effect traits' (how organisms influence their environment and ecosystem processes) (Koide et al. 2014). The evolution of greater mycelium production either intra or extra-radically could reflect an adaptive response to increased susceptibility to soil disturbances (response trait) rather than directly enhancing soil P acquisition (effect trait). Considering these distinctions in a trait-based framework could help refine our understanding of AM fungal trait functionality on soil, hosts and AM fungi. Despite these advances towards better consistency in predicting functional outcomes from morphological and taxonomy data, we argue that only by integrating into databases morphological, physiological, and genetic trait data obtained across environmental conditions can we establish a basis for more accurately predicting the functions of these fungi.

Previous studies lacked a comprehensive 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). This may not reflect the reality in natural environments. Both the study by Sikes et al. (2009) investigating differences in plant pathogen protection between AM fungal taxa, as well as that by Lerat et al. (2003) on C-sink strength among different AM fungal families suggest 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 (see Chaudhary et al. 2022). This brings an additional layer of complexity to the study of AM fungal ecophysiology and 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 and Scheublin (2007) conducted the first comprehensive review of AM fungal traits to predict plant growth and ecosystem functioning. They provided a list of 13 AM fungal functional traits categorized into morphological traits (*e.g.,* hyphal length, rate and extent of root colonization, spore production) and physiological traits (*e.g.*, fungal C acquisition, host preference, nutrient uptake efficiency, exudation of compounds into the hyphosphere). Subsequently, Behm and Kiers (2014) noted substantial intraspecific trait variation among AM fungal species (also see Koch et al. 2017; Schoen et al. 2021), 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). 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 those formed by mycorrhizal fungi. 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, categorizing traits into three main groups: morphological, physiological, and phenological. Within each of these categories, they pinpointed distinctive AM traits specific to both the host plant (*e.g.,* root:shoot ratio, growth form, photosynthetic pathways) and the fungal partner (*e.g.*, spore size, hyphal length, and melanin content). Beyond these discrete traits for plants or fungi, they introduced the concept of mycorrhizal traits as unique attributes that emerge during symbiosis and are co-dependent on both partners. These 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 plant natural communities. By allowing speculative connections to be made regarding potential linkages between fungal traits (*e.g.*, hyphal fusion, sporulation phenology, C sink strength, growth rates) 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 struggles to predict AM fungal responses in complex multi-stress scenarios (Heuck et al. 2024). In addition, it fails to consider several AM fungal families (*e.g.*, Pacisporaceae, Entrophosporaceae, Diversisporaceae, or more basal lineages like Paraglomeraceae, Archaeosporaceae, and Ambisporaceae). 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 merely as 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 two significant barriers to achieving a more predictive understanding of AM fungal ecology. First, discrepancies among studies often arise due to non-standardized experimental approaches. Second, the absence of a comprehensive database on AM fungal traits further complicates progress in this field (TraitAM is expected to become publicly available in 2025; Chaudhary, personal communication). 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 plant growth responses or effects on ecosystems.

Traits and function

Arbuscular mycorrhizal fungal traits, including for example hyphal length, arbuscule morphology, or the robustness of hyphal and 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 Fig. 1 and 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 serve as indicators of mycorrhizal function and depend on the morphological, physiological, or phenological characteristics of the fungal partner (Chaudhary et al. 2022). In addition, genetic traits are becoming increasingly well understood (see below). In this context, AM fungal traits are most likely instrumental in defining ecosystem resistance, resilience and adaptability to environmental stress, as certain fungal isolates with specific traits may demonstrate superior robustness or flexibility under changing conditions.

Morphological traits

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 (or other propagules) are activated, germinate and explore the soil for a compatible host, and (2) the symbiotic phase, which includes four stages: a) initiation of root colonization; b) formation of structures within the root cortex; c) extension of mycelium into the soil matrix and possibly other hosts; and d) 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 (MacLean et al. 2017; Lanfranco et al. 2018). Hyphae emerging from these propagules perceive a host root, adhere to its surface, and commence root colonization. A swollen hyphopodium forms, from which a single hypha penetrates the root epidermis to access the cortex. A series of morphogenetic and molecular processes come into play at these initial stages, enabling the plant to recognize the presence of the AM fungus (as reviewed by Bonfante and Perotto 1995; Gianinazzi-Pearson et al. 2007; Bonfante and Genre 2010; Luginbuehl and 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 and intraradical spores. Upon attaining a certain threshold of root colonization, hyphae extend beyond the root system into the soil matrix, forming the extraradical mycelium (ERM), which consists of runner hyphae, branched absorbing structures (BAS), spore associated BAS, and spores. The expansive hyphal network, comprising IRM, ERM, embodies the traits that underpin several ecosystem-level processes attributed to AM fungi (*e.g.*, nutrient cycling, soil C sequestration, water regulation, soil formation, pathogen regulation, etc.). As we will explore next, these traits impact not just the host plants and soil environment, but also the fungal organism itself.

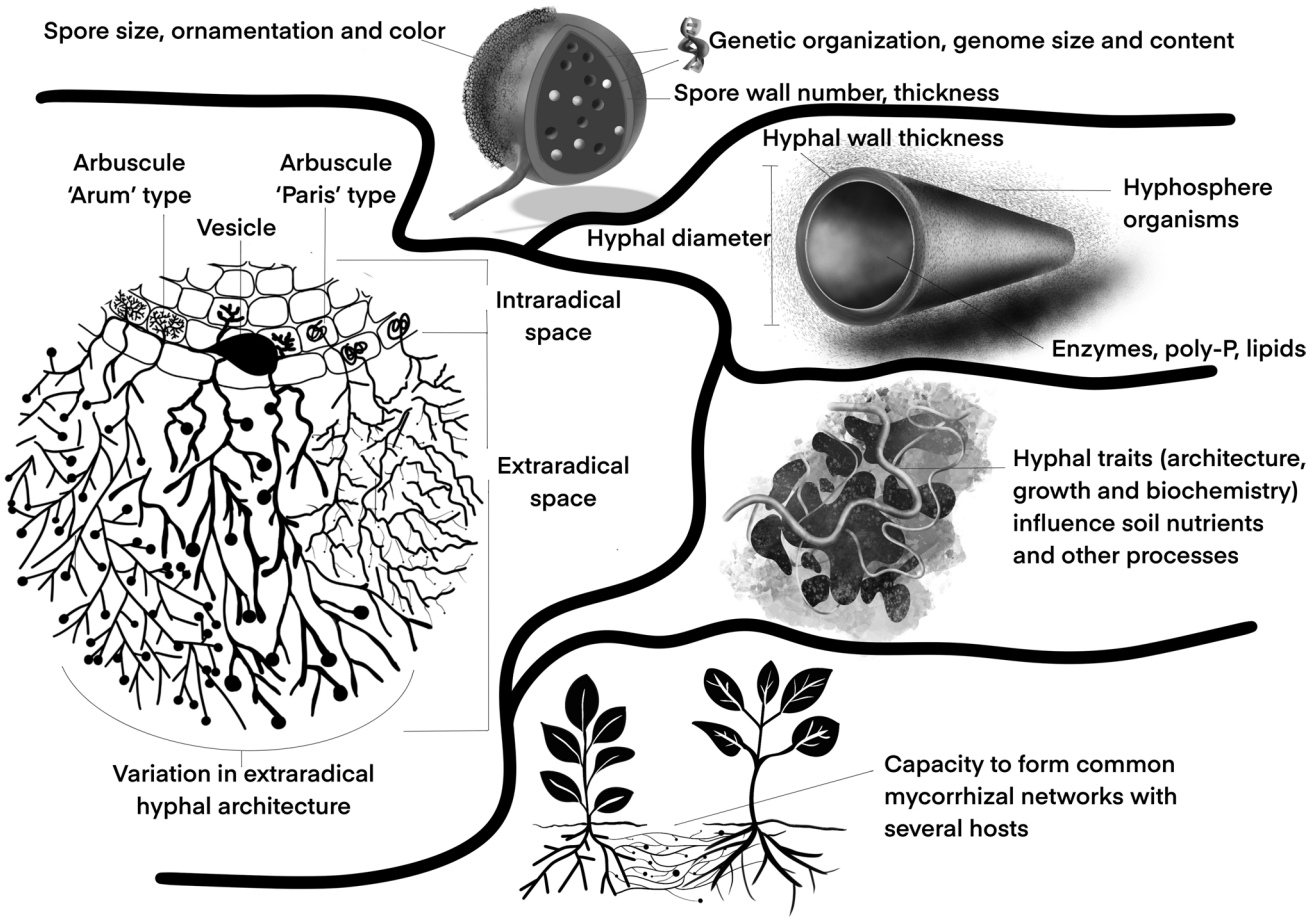

Fig. 1 Visual representation of key arbuscular mycorrhizal fungal traits, highlighting their morphological (*e.g.*, spore wall number, hyphal architecture), physiological (*e.g.*, enzymes, poly-P, lipids), and genetic features (*e.g.*, G + C content, genome size, homo- versus dikaryosis). The figure provides an overview to help readers connect these traits to their functional roles. Note that the illustration is not exhaustive and further details can be found in Table 1. Illustration created by Pedro M. Antunes using Procreate

AM fungal spores

Arbuscular mycorrhizal fungal spores are among the largest (Aguilar-Trigueros et al. 2023) and most multinucleated spores (Cooke et al. 1987; 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 hypha 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, inside the neck, or within 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)). Spores have been observed to form individually in the soil, in loose clusters, or within small to large compact sporocarps. The spores' cytoplasm contains not only nuclei (ranging from hundreds to thousands) but also lipid reserves that assist in germination and early colonization. Based on spore ontogeny, three main phenotypic characteristics are observed in AM fungal spores: spore wall, germinal walls, and germination structure, with the latter two absent in many species (Morton et al. 1995). Additionally, the spore walls of many species exhibit different types of ornamentations. Some AM fungi produce sporocarps (*i.e.*, aggregations of spores) that function in reproduction (Yamato et al. 2022) and dispersal, including dispersal by mammals (Mangan and 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 vectors), survival in the absence of the host (*e.g.*, resistance to desiccation and pathogenesis) and early colonization strategies (Chaudhary

Table 1 Key traits of arbuscular mycorrhizal (AM) fungi, their hypothesized function, and methods for trait measurement. Checkmarks (x) in the AM fungi, Plant, and Soil columns indicate where a trait's function is evidenced or has functional outcomes

Fungal and mycorrhizal traits	Hypothesized symbiotic effects	AM fungi	Plant	Soil*	Qualitative/quantitative (unit)	Method	References**
MORPHOLOGICAL TRAITS							
Spores							
Number	- fitness/competitive ability - dispersal - carbon storage	x x x		x	Quantitative: number of spores/g soil; Number of spores/meter of mycelia	Spores extracted by wet-sieving (Gerdemann and Nicolson 1963) and sucrose gradient centrifugation (Daniels and Skipper 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 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 and Schenck (1991); Spores over filter paper in a soil-filled Petri plate (Koske 1981)	Tommerup 1983; Maia and 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 and 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

Table 1 (continued)

Fungal and mycorrhizal traits	Hypothesized symbiotic effects	AM fungi	Plant	Soil*	Qualitative/quantitative (unit)	Method	References**
Sporocarps	- dispersal	x		x	Quantitative: sporocarp size Qualitative: presence of fungal peridium on the sporocarp surface	Sporocarps are measured under a microscope for size (Redecker et al. 2007) and presence of peridium (Schüßler et al. 2011)	Mangan and Adler 2002
Spore nuclear content	- spore viability and germination - colonization ability after dispersal	x x	x x	x	Quantitative: 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 Mycelium							
Length	- nutrient/water acquisition - carbon storage - soil aggregation - plant productivity - resistance to abiotic and biotic stress	x x x x	x x x x x	x x x x x	Quantitative: hyphal length in m/g dry soil	Miller et al. 1995 Baláz and Vosátka 2001	Wilson et al. 2009; Johnson et al. 2015
Architecture (branching rate, anastomoses rate, absorptive/runner hypha)	- nutrient/water acquisition - carbon storage - soil aggregation - plant productivity - resistance to abiotic and biotic stress	x x x x x	x x x x x	x x x x	Qualitative: architecture description Quantitative: Using image analysis in in vitro systems	Friese and Allen (1991) or Bago et al. (1998b) in monoxenic conditions Hammer et al. 2024	Knowledge gap (*e.g.*, is hyphal architecture akin to root architecture for nutrient acquisition? how does hyphal architecture influence soil aggregate stability?)
Inter-host connection (Common mycorrhizal network, CMN)	- transfer of nutrient/water/signals/cues among hosts - resistance to abiotic and biotic stress	x x	x x	x	Quantitative: amount of nutrient/signal/cue transferred Number of hosts connected by the same fungus	Weremijewicz and Janos 2019; Frey and Schüepp 1993	Knowledge gap (*e.g.*, are there fungi that interconnect more hosts than others? can 'Common Mycorrhizal Networks' trigger additional pathogen protection to hosts in the network?)
Hyphal diameter	- carbon storage - resistance to abiotic and biotic stress - palatability	x x x	x	x	Quantitative: in µm	Friese and Allen 1991	Klironomos and Kendrick 1996
Growth rate	- nutrient/water acquisition - carbon storage - resistance to abiotic and biotic stress	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

Table 1 (continued)

Fungal and mycorrhizal traits	Hypothesized symbiotic effects	AM fungi	Plant	Soil*	Qualitative/quantitative (unit)	Method	References**
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	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 and Schüepp 1993; Jakobsen et al. 1992a	Frey and 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 more melanized darker hyphae more resistant to fungivores?). > melanin content in dryer soils (Deveautour et al. 2020)
Wall/membrane chemical composition	- resistance to abiotic and biotic stress - nutrient/water acquisition - fungal recognition (anastomosis) - palatability	x x x x	x	x x	Quantitative: in µg	Bethlenfalvay et al. (1981) for chitin; Frey et al. (1992, 1994) for chitin and ergosterol; Butler and Lachance (1986) for melanin; Harrison and Vanbuuren (1995) for P transporters	Deveautour et al. 2020
Pattern of anastomosis	- fungal recognition - fitness	x 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
Intraradical Mycelium							
Hyphal thickness	- resource flux/exchange - resistance to abiotic and biotic stress	x x	x x		Quantitative: in µm	Abbott 1982	Knowledge gap (*e.g.*, are thicker hyphae more resistant to pathogens? Is there a trade-off between nutrient transfer and biotic resistance in terms of hyphal thickness?)
Pattern of colonization (localized / widespread)	- resource flux/exchange - resistance to abiotic and biotic stress	x x	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?)
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 - resistance to abiotic and biotic stress	x x	x x		Qualitative	Dickson 2004	van Aarle et al. 2005

Table 1 (continued)

Fungal and mycorrhizal traits	Hypothesized symbiotic effects	AM fungi	Plant	Soil*	Qualitative/quantitative (unit)	Method	References**
Turnover rate	- resource flux/ exchange	x	x		Quantitative: number of days	Alexander et al. 1989; Toth and 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 and 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?
PHYSIOLOGICAL TRAITS							
Acid and alkaline phosphatases	- nutrient/water acquisition - biological activity	x x	x		Qualitative: SDS-PAGE for activity Quantitative: hydrolysis of *p*-nitrophenol and spectrophotometry (µmol *p*-nitrophenol g^{-1}dry weight min^{-1})	Sato et al. 2015 Joner and Johansen 2000	Sato et al. 2015 Joner and Johansen 2000
N metabolism enzymes	- nutrient acquisition	x			Quantitative: enzyme activity (µmol mg^{-1} protein h^{-1})	- Synthetase assays, substrate disappearance, spectrophotometry, according to each enzyme (Cruz et al. 2007)	Cruz et al. 2007

Table 1 (continued)

Fungal and mycorrhizal traits	Hypothesized symbiotic effects	AM fungi	Plant	Soil*	Qualitative/quantitative (unit)	Method	References**
Inorganic polyphosphate (Poly-P)	- nutrient acquisition	x	x		Quantitative: (μg of P g^{-1} fresh weight of hypha) Qualitative: in polyacrylamide gel-electrophoresis	- Extraradical hyphae collected by wet-sieving and decanting, poly-P fractions extracted in aqueous solution and detected by reaction of toluidine blue (Solaiman et al. 1999) - Hyphal extracts desalted in gel-filtration column, incubated and transferred to 10% polyacrylamide gel (Ezawa et al. 2004)	Solaiman et al. 1999 Ohtomo and Saito 2005
Phosphate transporters	- nutrient acquisition	x	x		Qualitative: southern-blot and PCR to amplify phosphate transporter gene from spores	Harrison and Vanbuuren 1995	Sun et al. 2022
Lipid and fatty acid composition	- carbon storage	x			Qualitative: proportion of different types of fatty acids Quantitative: extraction, purification and analyses by gas chromatography (nmol mg^{-1} of mycelium/spore)	Extraction and separation via gas chromatography (Bentivenga and Morton 1996; Olsson and Johansen 2000)	Olsson et al. 1997
Spore/hyphal wall chemical receptors	- perception of host recognition and host/soil environmental signals/cues affecting spore germination	x	x			Knowledge gap (develop a method to identify specific receptors or use similar methods as those used in plant-AM fungal receptors)	Knowledge gap (are there specific receptors on the spore wall that trigger germination?)
GENETIC TRAITS							
Genome size	- reproductive rate - survival - plasticity and/or adaptive capacity	x x x x	x x x	x x x	Quantitative: size of genome in Mb (megabase)	Whole genome sequencing Flow cytometry (*e.g.,* Tisserant et al. (2013)	Sperschneider et al. 2023 Hosny et al. 1998
G+C content of the genome	- mycorrhizal host response - host preference	x	x x	x	Quantitative: % of GC content of the entire genome	Whole genome sequencing Knowledge gap (missing genomes across phylogeny)	Malar et al. 2022
Spore nuclear content	- spore viability and germination - colonization ability after dispersal	x x	x x	x	Quantitative: Number of nuclei per spore	Confocal microscopy, Flow cytometry	Kokkoris et al. 2020, 2021; Bianciotto et al. 1995; Marleau et al. 2011

Table 1 (continued)

Fungal and mycorrhizal traits	Hypothesized symbiotic effects	AM fungi	Plant	Soil*	Qualitative/quantitative (unit)	Method	References**
Genetic organization/ (homokaryon/dikaryon)	- fitness	x	x	x	Quantitative ddPCR: number of nuclei	Droplet Digital PCR (ddPCR) (Cornell et al. 2022)	Cornell et al. 2022; Serghi et al. 2021
	-nutrient/water acquisition	x	x	x			
	- carbon storage	x	x	x			
	- soil aggregation	x	x	x			
	- plant productivity	x	x				
	- resistance to abiotic and biotic stress	x	x				
	- hyphal network interconnectedness	x	x				
	- plasticity and/or adaptive capacity						
rDNA copy number	- survival and fitness	x	x	x	Quantitative: copies per genome	PacBio SMRT sequencing and Illumina sequencing for validation (Maeda et al. 2018)	Maeda et al. 2018
	- symbiotic efficiency	x	x	x			
	- plasticity and/or adaptive capacity	x					

*abiotic and/or biotic

**Examples/papers showing relationship(s) between trait measurement(s) and biological/ecological function(s). References might not necessarily represent best practice

et al. 2018). While the potential functions of most spore traits remain poorly understood (*e.g.*, we could not find a study exploring the potential functional implications of spore ornamentation; see Table 1 for more examples), trait-based studies are starting to emerge. For example, traits such as color, size, and abundance, can mediate the effects of disturbances like fire and grazing (Hopkins and Bennett 2023).

Intraradical mycelium (IRM)

The AM fungal mycelial system colonizes two distinct environments: the IRM which develops within plant roots in a consistent environment, and the ERM which extends into the soil, where it encounters, by comparison, highly variable environmental conditions (Smith and 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). Why these two types or arbuscules exist and can be formed by the same AM fungus is not well established. Studies making direct comparisons of trait efficiency under varying environments are needed to address this knowledge gap. 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 of the Gigasporaceae family (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 and 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 and Read 2008). They are not formed by members of Gigasporaceae, and there is some evidence that the same is true for basal families such as Ambisporaceae, Archaeosporaceae, and Paraglomeraceae. There is a paucity of studies solely investigating the ecological role of vesicles, particularly in symbiotic efficiency under environmental stress. However, given that they serve as energy reserve structures, understanding the role of vesicle formation on edaphic processes such as C turnover is an important knowledge gap.

Extraradical mycelium (ERM)

The 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 soil nutrient uptake and translocation to the host (Friese and 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). However, ERM morphology, encompassing hyphal diameter and architectural configuration, which, for example, regulate nutrient and C transport efficiency, is poorly understood. We posit that this is largely governed by fundamental physical principles. Hyphae with smaller diameters possess higher surface-area-to-volume ratio, potentially enhancing their capacity for nutrient absorption. However, according to the Hagen–Poiseuille law (Sutera 1993), smaller diameters increase resistance to fluid flow, thereby reducing efficiency in long-distance transport. Conversely, hyphae with larger diameters exhibit reduced internal resistance to flow but this advantage is offset by a lower surface-area-to-volume ratio, which may diminish nutrient uptake efficiency. Additionally, hyphal architecture is likely to determine fluid transport efficiency; simple, linear structures minimize resistance for direct transport, while highly branched hyphae enhance nutrient scavenging capabilities, but this might increase internal transport resistance.

The extraradical mycelium can form Common Mycorrhizal Networks (CMNs), where a single AM fungus can interconnect multiple plant hosts, facilitating resource exchange and, possibly, communication among hosts and AM fungi (Barto et al. 2011; Babikova et al. 2013). Perhaps the capacity of an AM fungus to form CMNs can be considered a fungal trait (Karst et al. 2023; Lehmann and Rillig 2024). Furthermore, novel traits specifically associated with CMNs could emerge. For example, hosts in a CMN can detect when one of the hosts in the network is under stress (*e.g.*, herbivory) (Babikova et al. 2013; Song et al. 2014); however, the mechanisms for this remain elusive. It can be hypothesized that AM fungi could gain an advantage by actively producing warning signals (Scott and Kiers 2025). However, the cost–benefit of producing such signals is unclear, considering competition among AM fungi forming CMNs and the fact that plants in communities may associate with different AM fungi (Schamp et al. 2025). A more plausible hypothesis for plant alert mechanisms via AM fungi is that these fungi simply act as passive conduits for unavoidable host cues, in which case such "signalling communication" may not be a trait *per se*. More research is certainly necessary to clarify the role of CMNs in natural ecosystems and to assess the extent to which the ability to form CMNs can be considered a fungal trait (Karst et al. 2023; Lehmann and Rillig 2024).

Physiological traits

The form and function of the main components of AM fungi (*i.e.*, spores, IRM, and ERM) are intrinsically associated with physiological traits. These consist mainly of mechanisms (*e.g.*, signaling) involved in spore germination and host recognition, enzyme activity, membrane transporters, and a wide range of biomolecules. Ultimately, they determine the resistance and resilience of AM fungi to specific environmental conditions and influence host and soil responses. For example, spores capable of germinating under extreme conditions or of storing energy to sustain asymbiotic growth for extended periods can be particularly important in some ecosystems. Despite their relevance, the physiological traits involved, and potential trade-offs remain poorly understood (see Akiyama and Hayashi 2006; Martin and van der Heijden 2024; Klein et al. 2024).

Emerging research links AM fungal physiological traits to enzyme production for soil nutrient acquisition and storage. Perhaps the most relevant and widely studied are acid and alkaline phosphatases secreted by hyphae, which facilitate the release of inorganic P from organic compounds. This is a critical trait particularly in low-P soils (Joner et al. 2000; Plassard et al. 2019). For example, the proportion of arbuscules exhibiting alkaline phosphatase activity showed a positive correlation with shoot weight and P content (Joner et al. 2000). AM fungi can also enhance the uptake, transport, and assimilation of soil NO_3^- and NH_4^+ into amino acids through the action of various enzymes (reviewed by Govindarajulu et al. 2005 and Jin et al. 2012). As experimental techniques such as in vitro root organ cultures, isotope labeling, and biochemical analyses of enzyme activity continue to evolve, so does the potential to link these physiological traits to AM functional roles in soil processes, plant community dynamics, and ecosystem function (*e.g.*, Hestrin et al. 2022).

A particularly relevant physiological trait is the ability of AM fungi to transport and store inorganic polyphosphate (Poly-P) (Ezawa et al. 2004). The uptake of inorganic phosphate (Pi) from the soil by the AM fungal mycelium is mediated by high-affinity Pi/H^+ transporters, which have been identified in *R. intraradices*, *F. mosseae*, *Gigaspora margarita*, and *Diversispora versiformis* (reviewed by Rui et al. 2022). Once Pi is transported into the fungal cytoplasm, it is incorporated into Poly-P and subsequently translocated to the IRM and arbuscules. Poly-P molecules may serve as a reservoir when soil P is abundant, which can then be used by the fungi when P becomes scarce, possibly driven by source-sink relations with host(s) (Bunn et al. 2024). The capacity of different AM fungi to rapidly store and transport Poly-P

can be seen as a key functional 'response' trait, reflecting both fungal resilience and variations in phosphate metabolism among different fungal taxa. For instance, Boddington and Dodd (1999) observed accumulation of Poly-P in the ERM of *Gigaspora rosea* but not in *R. manihotis* after a 10-week period, which could be related to different life-cycle strategies. Given the key roles of Pi transporters and Poly-P in the AM symbiosis, comparative studies should examine Pi transporter expression and Poly-P storage and transport in the IRM and ERM across Glomeromycota taxa.

AM fungi have distinct lipid and fatty acid metabolism compared to saprotrophic fungi and rely on host-derived lipids for growth and development (Luginbuehl et al. 2017). The lipid and fatty acid profiles are unique AM fungal traits used as independent criteria for testing phylogenetic hypotheses (Bentivenga & Morton 1996) and to estimate mycelium biomass in soil and roots (Olsson et al. 1995). Thus, fatty acid content is an indicator of C allocation to storage (Olsson et al. 1997). Recently, the phospholipid fatty acid 16:1ω5 has been used as an indicator of the presence of AM fungi; however, studies should also include non-mycorrhizal plants or mycelium-free compartments (Olsson & Lekberg 2022). While lipid and fatty acid profiles have been primarily characterized in Glomeraceae and Gigasporaceae, expanding investigations to other taxa is essential for a more comprehensive understanding of these physiological traits.

Another idea stemming from nutrient-storage processes may be the development of traits associated with post-mortem (necromass) ecological consequences of AM fungal traits. Koide et al. (2014) introduced the response-effect trait framework, which could be relevant for understanding how AM fungal traits influence ecological processes after fungal and plant death. For example, the potential roles of melanization in the survival of AM fungi (*e.g.*, protection against UV radiation) could be relevant here (Deveautour et al. 2020). Moreover, this trait could play a role in persistence post-mortem (Fernandez and Kennedy 2018).

A recent study on saprobic fungi providing a structured framework to understand physiological traits, can also help understand AM fungi (Camenzind et al. 2024a). Specifically, the authors posit that stoichiometric flexibility in saprobic fungi is a key trait to maintain growth under resource-limited conditions. Stoichiometric flexibility could also apply to the ability of AM fungi to adjust nutrient exchange rates with host plants or reallocate resources to support hyphal networks under stress (Camenzind et al. 2024b). For example, in P-deficient but nitrogen (N)-rich soils, AM fungi may increase P uptake efficiency by expressing more P transporters and phosphatases, and extending hyphae into nutrient-depleted zones. However, this change alters source-sink relations between symbionts with AM fungi requiring more C and the host more of the scarce P resources. Under drought, stoichiometric flexibility could enable AM fungi to mobilize stored resources (*e.g.*, specific lipids, polyP) to sustain existing hyphae over growing new ones. Tolerance to drought, heavy metals, and fungicides involves various physiological traits in saprotrophic fungi, which in some aspects may be analogous to AM fungi (*e.g.*, Hage-Ahmed et al. 2019; Riaz et al. 2021; Oliveira et al. 2022). We consider that further comparative studies between saprobic and AM fungi could help refine trait-based frameworks including physiological traits associated with nutrient acquisition, transfer and storage, C metabolism and symbiotic interaction traits such as those involved in host recognition.

Genetic traits

We define genetic measurements that have been proven or have the potential to reflect differences in life history strategies as "genetic traits". Among these traits are the genetic organization of AM fungal strains, spore nuclear content, genome size, rDNA copy number and G + C content of the genome (*i.e.*, percentage of nitrogenous bases in the DNA that are either guanine, G, or cytosine, C). 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) or two nuclear genotypes (*i.e.*, dikaryotic (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-history strategies or different life stages if the same is shown in multiple species (Serghi et al. 2021). Homokaryotic strains exhibit higher and faster germination rates compared to the lower germination rates observed in dikaryotic strains. Conversely, dikaryotic strains demonstrate faster growth and produce larger, more interconnected ERM compared to their homokaryotic counterparts. This difference in nuclear organization can significantly influence 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 functional trait, until homo- versus 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 AM fungal taxa.

The spore's nuclear content also seems to be associated with life history-traits, although further experimental evidence is needed. The range of nuclei present in spores correlates with spore size, ranging from 35,000 nuclei for spores of *Gigaspora decipiens* which have an average diameter of 400 µm, to 130 nuclei for the spores of *Glomus cerebriforme* with an average diameter of 80 µm (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* species. It has been hypothesized that the numerous nuclei could serve as resource reserve via nucleophagy when facing starvation, a phenomenon observed in other fungi (Shoji et al. 2010; Kokkoris et al. 2020).

Despite the number of genotypes and the number of nuclei present in AM fungal networks and spores, the overall genome size might influence the reproductive rate, environmental adaptability and 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 in plant community ecology. Grime and Mowforth (1982) linked plant genome size to climate growth conditions, Veselý et al. (2012) to early flowering events and preference for humid conditions, and Bhadra et al. (2023) 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*) 116 Mb (Kokkoris et al. 2020). Expanding our datasets with this information is important for uncovering connections between genome size and the morphological, physiological, and phenological traits of AM fungi.

A lesser explored but potentially significant genomic trait in AM fungi is the ribosomal DNA (rDNA) copy number per genome. In bacterial communities, variation in ribosomal RNA operon copy number has been linked to ecological strategies, where bacteria with low copy number are slow-growing but well adapted to resource-limited environments. In contrast, individuals with higher copy numbers tend to thrive in nutrient-rich conditions due to their rapid growth potential (Roller et al. 2016). Similarly, fungi can vary considerably in the number of rDNA copies they carry in their genomes, with previous estimates ranging from 14 to more than 1400 copies across 91 taxa exhibiting a strong phylogenetic signal but no clear correlation to ecological lifestyle (Lofgren et al. 2019). In AM fungi, the copy number of rDNA has been examined only for *R. irregularis* where a single genome contains up to 11 copies of rDNA (Maeda et al. 2018); an extremely low number when compared to other fungi and eukaryotes. Eukaryotes with exceptionally low rDNA copy numbers often share similar characteristics, such as symbiotic and asexually reproductive lifestyles, indicating niche preference (e.g., Dalrymple 1990; Gardner et al. 2002). It would be of interest to examine the rDNA copy variation across the AM fungal phylogeny to determine whether differences could provide insights into whether certain AM fungal species are more specialized for high- or low-nutrient environments or correspond to rhizophilic versus edaphophilic taxa, potentially linking rDNA copy number to habitat preference, colonization efficiency, and symbiotic compatibility.

Finally, a particular genetic trait to be considered is the G + C content of genomic DNA, which has the potential to reflect ecological niche or pathogenicity in fungi (Yoder and Turgeon 2001). Once again, despite limited data as few complete genomes are available, substantial variation in G + C content exists in AM fungi (range from 25 to 36%), which could potentially reflect differences observed in mycorrhizal response and host preference (Malar et al. 2022). With recent advancement in single-cell genomics, collection and characterization of genetic traits becomes increasingly more feasible, even for environmentally derived samples (*e.g.*, single spores).

The way forward on AM fungal traits research

Considering the above discussion, 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) *Share trait data through a centralized database of AM fungal traits*. This is a keystone task to integrate data into analyses aimed at predicting plant responses and ecosystem processes. Published databases of AM fungal traits include FUN^FUN, which describes spore size and shape (Aguilar-Trigueros et al. 2019), or are limited to mycorrhizal type and root colonization primarily at early stages of plant development (Soudzilovskaia et al. 2020). Certain trait data are also collated along with AM fungal culture collections (*e.g.*, INVAM, CICG, CCAMF, BEG), though public accessibility of such data is limited. Currently these trait datasets are not interoperable or harmonized. A new trait database, TraitAM, expands on FUN^FUN by incorporating additional spore traits, new calculated indices, and an updated phylogenetic tree to examine trait conservatism. It is expected to be pub-

licly accessible and downloadable in full format in 2025 and could provide a generic structure for which to build on future AM fungal trait data efforts (Chaudhary, personal communication). We propose either a new database or the integration of a generic structure for individual AM fungal taxa stemming from the traits proposed in Table 1, consistent with the principles of 'Observation and Measurement Ontogeny', into existing ones (Madin et al. 2007). The central focus of this database is the taxonomy at the species and strain levels, along with accession codes, if available. Ancillary data relate to: a) the site of origin, including latitude, longitude, date, and observer; b) the source, whether 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 (spores, mycelium, arbuscules, vesicles) and its measurement values and units.

2) *Determine traits at fine levels of taxonomic resolution*. Results from distinct experimental approaches indicate that AM fungal traits exhibit some conservation at the family level, although variation within these clades was also observed (Hart and Reader 2002b; Maherali and Klironomos 2012). Seemingly inconsistent with this finding, high intraspecific variability in root colonization, ERM 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.
3) *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, harboring 76% of the total number of species in the Glomeromycota. Other families such as Diversisporaceae, Entrophosporaceae, and basal families such as Paraglomeraceae and Archaeosporaceae are rarely included in experiments and there is very little information on their traits, despite being commonly present in communities. Glomeromycota comprise 12 families and 357 species but likely surpass that number by a factor of 5 to 15x (Öpik et al. 2010; Lutz et al. 2025). However, we estimate that only *ca.* 88 species are represented in culture collections worldwide.
4) *Measure and report AM fungal traits using standardized experimental approaches*. AM fungal traits reported in the literature have been assessed using diverse methodologies, complicating direct comparisons across studies. To address this challenge, we propose a standardized set of minimum parameters for studying AM fungal traits (see 'Experimental approaches' section below). A priority will be to validate the reproducibility of AM fungal trait measurements across different research teams, using the same starting inoculum material.
5) *Determine the variability (plasticity) of AM fungal trait expression*. Our understanding of AM fungal traits is limited by insufficient knowledge of their consistency under varying environmental conditions. Targeted experiments are needed to assess how specific environmental factors affect these traits. Furthermore, studying trait variation across traits and taxa could reveal whether some traits are more conserved or show greater variability within certain taxa.
6) *Utilize AM fungal isolates deposited in culture collections.* Culture collections worldwide uphold a considerable variety of AM fungal isolates cultivated in a mineral substrate and root organ cultures. These isolates are well characterized taxonomically, thereby representing important resources for comparative studies of trait variation. These centers play a key role in training personnel through specialized workshops that offer both hands-on experience and theoretical knowledge on measuring AM fungal traits.
7) *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. 2023a)), it is conceivable to conduct experiments (physical disturbance, nutrient additions, drought etc.) on whole natural AM fungal communities and examine 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 of AM fungi (*e.g.,* Violle et al. 2014).
8) *The use of microphotography, artificial intelligence (AI) and machine learning:* The integration of microphotography, AI and machine learning algorithms could not only help standardize and accelerate trait quantification and data integration, thereby eliminating the subjectivity of the observer, a common issue entangled with our current quantification approaches. Successful integration of the three 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 colo-

nization 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 above, key ecological functions of the AM fungal symbiosis (*e.g.*, host plant growth promotion), depend on both the fungal isolate's traits and its interaction with the host. While this may limit some trait measurements, the hypothesis-driven approach of linking trait and function(s) outlined here, though not addressing all ecological questions about AM fungi, marks a significant step forward.

Experimental approaches

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** (Fig. 2A) approach to examine 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 and data encompassing external hyphal length and spore numbers are gathered periodically. Other than the "sterile mesh bag" host biomass, root colonization and other variables can be measured. The approach allows measuring AM 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 over time, correlations between root colonization, spores, and external hyphae are more likely to represent realistic relationships among these traits. Nonetheless, this approach has potential limitations, including the need for propagules to penetrate the mesh bag, which may pose challenges for certain taxa (*e.g.*, Thonar et al. 2011). Additionally, analyzing multiple isolates or species simultaneously can be time-consuming and labor-intensive.

Direct measurement of fungal traits may require separating hyphae from the substrate altogether, which can be achieved using a slight variation of the sterile mesh bag. Zhang et al. (2023a) used **hyphal in-growth bags** (Fig. 2A) (glass beads with silt/clay to which a dilute nutrient solution was added, enclosed in 38 µm nylon mesh) to harvest mycelium for C, N, and P analysis after eight weeks (Neumann and George 2005). Bags (2 × 10 cm) containing 40 g of the mixture were buried in the top 10 cm of pots. The mesh blocked *Medicago sativa* roots but not *Festuca arundinacea* root hairs, which required 10 µm mesh (Zhang et al. 2021). Soil particles adhering to hyphae must be removed before analysis. This method, unlike sterile mesh bags, better suits physiological studies.

A third approach was used by Jakobsen et al. (1992a) to study AM fungal hyphae abundance in soil and P uptake into roots. **Root compartment bags** (Fig. 2B) consist of cylindrical (60 mm diameter) bags constructed using a 25 µm nylon mesh filled with AM fungal inoculum. Bags are placed in 1.5 L pots with steamed sterilized soil, and pre-germinated seeds are transplanted into each bag, confining roots within the bag while allowing AM fungal hyphae to extend into the surrounding soil. After 25 days, the bags are transplanted into rectangular PVC boxes (300 × 185 × 130 mm) containing 7 kg of steamed dry soil. Hyphal growth is measured by collecting 10 mm soil cores on five dates at various distances from the root compartment. The root compartment method, like the sterile mesh bag approach, enables hyphal length

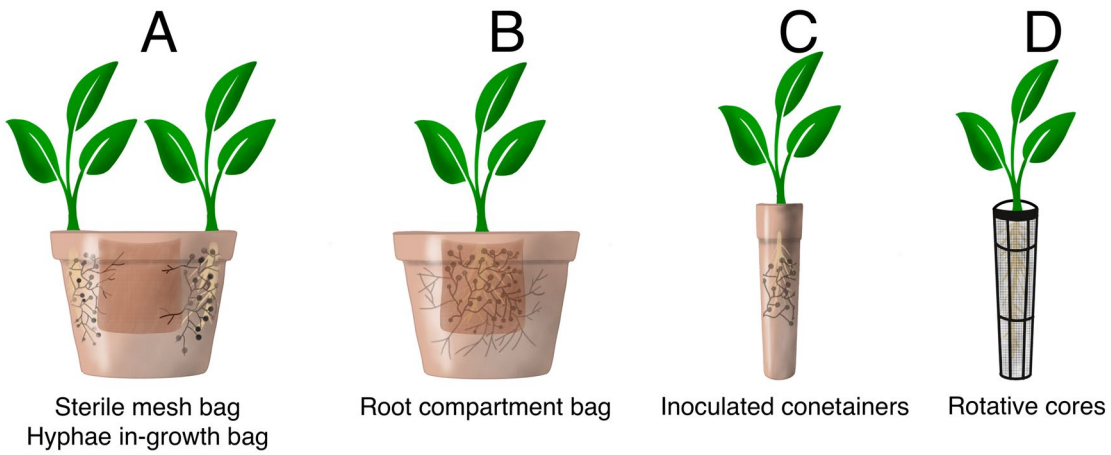

Fig. 2 Illustration of various approaches to study AM fungal traits, which can be used either in the greenhouse or the field. Illustration created by Pedro M. Antunes using Procreate

measurements in a mycorrhiza-free environment. Transplanting the compartment into large boxes makes it ideal for studying AM fungal hyphal spread and comparing fungal taxa; however, it requires a substantial amount of soil. A variation of the method including a trap plant allows measurements of resource movement between hosts in a community (Mikkelsen et al. 2008) or compartments separated with an air gap for measurements of water transport when combined with stable isotopes and dyes (Kakouridis et al. 2022).

A fourth approach, named **inoculated containers** (Fig. 2C)**,** was used by Hart and Reader (2002b) to establish the taxonomic basis for the variation in root colonization strategies observed among AM fungal 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 it has been shown that AM fungi do not produce ergosterol (Olsson et al. 2003; Olsson and Lekberg 2022). Containers (also known as cone-tainers) (4 cm diameter × 20.5 cm deep) are $^{2}/_{3}$ filled with soil, inoculated with spores, hyphae, and colonized root fragments, and sown with leek as a surrogate host. After 30 days, shoots are harvested, and soil undergoes experimental treatments with different hosts. Containers are harvested six times over 12 weeks to measure root and soil colonization. This small-container approach enables studying multiple isolates over time, with standardized fungal biomass allowing direct taxonomic comparisons. However, as hyphal abundance is measured in the same container as the inoculum, distinguishing new hyphae from the original ones is not possible.

Johnson et al. (2001) introduced a method using **rotative cores** (Fig. 2D) to study CMNs. Conical containers (270 mL) with a 2 × 5 cm slot covered by 40 µm nylon mesh (although the whole container can be covered with a mesh) or a hydrophobic membrane are filled with soil-sand mixtures, inoculated with AM fungi, and seeded with a host plant. After 2–3 months of CMN establishment, treatments are applied by either keeping containers static (undisturbed CMNs) or rotating them to sever hyphal networks. Despite being labor-intensive, this method is effective for studies on CMNs and effects of hyphal disruption on soil (*e.g.*, bacterial structure, aggregation) and plant (*e.g.*, biomass, nutrient allocation) (Babikova et al. 2013). It can also be used to assess physiological aspects of the ERH by adding tracers that only hyphae have access to (Lekberg et al. 2024).

The study of genetic traits typically requires the use of isolation techniques into in vitro culture (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 soil biota (Faghihinia et al. 2023; Vieira et al. 2025).

Despite extensive research, a comprehensive understanding of AM fungal traits across taxa remains elusive due to experimental variability (*e.g.*, host plants, soil type, fertilization, environment). We propose standardizing key experimental items while collecting non-standardizable factors (*e.g.*, soil type, lighting) as metadata. This approach enables experiments across labs using the same AM fungal taxa under varied conditions (*e.g.*, disturbance, salinity, drought, CO_2, temperature, light) to assess trait conservation and prediction accuracy based on taxonomy.

Standard plant-growth conditions

Standardizing mycorrhizal fungal trait quantification improves data quality, reduces bias, and enhances study comparability and reproducibility. This consistency strengthens meta-analyses and fosters collaboration, advancing our understanding of the AM symbiosis. Based on the authors' expertise, we propose guidelines for standardizing trait measurements. However, potentially necessary deviations from these recommendations, if well-documented, remain valuable for understanding AM fungal life histories.

a) Pot Size and Type: Measuring AM fungal traits often involves numerous experimental units. To ensure feasibility, pot size and substrate are crucial considerations. For mesh bags, pots over 2 L are recommended to avoid root bounding issues. For inoculated containers or rotative cores placed in a larger pot, conical containers (4 cm diameter × 20.5 cm deep) with open bottoms are ideal (Weremijewicz and Janos 2019).
b) Soil texture: Soil texture influences AM fungal mycelium production and sporulation by affecting pore space for growth. Inert media like sand:expanded clay could be used to standardize the substrate. While these media have the advantage of not containing AM fungal propagules and facilitate spore and ERM hyphal extraction at the end of the experiment, they hardly represent the common habitat of AM fungi. We recommend using sterilized loam soil for standard experiments, as it is easier to handle, facilitates root washing, and provides a more representative soil type. If unavailable, adding quartzite or coarse river sand to adjust texture is suggested to bring the texture closer to that of 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 24 h interval. However, if possible, gamma radiation is a useful but costly alternative that limits chemical alteration of organic matter and downstream consequences on dissolved organic C,

aggregate stability and manganese toxicity (Boyd 1971; Berns et al. 2008).

d) Nutrient solution: Plant nutrition is an important aspect to be considered as it impacts the establishment and functioning of the AM symbiosis. We suggest the use of a low P (*i.e.*, 0.4 mM of KH_2PO_4) half-strength Hoagland's solution for monocots to provide the minimum amount of macro and micronutrients to the host.

e) A microbial wash, prepared by mixing AM fungal inocula with water followed by filtration through a 20 µm sieve, is typically added to test pots (Ames et al. 1987). As this step is challenging to standardize, we recommend including a control treatment without the wash to evaluate the microorganisms' impact 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 root colonization and hyphal growth. Host preference is another factor to consider, as it impacts 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 AM fungal germplasm in culture collections (Morton et al. 1993); b) it has a fasciculate 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.

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Author contributions PMA and SLS developed the manuscript's concept and co-wrote the manuscript. All authors actively participated in the editing and revision process, provided final approval for publication, and agreed to take responsibility for the integrity and accuracy of the work presented.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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