



Exploring the secrets of hyphosphere of arbuscular mycorrhizal fungi: processes and ecological functions

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Abstract

Background Most plants have a hyphosphere, the thin zone of soil around extraradical hyphae of arbuscular mycorrhizal (AM) fungi, which extends beyond the rhizosphere. This important interface has critical roles in plant mineral nutrition and water acquisition, biotic and abiotic stress resistance, mineral weathering, the formation of soil macroaggregates and aggregate stabilization, carbon (C) allocation to soils and interaction with soil microbes.

Scope This review focuses on the hyphosphere of AM fungi and critically appraises the important findings related to the hyphosphere processes, including physical, chemical and biological properties and functions. We highlight ecological functions of AM fungal hyphae, which have profound impacts on global sustainability through biological cycling of nutrients, C sequestration in soil, release of greenhouse gas emissions from soil and

the diversity and dynamics of the microbial community in the vicinity of the extraradical hyphae.

Conclusions As a critical interface between AM fungi and soil, hyphosphere processes and their important ecological functions have begun to be understood and appreciated, and are now known to be implicit in important soil processes. Recent studies provide new insights into this crucial zone and highlight how the hyphosphere might be exploited as a nature-based solution, through understanding of interactions with the microbiome and the impacts on key processes governing resource availability, to increase sustainability of agriculture and minimize its environmental impact. Uncovering hyphosphere chemical and biological processes and their subsequent agricultural, ecological and environmental consequences is a critical research activity.

Keywords Arbuscular mycorrhizal (AM) fungi · Hyphosphere · Extraradical hyphae · Physiochemical process · Biological interaction · Nutrient cycling

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Introduction

It is well-known that the rhizosphere, defined by Hiltner (1904) as the thin layer of soil surrounding the living roots, is a critical and active zone due to the physical, chemical and biological changes induced by roots (Marschner 1995). Subsequently, a large number of studies about rhizosphere processes have demonstrated that the rhizosphere differs from the bulk soil regarding pH (George et al. 2002), water and redox potential (York et al. 2016), porosity (Koebernick et al. 2017, 2019), concentrations of soluble carbohydrates and nutrients (Lambers et al. 2006, 2009; Lambers 2022), biochemical properties (Zhang et al. 2010), and the structure and functioning of microbial communities (Besset-Manzoni et al. 2018). However, this active zone of soil is not influenced only by plant roots.

Arbuscular mycorrhizal (AM) fungi are obligate biotrophs that colonize more than two thirds of terrestrial plant species to establish a symbiotic relationship (Smith and Read 2008); they have been forming relationship with plants ever since plants first colonized land approximately 450 million years ago (Delaux and Schornack 2021). As pivotal members of the soil microbiome, AM fungi provide multiple services to their host plants, such as mineral nutrition (especially phosphorus uptake), water acquisition, biotic and abiotic stress resistance, and other ecological functions, such as improving ecosystems diversity and productivity (van der Heijden et al. 2015). Importantly, AM fungi provide an alternative and important nutrient acquisition pathway to that of plant roots from soil (Bucher 2007; Smith et al. 2011; Smith and Smith 2011). For example, in many cases, the contribution of the mycorrhizal pathway to total P uptake is greater than that of direct root pathway (Chu et al. 2020; Nagy et al. 2009), and can even completely replace the direct root pathway (Smith et al. 2003, 2004).

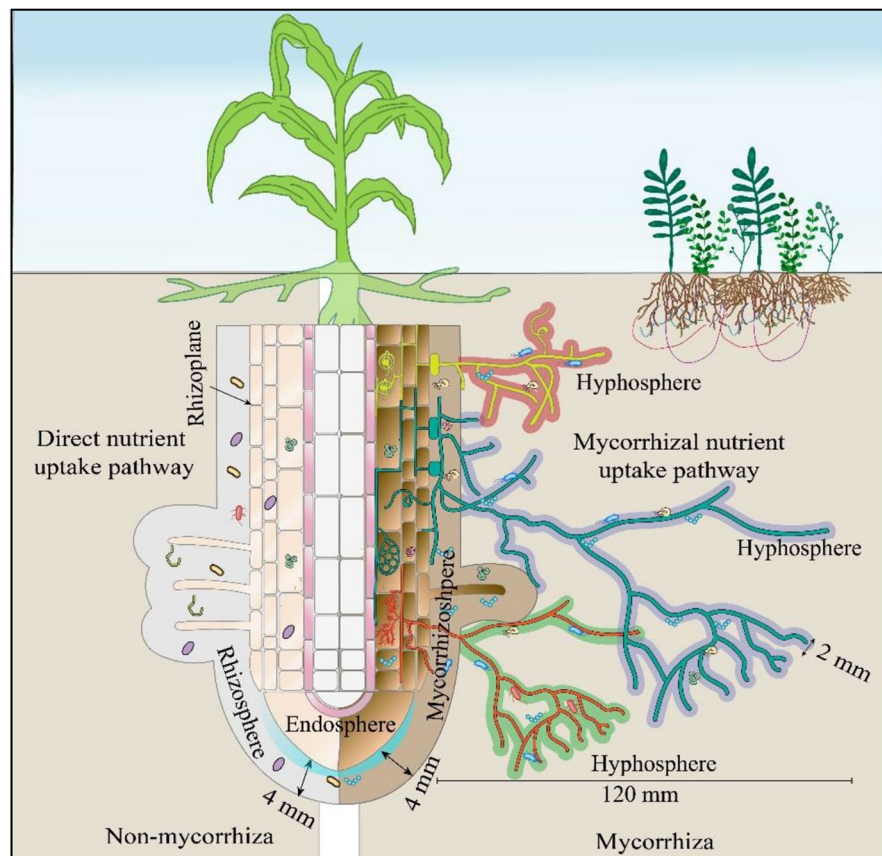
When plant roots are colonized by AM fungi, the rhizosphere includes not only roots but also mycelium of AM fungi, whereby mycelial activity significantly changes the rhizosphere properties; moreover, the mycelium extends beyond the rhizosphere, thus influencing soil properties in the zones beyond the reach of the root impacts. It has been demonstrated that colonization of roots with AM fungi influences plant metabolism and causes changes in plant assimilates, which strongly inhibits activity of manganese (Mn)-reducing bacteria, for example, at the

interface between soil and the fungi-root symbionts and decreases Mn concentration in plants (Arines et al. 1992; Kothari et al. 1991b; Posta et al. 1994). A large number of researches further demonstrated that the biodiversity, abundance and assembly of microbial community located in the inner root space, rhizosphere and hyphosphere are completely different (Bai et al. 2015; Bulgarelli et al. 2012; Toljander et al. 2007). Moreover, different AM species co-colonizing a single plant root system recruit distinct microbiomes in the hyphosphere (Zhou et al. 2020). Therefore, it is necessary to re-examine the definitions of the soil zones/volumes that are directly influenced by plant roots or AM fungal hyphae or where they interact. The following terms are suggested as a nomenclature for the range of habitats in and around roots (Table 1). The endosphere is defined as inner root tissues (including rhizodermis, cortex and stele) inhabited by microorganisms. Rhizosphere is defined as the thin layer of soil surrounding the living roots not colonized by mycorrhizal fungi. This zone (including the root surface and the adjoining soil) is directly influenced by root exudates. Mycorrhizosphere is defined as a zone of soil surrounding the living roots colonized by mycorrhizal fungi. This zone (including the root and hyphal surface, and the soil adjoining the roots and hyphae) is directly modified by mycorrhizal fungi mediating root exudates and hyphal exudates. The unique feature of the mycorrhizosphere is an overlap of mycorrhized roots and hyphae. The width of mycorrhizosphere was reported to be broader than that of rhizosphere, and the biochemical activities were stronger than those in rhizosphere, at least in terms of phosphorus depletion and pH changes (Li et al. 1991a, c; Joner and Jakobsen 1995; Song et al. 2000). Hyphosphere is defined as a critical and active zone of soil surrounding the living extraradical mycelium of mycorrhizal fungi beyond the rhizosphere and mycorrhizosphere. This zone (only including the hyphal surface and the soil adjacent to the hyphae) is directly influenced by exudates of mycorrhizal fungi (Fig. 1, Table 1). This more precise nomenclature will allow us to highlight the specific effects of AM fungi on the metabolism of plant roots and will be helpful to understanding of the in situ biological, physical and chemical process in the plant root-mycorrhizal-microbe-soil continuum.

Another important consideration is that the diameter of AM hyphae (2–10 μm) is up to two

Table 1 The definition of the different spheres

Glossary	Definition
Endosphere	inner root tissues (including rhizodermis, cortex, and stele) inhabited by microorganisms
Rhizosphere	the thin layer of soil surrounding the living roots not colonized by mycorrhizal fungi. This zone (including the root surface and the adjoining soil) is directly influenced by root exudates
Mycorrhizosphere	a zone of soil surrounding the living roots colonized by mycorrhizal fungi. This zone (including the root and hyphal surface, and the soil adjoining the roots and hyphae) is directly modified by mycorrhizal fungi mediating root exudates and hyphal exudates
Hyphosphere	a critical and active zone of soil surrounding the living extraradical mycelium of mycorrhizal fungi beyond the rhizosphere and mycorrhizosphere. This zone (only including the hyphal surface and the soil adjacent to the hyphae) is directly influenced by exudates of mycorrhizal fungi

Fig. 1 Definition of the range of spheres influenced by root or by AM fungi in soil, forming the endosphere, rhizosphere, mycorrhizosphere and hyphosphere

orders of magnitude thinner than that of fine roots (around 0.2 mm or less); hence, the hyphal length density in soil (exceeding 10^8 m/m³) is much greater than that of roots (*ca.* 10^4 m/m³) (Allen 2007; Miller et al. 1995) (Table 2). Moreover, hyphae of AM fungi produce numerous branches (Friese and Allen 1991), thus enlarging the effective root surface area and its contact with the soil

(Lanfranco et al. 2018). Compared with the root, morphological advantages of mycelia imply that the hyphosphere, in addition to rhizosphere/mycorrhizosphere, is a critical and active soil zone providing a hotspot of activity in which physical, chemical and biological changes and functions are controlled mainly by the hyphae of AM fungi.

Table 2 Comparison of vital properties of the rhizosphere and hyphosphere

Properties	Root/Rhizosphere	Hyphae/Hyphosphere	References
Morphological	Fine root diameter less than 0.2 mm; <i>ca.</i> 10^4 m/m ³ in root length density	Hyphae diameter 2–10 μ m; > 10^8 m/m ³ in hyphal length density	Allen 2007; Miller et al. 1995
Effective zone	0–4 mm from root surface and up to meters in depth down the profile	0–2 mm from hyphal surface and extending 120 mm beyond root surface, and up to meters in depth	Gai et al. 2015; Kuzyakov and Razavi 2019; Li et al. 1991a, b, c; Manschner 1995; Oehl et al. 2005
Physical	Penetration and biopore distribution in soil; Water flow and root hydraulic lift; Rock weathering	Hyphal entanglement or stickiness; Soil aggregate formation and water stability; Water flow and hyphal hydraulic lift; Common mycorrhizal network mediated water redistribution of root hydraulically lifted water; Highway for bacteria movement; Rock weathering	Egerton-Warburton et al. 2008; Jiang et al. 2021; Rillig and Mummey 2006; Thorley et al. 2015; Verbruggen et al. 2021; Zhang et al. 2010
Chemical	pH or/and Eh changes; Nutrient depletion and mobilization; Mineral weathering	pH or/and Eh changes; Nutrient depletion and mobilization; Mineral weathering	Ding et al. 2011; Hinsinger 2001; Kothari et al. 1991b; Li et al. 1991b; Wang et al. 2013; Zhang et al. 2016, 2018a, b
Biological	C deposition and root exudates; Signalling; Priming effects and nutrients cycling; Governing microbiome structure and root-microbiome interactions	C deposition and hyphal exudates; Signalling; Priming effects and nutrients cycling; Governing microbiome structure and root-microbiome interactions	Bharadwaj et al. 2012; Emmett et al. 2021; George et al. 2002; Jakobsen et al. 2021; Jansa and Hodge 2021; Richardson et al. 2009; Scheublin et al. 2010; Svenningsson et al. 2018; Tisserant et al. 2012

Even though the ecology of the rhizosphere has been investigated for more than 100 years, exploring the nature of hyphosphere interactions has received attention only in the last 30 years. Nevertheless, many ecological processes and functions in the hyphosphere have been characterized using innovative research methodologies (e.g. rhizoboxes, root and AM fungi dual culture, ^{13}C -SIP-DNA/RNA analysis, NanoSIMS, quantum dot labelling, microfluidic chip, etc.). This Marschner review aims to critically evaluate the current knowledge on the processes, ecological functions and importance of the AM hyphosphere.

Hyphosphere processes

Until recently the limitations of research methods meant it was difficult to differentiate the effects of AM fungi and plant roots in the mycorrhizosphere because the fungi and roots grew in the same compartment. In the last 30 years specific techniques have been employed to disentangle the effects in the rhizosphere/mycorrhizosphere and hyphosphere. For example, development of compartmented systems (Kothari et al. 1990; Li et al. 1991a, b, c), using nylon mesh to separate the physical spaces that plant roots and AM fungal hyphae can occupy, has provided new insights into how the extraradical mycelium mediates soil processes. The most compelling findings show that the fungal hyphae, invisible to the naked eye, can cause a range of profound physical, chemical and biological changes to the hyphosphere. Moreover, these changes are of the sufficient magnitude for the hyphosphere to be considered significant within the context of the global carbon (C), nitrogen (N) and phosphorus (P) cycles and their consequent environmental impacts.

Hyphosphere: physical processes

Soil macroaggregate formation and stabilization

Hyphae of AM fungi can promote soil aggregate formation and stabilization by mechanical entanglement or by promoting stickiness (He et al. 2020; Rillig and Mummey 2006) through the production of gelling exudates (Table 2, Fig. 2), independently of the soil organic C (Leifheit et al. 2014; Peng et al. 2013), soil texture and carbonate content (Kohler

et al. 2017). In a similar fashion to plant roots, extraradical hyphae of AM fungi serve to enmesh and entangle soil particles, organic materials and small aggregates, facilitating macroaggregate (> 250 μm diameter) formation and enhancing soil structure stabilization (Morris et al. 2019; Rillig et al. 2010). For example, Degens et al. (1996) reported that increases in aggregate stability could be attributed largely to the increased growth of hyphae in a sandy soil. The stabilization of soil aggregates was apparently increased with the increasing proportion of macroaggregates when the hyphae of six different species of the genus *Glomus* were present in the hyphosphere (Bearden and Petersen 2000). The recent results from a two-compartment system showed that greater hyphal length contributed to the formation and stability of soil water-stable macroaggregates under both well-watered and drought stress conditions (Ji et al. 2019).

Some compounds (e.g. glomalin or glomalin-related soil proteins (GRSP), mucilage, polysaccharides, etc.) released by extraradical mycelia of AM fungi are involved in soil aggregate formation and stabilization (Rillig 2004; Rillig and Mummey 2006; Rillig et al. 2001; Singh et al. 2013) (Fig. 2). Although these compounds are not uniquely produced by AM fungi, they are regarded as “glue” tightly binding to soil particles and play a dominant role in converting clay mineral to structured aggregations. For example, exudation of glomalin (a specific immunoreactive glycoprotein secreted into the soil by hyphae and spores of AM fungi, Wright and Upadhyaya 1998) or GRSP correlated positively with soil aggregate stability in natural systems. A recent review suggested that glomalin is a 60 kDa heat shock protein (HSP60), both AM fungi and other soil organisms can produce HSP60 (Irving et al. 2021). Path model analysis showed that the easily extractable GRSP and total GRSP exhibited significant positive effects on mean weight diameter of water-stable aggregates (Ji et al. 2019). Additionally, total GRSP content and the proportion of soil water-stable macroaggregates > 5 mm in diameter were significantly increased in an experimental hyphal compartment under both well-watered and drought stress conditions (Ji et al. 2019). In brief, GRSP can improve aggregate formation and enhance soil stability by binding to soil particles.

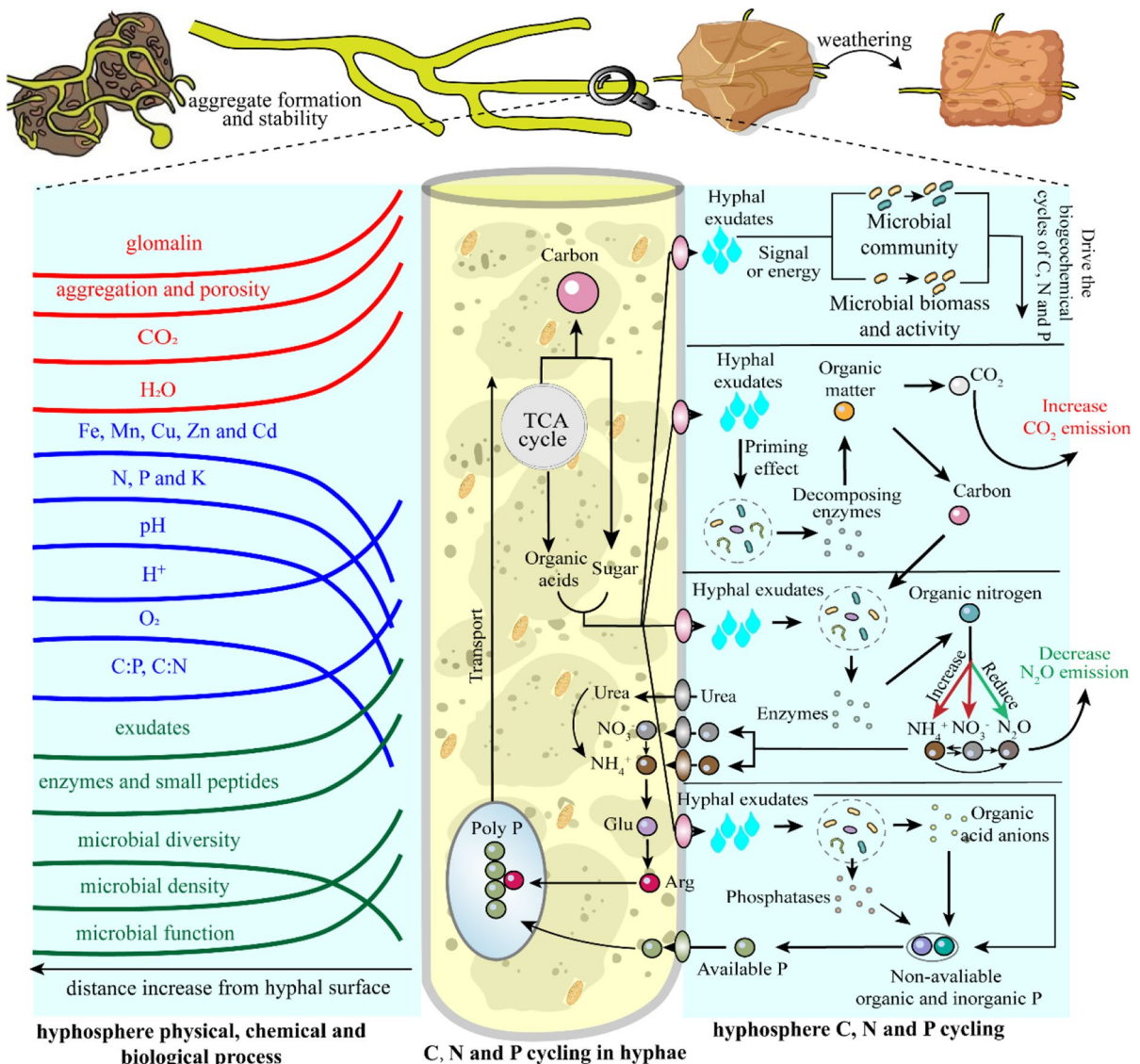


Fig. 2 The AM hyphae-mediated physical, chemical and biological changes and nutrient cycling in the hyphosphere. The lines with different colors on the left depict physical, chemical and biological processes at the hyphae-soil interface. Physical processes (red line) include soil aggregate formation and stabilization, physical weathering, acquisition, utilization and redistribution of soil water. Chemical processes (blue line) involve metal cation adsorption, nutrient depletion, acidification and oxidation–reduction. Biological processes (green line) include exudates, enzymes, small peptides and microorganisms. The cylinder in the centre depicts the hyphae and contains information on the internal carbon, nitrogen and phosphorus cycling mediated by transporters, permeases and metabolic pathways in the extraradical hyphae. The four zones on the right depict carbon, nitrogen and phosphorus cycling and their subsequent

environmental consequences in the hyphosphere mediated by exudates of extraradical hyphae. These mycelial exudates contain low-molecular-weight compounds, such as glucose, fructose, oligosaccharides and amino acids, and they could serve as a C sources substrate or signalling molecules to stimulate growth and activity of AM fungi-associated bacteria. These bacteria are involved in decomposing organic matter through decomposing enzymes, and consequently release CO_2 . The N cycle in the hyphosphere is primarily controlled by microorganisms (saprotrophic fungi, bacteria, protists, etc.), and they are involved in transformation of inorganic and organic N forms by different pathways. The AM fungi-associated bacteria stimulated by mycelial exudates secrete phosphatase and organic acid anions to mobilize sparingly soluble inorganic and organic P, and consequently promote plant P uptake

Mineral weathering by AM hyphae

AM fungal hyphae can play an active role in parent mineral weathering (Koele et al. 2014; Quirk et al. 2012; Thorley et al. 2015; Verbruggen et al. 2021) (Table 2, Fig. 2). Fossil evidence for biotite etching and weathering by AM fungal hyphae in the Miocene (5–23 Mya) was found (Sanz-Montero and Rodríguez-Aranda 2012). Moreover, laboratory experiments have also demonstrated that inoculation with AM fungi accelerated biotite weathering and formation of low-potassium clay minerals (Arocena et al. 2012).

The mechanisms of mineral weathering by AM hyphae are diverse (Verbruggen et al. 2021). On the one hand, they can trace the contours of particle surfaces or strongly adhere to mineral surfaces and exert forces using high turgor pressures, subsequently entering pores and fissures in minerals (Thorley et al. 2015) and acting as efficient sinks for ions released by the mineral weathering (Quirk et al. 2015). On the other hand, AM hyphae may also form weathering trenches in silicate mineral surfaces buried in proximity to roots of trees (Quirk et al. 2012, 2014). However, hyphal tunnelling appears to be slow and contributes only modestly to weathering, accounting for around 2% of total feldspar weathering in a temperate coniferous forest (Smits et al. 2005). Additionally, GRSP secreted by extraradical mycelia of AM fungi (Wright and Upadhyaya 1998) may play important roles in mineral weathering by increasing the amount of soil aggregates, or by enhancing biological and chemical weathering of small soil particles.

AM fungal mycelium mediated redistribution of soil water

Arbuscular mycorrhizal fungi are well known for improving water acquisition and utilization by plants, enhancing drought tolerance of plants through extension of root growth and greater hyphal water absorption rate (Allen 2007). They therefore play a major role in water redistribution processes in the soil–plant system (Table 2, Fig. 2). The external hyphae can also directly absorb and transport water to their host plants from the soil (and even from the bulk soil beyond the influence of roots by extension of the extraradical mycelium) via penetration into soil pores and enlargement of the absorptive surface area (Allen 2007; Faber et al. 1991; Querejeta 2017), although

contribution from extraradical mycelium to plant water metabolism is relatively smaller than roots (Hallett et al. 2009; Khalvati et al. 2005; Püschel et al. 2020; Ruiz-Lozano and Azcon 1995). Using mycorrhiza-resistant mutants and wild type plants, Bitterlich et al. (2018) found that arbuscular mycorrhiza improved hydraulic conductivity of the substrate (with root excluded) in the range of plant-relevant water availabilities.

Hyphosphere: chemical processes

Precipitation–dissolution and adsorption–desorption

In neutral or alkaline soils, P ions precipitate as calcium phosphates, whereas Fe and Al have a high capacity to combine with P ions under acidic conditions. The dissolution of these types of P minerals depends on the soil pH and anion exchange reactions. Decreased soil pH is associated with increased solubility of calcium phosphates, whereas soil alkalization results in Fe–P and Al–P solubilization (Hinsinger 2001). Mycorrhizal hyphae are known to change the pH of the hyphosphere and thus influence P availability. By contrast, there is no convincing evidence to demonstrate that AM fungi have a capacity to mobilize sparingly soluble phosphates by releasing organic ligands or anions to compete with P ions for adsorption on the soil exchange complex.

Arbuscular mycorrhizal hyphae are implicated in detoxification of metals and have strong metal adsorption capacity relative to other microorganisms (Table 2, Fig. 2). The competition between Cd^{2+} , Ca^{2+} and Zn^{2+} ions for adsorption sites on AM hyphae seems to favour Cd^{2+} over Ca^{2+} and Zn^{2+} (Joner et al. 2000). This may be due to the presence of cysteine ligands in AM fungi, forming a class of "metallothionein" binding substances. In addition to these organic ligands, metals were also adsorbed to inorganic binding sites in the cell walls of AM fungal hyphae. Some free amino acids and carboxyl, hydroxyl, phosphate and sulfhydryl groups in mycelium cell walls are all negatively charged and can adsorb metal cations, thus preventing them from entering the fungal cells (Meier et al. 2012). Another possibility is that the mycelia of AM fungi immobilize Cd and consequently reduce Cd concentrations and accumulation in shoots (Luo et al. 2017; Rask et al. 2019). In this way, AM fungi substantially reduce the harmful effects of Cd for

human health. Additionally, the glomalin was incorporated in the cell wall of AM mycelia and spores. Upon release of glomalin into soil, it formed GRSP (Rillig 2004). The content of GRSP in soil was significantly positively correlated with the concentration of metals (Vodnik et al. 2008). In metal-contaminated soils, GRSP can adsorb metals and decrease their availability. This has implications for the increasing use of poor-grade rock-phosphates as fertilizer, which tend to have relatively high metal contamination. By promoting crops colonized with mycorrhizal hyphae, there is opportunity to increase the utilization of the remaining sources of rock phosphate and extending the timespan of global phosphate reserves.

Chemical weathering of parent material

Most minerals are dissolved in acidic microenvironments, and acidification is one of mechanisms underpinning mineral weathering by AM fungi and leads to the replacement of cations with protons on the mineral surface. Arbuscular mycorrhizal hyphae can release protons (Wang et al. 2013), influencing mineral weathering. Alkali cations such as Na^+ , K^+ and Ca^{2+} on rock surfaces can be released via exchange with protons (Table 2).

Other mechanisms by which AM fungi stimulate weathering are chelation reactions. Some organic acid anions chelate metal ions (Fe^{3+} , Al^{3+} , Cu^{2+} , Zn^{2+} , Mn^{2+} , etc.), significantly increasing the mobility of these elements (Landeweert et al. 2001). Arbuscular mycorrhizal hyphae can exude sugars and organic acid anions (Bharadwaj et al. 2012; Luthfiana et al. 2021; Toljander et al. 2007) that can act as chelators to destabilize mineral surfaces, increasing weathering potential. Moreover, the turnover of organic compounds, hyphae and bacteria associated with them increases respiration on mineral surfaces, which further stimulates silicate weathering (Verbruggen et al. 2021).

pH variation

It is generally recognized that acidification is one of the important mechanisms of mobilization of sparingly soluble phosphates in the rhizosphere (Andersson et al. 2015, 2016; Ding et al. 2011) or hyphosphere (Li et al. 1991b; Wang et al. 2013) in neutral-to-alkaline soils (Table 2, Fig. 2). Many studies have reported that the imbalanced uptake of anions and cations by

plant roots or AM mycelia induce variation of pH in the rhizosphere (Andersson et al. 2016) or hyphosphere (Wang et al. 2013). Reduction of soil pH (by 0.2–0.5 units) was detected at both the root-soil and the AM hyphae-soil interfaces when the soil was supplied with NH_4^+ under axenic conditions (Bago et al. 1996; Ding et al. 2011; Li et al. 1991b; Villegas and Fortin 2001, 2002; Villegas et al. 1996; Wang et al. 2013). The fungi prefer ammonium to nitrate because of the greater costs of metabolic energy associated with absorption and assimilation of nitrate compared to ammonium (Hawkins et al. 2000). Acidification in the hyphosphere induced by NH_4^+ uptake enhanced mobilization of sparingly soluble inorganic phosphates through releasing phosphate ions combined with Ca^{2+} or mineralization of organic phosphates by increasing phosphatase activity in calcareous soils (Li et al. 1991b; Wang et al. 2013).

Redox reactions

For some metal elements with polyvalent states, such as Mn and Fe, their availability in soil usually depends on a soil oxidation–reduction potential and protons and electron-carrying reducing agents excreted by plant roots and microorganisms (Table 2, Fig. 2). The reduced forms of these elements are available to plants (Marschner 1988). Generally, oxidation reactions are mostly biological, but Mn reduction may be either biological or chemical in nature (Rengel 2000).

Depletion of oxygen in the growing medium changes the redox potential, with Mn and Fe serving as alternative electron acceptors for microbial respiration and are transformed into reduced ionic forms. This process increases the solubility and availability of Mn and Fe, but their availability is a complex variable that depends on plant genotypes, soil chemistry and microbial activity (Rengel 2015). For instance, an increase in the number of Mn-oxidizing bacteria in the wheat mycorrhizosphere affected quantity of exchangeable Mn rather than available Mn (Arines et al. 1992). Similarly, Nogueira et al. (2004) reported that stimulation of Mn-oxidizing bacteria and suppression of Mn-reducing bacteria in the rhizosphere of mycorrhizal (*Claroideoglossum etunicatum* or *Glossum macrocarpum*) soybean plants may have contributed to the lower Mn concentration in these plants. However, Nogueira et al. (2007) found the Mn-reducing bacteria were stimulated in the rhizosphere

of mycorrhizal soybean plants, but Mn-oxidizing bacteria were diminished, suggesting exudation of carbohydrates from mycorrhizal roots may have been responsible for such an opposite effect. For the hyphosphere, AM fungi were found to decrease the number of Mn-reducing bacteria in the hyphal compartment (Kothari et al. 1991b; Posta et al. 1994), thereby indirectly diminishing the reducing potential and availability of Mn in the mycorrhizosphere or hyphosphere. This was thought to be the main cause of AM-colonized plants having relatively low Mn concentration (Arines et al. 1989).

The capacity of Fe-reducing bacterium *Klebsiella pneumoniae* strain L17 to enhance Fe(III) reduction in the mycorrhizosphere was up-regulated by exogenous application of labile C added as a pulsed input under the dual inoculation of Fe-reducing bacterium and arbuscular mycorrhizal fungus *Funneliformis mosseae* (Ding et al. 2014), suggesting that organic molecules can shuttle electrons from organisms to solid phase Fe(III) minerals and therefore affect Fe(III) reduction rates. Hence, the release of Fe–P was closely linked to the reduction of Fe oxide by Fe-reducing bacteria.

Hyphosphere: biochemical and biological processes

Metabolites in AM hyphal exudates

Symbioses between plants and AM fungi are based predominantly on an exchange of plant C for soil P (Genre et al. 2020; Smith and Read 2008). Isotopic tracing studies showed that AM fungi utilize 5–20% of the net C fixed by plants (Jakobsen and Rosendahl 1990; Pearson and Jakobsen 1993), and the flows of C to extraradical mycelia via roots have been determined by several studies. Two-compartment microcosms were employed to trace the C fluxes from host plant to the extraradical mycelial network of AM fungus in situ using pulse labelling by $^{13}\text{CO}_2$. This demonstrated for the first time the C flux from plants to the AM mycelium-associated phosphate-solubilizing bacteria (Kaiser et al. 2015; Zhou et al. 2020), increasing the $\delta^{13}\text{C}$ of hyphosphere soil from -22.1‰ to $+176.6\text{‰}$ (Wang et al. 2016). Therefore, exudation of carbohydrates by AM fungal extraradical mycelia may be an important source of energy for microorganisms associated with hyphae (Table 2, Fig. 2).

By combining the mycorrhizal root organ culture in the split Petri dish system and proton nuclear

magnetic resonance spectrometry, low-molecular-weight compounds, such as formate, acetate, glucose and oligosaccharides, were detected in mycelial exudates (Toljander et al. 2007). Similarly, exudates of *Rhizoglossum irregulare* extraradical mycelium contained carbohydrates, amino acids and unidentified compounds, and all these C sources could serve as a substrate to stimulate growth of AM fungi-associated bacteria (Bharadwaj et al. 2012; Luthfiana et al. 2021). In addition, fructose exuded by the extraradical hyphae of *Rhizophagus irregularis* may be a key signalling molecule (Zhang et al. 2018a). Even though these studies provide evidence that the metabolites detected in hyphal exudates have a positive effect on growth of mycelium-associated bacteria, the full suite of chemical forms and functions of hyphal exudates is poorly understood due to technical difficulties in collecting and quantifying the hyphal exudates (Table 3).

Activity of microorganisms

Studies on microorganisms in the hyphosphere began with microscopic inspection and revealed a number of important findings. First, soil bacteria, such as *Paenibacillus brasilensis*, *Pseudomonas fluorescens* and *Bacillus cereus*, were shown to be attached to AM hyphae (Artursson and Jansson 2003; Battini et al. 2016; Scheublin et al. 2010; Toljander et al. 2006) and spores (Levy et al. 2003; Roesti et al. 2005), and formed biofilms on the surface of AM hyphae (Lecomte et al. 2011). Moreover, a recent study showed that bacteria could move in a thick water film formed around extraradical hyphae of AM fungi, which suggests that AM hyphae can act as a “highway” for bacteria to move along to reach organic P patches, therefore enhancing utilization of these discrete sources of organic P (Jiang et al. 2021) (Fig. 3c, d). Second, the hyphae-associated bacteria, called mycorrhiza helper bacteria (MHB), have been shown to stimulate mycelial growth, spore germination and mycorrhization (Artursson et al. 2006; Frey-Klett et al. 2007; Xavier and Germida 2003). Third, hyphae-associated bacteria have also been shown to inhibit growth and activity of extraradical mycelium (Leigh et al. 2011; Svenningsen et al. 2018). In return, AM hyphae or hyphal exudates were demonstrated to stimulate bacterial growth and activity (Filion et al. 1999; Mansfeld-Giese et al. 2002; Toljander et al. 2007), and alter the bacterial community

Table 3 Critical areas for further research in the hyphosphere

Research areas	Specific aims of future research
Methodology	Quantify the physical, chemical and biological processes in both the direct and mycorrhizal nutrient uptake pathways; Quantify the dynamics of AM fungal biomass and content of C, N, P, etc.; Develop new in situ visualization and analysis methods for exploring the interactions between AM hyphae and hyphosphere microbiota; Confirm whether AM fungi have capacity to secrete phosphatase; Collect and quantify the hyphal exudates
Mechanisms	Quantify the recruitment of distinct bacterial communities by different AM fungal species and host plants; Understand how AM fungi and bio-/abiotic environmental factors interact; Quantify AMF-promoted organic N cycling
Biological interactions	Develop understanding of the interactions in the AM fungi-microbiome-plant continuum
Ecological functions	Assess the impact on metabolites in exudates of AM hyphae due to variation in soil conditions and environment; Assess impacts of microbiome in the hyphosphere on AM performance; Quantify the cycling of key resources in plant-soil system, particularly the role of AMF in C sequestration and nutrient cycling; Establish how interactions between AM fungi and bacteria influence the growth and development of AM fungi and host plant
Environmental consequences	Assess the environmental impact on ecosystem function of AMF and the hyphosphere

structure (Nuccio et al. 2013; Scheublin et al. 2010; Toljander et al. 2007). There is a clear role for AM hyphae in influencing bacterial abundance and function in the hyphosphere, but the underlying mechanisms and ecological functions of AM fungi-bacteria associations are not well understood. Most of what is known has been derived from axenic experiments, which may not correctly reflect the actual interactions in the environment and their effects on plant and/or soil biological processes. Therefore, studies on AM fungal hyphae-associated bacteria and their function and interactions in soil are critical for understanding responses of agricultural and natural systems to environmental change and are likely to become a hot research topic in the future (Table 3).

Biological cycling of elements in the hyphosphere

Nutrient cycling

Nitrogen

The extraradical hyphae of AM fungi can effectively acquire nitrate (NO_3^-), ammonium (NH_4^+) and amino acids (AAs) from soil (Bonfante and Genre 2010; Jin

et al. 2012). It has been found that N uptake and transport in AM symbiosis is offset by a C flux from the plant, a similar reward mechanism to that described for P and C exchange (Fig. 2). In detail, various N sources are absorbed and transformed into arginine (Arg) by extraradical hyphae. Ammonium, nitrate and amino acid transporters and permeases in the extraradical hyphae of AM fungi participate in N absorption. The glutamine synthetase-glutamate synthase pathway (GS-GOGAT) is involved in the urea cycle and Arg biosynthesis. Arginine is then transferred from extraradical to intraradical hyphae combined with Poly P and catabolized through a urea cycle in the intraradical hyphae, releasing $\text{NH}_3/\text{NH}_4^+$ into arbuscules. Because of the acidic environment in the periarbuscular space, the NH_4^+ ion is deprotonated prior to its transport across the plant membrane via the ammonia channels and released in its uncharged NH_3 form into the plant cytoplasm (Govindarajulu et al. 2005; Jin et al. 2005, 2012).

The N cycle in soil is primarily controlled by bacteria and archaea (Rozmoš et al. 2022). Even though AM hyphae predominantly take up inorganic N as either NH_4^+ or NO_3^- (Hodge and Storer 2015; Tanaka and Yano 2005), they also have the capacity to acquire N from organic sources such as glutamate and glycine (Hawkins et al. 2000). In the presence

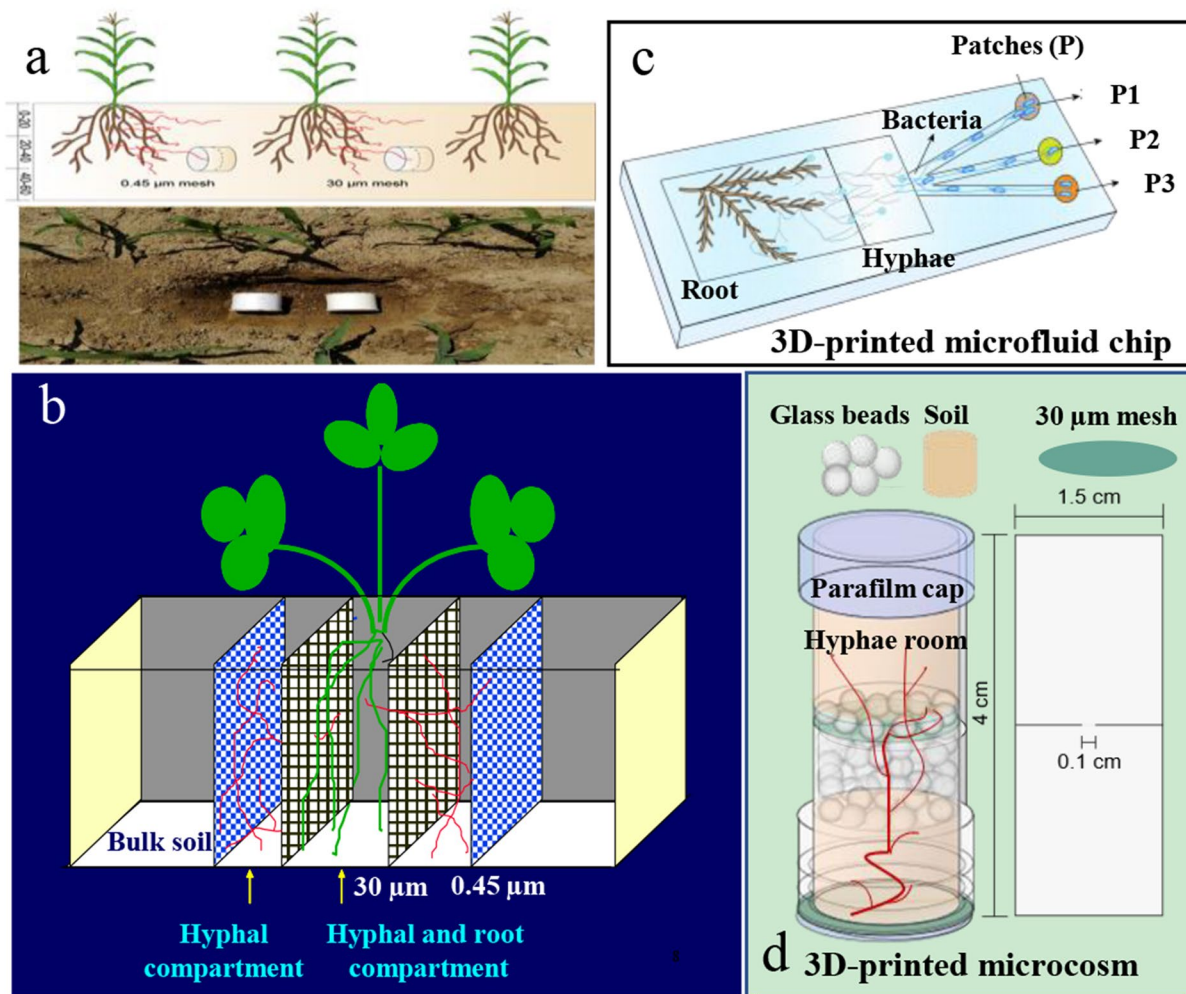


Fig. 3 The mesh-based microcosm for investigating AM hyphae-mediated ecological functions in the hyphosphere. (a) The in-growth tubes (10 cm in diameter, 6 cm in length), sealed with 30 μm mesh (permitting AM fungal hyphae but not roots to grow into) or 0.45 μm membrane (excluding both AM fungal hyphae and roots) at the two ends, were buried near the roots (in the layer 20–30 cm deep and 15 cm away from a maize plant) in the field to study the hyphosphere interaction effects (Zhang et al. 2018b). (b) Spatial separation of soil zones for root and hyphal growth and soil zones. The chamber

has five compartments, a central one for root growth (including mycorrhizal structures) separated from the two adjacent ones by 30 μm mesh. The bulk soil compartments are separated from the hyphal compartments by 0.45 μm mesh. (c, d) Schematic diagram describing the underground ‘hyphae highway’ formed by mycorrhizal network of AM fungi. The phosphate-solubilizing bacteria can quickly migrate toward an organic P patch along the hyphae highway (Jiang et al. 2021; Sun et al. 2021)

of oxygen, NH_4^+ can be oxidized to NO_3^- through the action of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), whereas in the absence of oxygen, NO_3^- can be used by many microbes as a respiratory electron acceptor in the process known as dissimilatory nitrate reduction to NH_4^+ during denitrification (Canfield et al. 2010; Kuypers et al. 2018). It seems that extraradical

hyphae of AM fungi could potentially compete for NH_4^+ and NO_3^- with other microorganisms involved in nitrification and denitrification (Bollmann et al. 2002). Therefore, the content of available NH_4^+ and NO_3^- in the hyphosphere is decreased; nitrification and/or denitrification may also be impaired with consequent reductions in NO_x emissions from soil (Table 2, Fig. 2).

Accumulating evidence indicates that extraradical mycelia of AM fungi can obtain substantial amounts of N from decomposing organic matter and increase N capture for host plants (Hodge et al. 2001; Hodge and Fitter 2010; Leigh et al. 2009, 2011). This phenomenon likely relies on the other microorganisms (saprotrophic fungi, bacteria, protists, etc.) influenced by AM fungal hyphae (Bukovská et al. 2018; Hestrin et al. 2019; Rozmoš et al. 2022) because AM fungi have no known saprotrophic capacity (Smith and Read 2008). Studies in soil have shown that plant N uptake from a litter patch was significantly increased when AM fungi accessed the patch compartment, and ^{15}N analysis of the plant material indicated that 2–4% of plant N originated from litter decomposed by bacteria associated with extraradical AM hyphae (Herman et al. 2012; Leigh et al. 2011). Recent *in vivo* experiments with ^{15}N isotope tracing provided direct evidence that organic N utilization by an AM fungus is mediated by a soil bacterium *Paenibacillus* sp. and a protist *Polysphondylium pallidum* (Rozmoš et al. 2022). Further research is therefore needed to understand the mechanisms and pathways of organic N cycling in the hyphosphere (Table 3).

Free-living diazotrophs, colonizing the surface of extraradical hyphae of AM fungi, can fix N_2 from the atmosphere (Shi et al. 2021), suggesting specific bacteria interacting with AM hyphae could improve the N status of host plants grown on a low-N soil. Results from a recent study featuring root- and AMF-exclusion plots around leguminous *Faidherbia albida* trees in smallholder fields using the ^{15}N natural abundance technique showed that one-third of tree-derived N in maize leaves was attributed to AMF-mediated N uptake from beyond the maize rooting zone (Dierks et al. 2022). Therefore, AM fungi quantitatively contribute to the crop uptake of legume-derived N and enhance agroecosystem functioning, which is indispensable in attaining sustainable agroecosystems.

Phosphorus

Extraradical mycelia enlarge the absorptive area of plant roots and profoundly influence biochemical properties of the hyphosphere, resulting in improved availability of the poorly mobile soil P and increased plant P uptake (Fig. 2, Fig. 3b). In addition to hyphal extension, the external hyphae of AM fungi also

exude protons to mobilize sparingly soluble inorganic P by acidification of the alkaline hyphosphere soil (Li et al. 1991b; Wang et al. 2013; Yao et al. 2001). However, for the hydrolysis of organic phosphates, it must be catalyzed in the presence of phosphatases to release free orthophosphate. Previous studies using either an *in situ* histochemical method (van Aarle et al. 2002; Feng et al. 2002), a split-dish *in vitro* carrot-mycorrhiza system (Koide and Kabir 2000; Sato et al. 2015, 2019) or a compartmented pot culture (Joner and Jakobsen 1995; Joner and Johansen 2000) were conducted to demonstrate that extraradical hyphae of AM fungi could release phosphatase, moreover, transcriptome analyses suggested that AM fungi had the potential to secrete phosphatases (Liu et al. 2013; Tisserant et al. 2012), but there is still no strong evidence that AM hyphae have capacity to secrete phosphatase. Therefore, organic P mineralization, particularly of inositol phosphates (the predominant form of organic P in soils), in the AM fungal hyphosphere is likely to be driven by microbially derived phosphatases and phytases.

Recently, several important findings regarding interactions between AM fungi and phosphate-solubilizing bacteria (PSB) to promote mineralization of soil P in the hyphosphere have been reported. For example, the AM fungus *Rhizophagus irregularis* was shown to interact with a phosphate-solubilizing bacterium *Pseudomonas alcaligenes* in the hyphosphere, increasing hyphosphere soil phosphatase activity and enhancing Na-phytate mineralization (Zhang et al. 2014). The photosynthates from ^{13}C -labelled maize were translocated to hyphosphere PSB via the extraradical AM hyphae, and extraradical mycelium-associated PSB enhanced mineralization and turnover of soil organic P in the hyphosphere (Wang et al. 2016).

The cooperation between AM fungi and PSB was influenced by the initial P availability and C:P ratio in the hyphosphere soil (Zhang et al. 2016). Fructose, one component of hyphal exudates, was shown to be not only a C source, but also a signal molecule to trigger mineralization of organic P mediated by a single PSB *Rahnella aquatilis* (Zhang et al. 2018a). In a rhizobox study in greenhouse, fructose was found to change the bacterial community by increasing the relative abundance of specific taxa (Zhang et al. 2020). However, the finding of various PSB on mycorrhizal hyphae *in vitro* (Taktek et al. 2015) suggest that a wide range of microorganisms

found in the rhizosphere and hyphosphere may have similar function. A recent study in the field demonstrated that AM fungi and their hyphae-associated microbiome play a role in promoting mineralization of soil organic P in the hyphosphere (Zhang et al. 2018b) (Fig. 3a).

Co-inoculations with AM fungus *Rhizoglyphus irregularis* strain QS69 and PSB belonging to the genus *Pseudomonas* in a two-compartment experimental system showed the potential for mobilization of inorganic and organic phosphate along with other plant growth-promoting traits (Sharma et al. 2020). Moreover, addition of fructose to the soil of hyphal compartment in a greenhouse set-up was shown to (i) act as an energy source to stimulate bacterial phosphatase activity by altering soil bacterial community structure and (ii) promote organic P mineralization, thus enhancing the utilization of P accumulated in organic forms in soils (Zhang et al. 2020). Interestingly, the extraradical hyphae may facilitate the transport of bacteria to soil nutrient patches. A phosphate-solubilizing bacterium was found to move in a water film along the AM fungal hyphae, being nourished by hyphal exudates on its way towards the phytate patch, where it extended the functional capacity of the fungus to utilize this otherwise inaccessible P source (Jansa and Hodge 2021; Jiang et al. 2021).

The interaction between AM fungi and other (non-PSB) bacteria to promote P nutrient turnover and plant uptake has also been reported. For example, extraradical hyphae of *Funneliformis mosseae* and Fe-reducing bacteria *Klebsiella pneumoniae* L17 in the hyphal chamber showed a competitive interaction in low-P soil, and a complementary, or possibly a C-dependent synergistic, function at high P availability (Zhang and Ding 2018). It is likely that P released from Ferralsols by Fe-reducing bacteria may be enhanced by additions of exogenous C.

Micronutrients

Arbuscular mycorrhizal fungi also play a crucial role in micronutrient acquisition by plants. The uptake of Cu and Zn by the external hyphae (in hyphal compartments) may account for about 53–62% of the total Cu uptake by white clover and 25% of the total Zn uptake by maize (Kothari et al. 1991a; Li et al. 1991c). Subsequently, Jansa et al. (2003) quantified

the contribution of the mycorrhizal pathway for uptake of Zn by maize plants inoculated with the AM fungus *Glomus intraradices* (syn. *Rhizophagus irregularis*) using the proportion of labelled Zn taken up. More recently, Watts-Williams et al. (2015) used ^{65}Zn to quantify the total amount of Zn and the relative contribution of Zn delivered via the mycorrhizal pathway of uptake in shoots of tomato plants inoculated with *R. irregularis*. Results showed that the highest relative contribution of the mycorrhizal pathway to Zn uptake was up to 24% in the low Zn concentration treatments. In more recent studies the mycorrhizal pathway of Zn accumulation in the edible portion of wheat and barley contributed up to 24% of total above-ground Zn in wheat, and up to 13% in barley. The greatest contribution by the mycorrhizal pathway was observed in barley at the lowest Zn addition, and in wheat at the highest one (Coccina et al. 2019). It is likely that the contribution by the mycorrhizal pathway to plant Zn uptake is highly dependent on the host plant species and soil Zn availability.

Mycorrhizal inoculation had a significant influence on the concentration of ^{59}Fe in shoots of sorghum plants, suggesting extraradical AM hyphae can mobilize Fe and promote both its uptake from the hyphal compartment and translocation to the host plant (Caris et al. 1998). By contrast, a decrease in Mn uptake by mycorrhizal plants could be due to decreased uptake and transport of Mn in the external hyphae or a decline in Mn availability due to decreased abundance of Mn-reducing bacteria (Kothari et al. 1991b).

Elements such as Zn and Fe are also essential micronutrients for humans, and studies indicate that AM fungi play a positive role on improving Zn and Fe concentrations in edible parts for a variety of crops (Ercoli et al. 2017; Lehmann et al. 2014). The role of AM fungi in food security therefore goes beyond just allowing more sustainable production of crops, but also by impacting the nutritional quality of food, promoting nutritional security by improving micronutrient nutrition and health for humans through the food chain.

Cycling of C between plants and hyphosphere

As obligate biotrophs, the AM fungi are an important sink for organic C fixed by the plant through

photosynthesis, with a portion of that C transferred by the AM fungus into the soil (Genre et al. 2020) (Fig. 2). In addition, AM hyphae can contribute to priming of soil organic matter mineralization (Patterson et al. 2016). Apart from stimulating litter decomposition, mycorrhizae can also stabilize litter C by reducing the abundance and activity of other soil microbes (Leifheit et al. 2015; Verbruggen et al. 2016). Nevertheless, AM hyphae may have a quick turnover, potentially acting as a rapid pathway for returning C to the atmosphere (Staddon et al. 2003). The AM hyphae not only accelerate decomposition of complex organic materials like grass litter, leading to enhanced CO₂ emission, but also contribute to additional C respired back to the atmosphere (Cheng et al. 2012); this net emission of CO₂ was likely due to increased C fixation by plants and transport of this C to the soil via AM fungi (Kowalchuk 2012).

Nitrous oxide emissions

Nitrous oxide (N₂O), a potent greenhouse gas, is a product of a disrupted N cycle caused by incomplete denitrification in soils. Nitrification and denitrification of inorganic N (NH₄⁺ and NO₃⁻) feed the N cycle to produce N₂O, and this process is driven by nitrifying and denitrifying bacteria (Hino et al. 2010). Recent meta-analysis has concluded that AM fungi can significantly decrease soil N₂O emission (Shen and Zhu 2021) (Fig. 2). For example, a microcosm experiment demonstrated that AM fungi suppressed N₂O emissions in sandy but not clay soils amended with rice straw (Zhai et al. 2021). The intense competition for inorganic N between AM fungi and nitrifying/denitrifying bacteria could decrease the availability of N substrates for N₂O producers and consequently lead to a reduction in N₂O emissions. In fact, AM fungi have been demonstrated to reduce N₂O emissions from soil through increasing the number of copies of *nosZ* gene that encodes nitrous oxide reductase, the enzyme that reduces N₂O to N₂ (Bender et al. 2014, 2015; Kuypers et al. 2018).

Recent studies with compartmented microcosms have demonstrated that extraradical AM hyphae also decrease N₂O emissions from soil. For example, Storer et al. (2018) found that production of N₂O from organic patches decreased due to diminished nitrification rates in the presence of AM hyphae. Gui et al. (2021) found that extraradical AM hyphae reduced N₂O emission by

decreasing the abundance of key genes responsible for denitrification (*nirK* and *nosZ*).

Bacterial community composition

There is evidence in the literature that root- or hyphae-associated bacteria utilize root or hyphal exudates as easily available C and energy sources for fast growth and reproduction, contributing to an increase in the number of bacteria in the rhizosphere and hyphosphere (Toljander et al. 2007; Zhang et al. 2016) (Fig. 2). In vitro, the addition of exudates produced by AM extraradical mycelia to a bacterial community extracted from soil not only stimulated bacterial growth and activity, but also changed community composition (Toljander et al. 2007). Similarly, in a chamber microcosm in situ under both greenhouse and field conditions, the presence of AM extraradical hyphae altered the soil bacterial community (Herman et al. 2012; Nuccio et al. 2013; Wang et al. 2019; Zhang et al. 2018b). However, when only extraradical AM hyphae had access to an organic patch in a compartmented microcosm, there was a limited impact on bacterial community structure due to bacterial competition for resource (Leigh et al. 2011).

Because hyphal exudates are found in smaller quantities than root exudates (Qin et al. 2022), the influence of extraradical hyphae on bacterial community in the hyphosphere is possibly less than that of roots in the rhizosphere. Qin et al. (2022) compared the differences in P-mobilizing bacterial community between rhizosphere and hyphosphere using compartmented rhizoboxes. The mycorrhizal regulation was greater in the mycorrhizosphere than the hyphosphere, and the P-mobilizing bacterial community in the hyphosphere was not the subset of that in the mycorrhizosphere. Likewise, results of alkaline phosphatase genes (*phoD*) and pyrroloquinoline quinone biosynthesis gene (*pqqC*) expression indicated that the P-mobilizing bacterial community in the rhizosphere differed from that in the hyphosphere. Consequently, the importance of bacterial community in the hyphosphere for promoting the P uptake of host plants should be investigated further.

Many bacterial phyla, such as Proteobacteria, Actinobacteria, Firmicutes, Gemmatimonadetes, Bacteroidetes, Chloroflexi, Acidobacteria, Cyanobacteria, Planctomycetes and Fusobacteria, have been identified in the hyphosphere. The hyphal effect on soil bacterial community resulted in the enrichment of

some bacterial phyla such as Proteobacteria (Emmett et al. 2021). These enriched bacteria played a positive role as mycorrhiza helper bacteria (Frey-Klett et al. 2007). Interestingly, the abundance of bacteria harbouring genes that encode enzymes involved in organic P mineralization [alkaline phosphatase (*phoD*) and β -propeller phytase (*BPP*)] increased due to the presence of extraradical AM hyphae (Wang et al. 2019; Zhang et al. 2018b).

The bacterial community composition and function were also influenced by interaction with some abiotic factors such as P forms (Wang et al. 2019) and concentrations of phytate (Zhang et al. 2018b) and fructose (Zhang et al. 2020) in the hyphosphere. Some biotic factors also influenced the hyphosphere bacterial community significantly. Using a split-root culture system and simultaneous inoculation with two different AM fungal species, Zhou et al. (2020) found that different AM fungi co-colonizing a single plant root system recruited distinct microbiomes. Emmett et al. (2021) also found that the host plant species strongly influenced the hyphosphere bacterial community. However, the mechanisms involved in the recruitment of these distinct bacterial communities by different AM fungal species and host plants are still unknown. In addition, how the interaction between AM fungi and bacterial community assemblage influenced the growth of AM fungi and eventually the host plant growth and development also warrants further studies (Table 3). Moreover, some AM fungi also harbour endobacteria that have important influence on the functions of AM fungi, which merits further research (Salvioli et al. 2016).

Future research perspectives

Although the AM hyphae are not visible to the naked eye, the biophysical, biochemical and biological reactions driven by AM fungi make the fungi-soil interface a unique critical zone affecting ecosystem functions that have global consequences. It has become clear in recent years that AM fungi, which form one of two nutrient acquiring pathways of plant in soil, not only influence the nutrient uptake efficiency of the mycorrhizal plants, but also have significant impacts on C balance and greenhouse gas emissions. However, many questions still remain about the full impact of this microscale interface harbouring highly complex ecological

processes. We propose several key areas for further research (Table 3).

First, we should integrate better the research on effects of mycorrhizosphere, rhizosphere (influenced by non-AM colonized roots) and hyphosphere in mycorrhizal plants. How plants balance different strategies in these ‘spheres’ to acquire resources based on the optimized C economy is a fundamental question in biology and ecology. Therefore, an integrated approach to quantify the trade-off between physical, chemical and biological processes in both the direct and mycorrhizal uptake pathways is needed in further studies.

Second, a single plant root system is usually colonized by diverse AM species simultaneously, forming common mycorrhizal networks with overlapping hyphospheres in the plant-soil continuum in nature. Different AM fungal species showed diverse capacity to influence hyphosphere functions (such as mobilizing sparingly soluble mineral nutrients or promoting soil aggregate formation) through recruiting distinct microbiome (Zhou et al. 2020). Despite this substantial ecological footprint, we know little about some fundamental aspects of the biology, ecology and agricultural significance of the hyphal exudates driving hyphospheric biological interactions, ecological functions and their subsequent environmental consequences. We need to shed more light on:

- (i) The molecular mechanisms through which the fungi and biotic/abiotic environmental factors interact;
- (ii) How the composition of AM hyphal exudates responds to soil conditions and environmental variation;
- (iii) How the hyphosphere microbiome plays a role of an extended genome and impacts AM performance; and
- (iv) How and to what extent the fungi-microbiome-plant interactions govern the cycling of key resources in plant-soil system and therefore the environmental impact of ecosystem functions.

Third, it is widely accepted that the Glomeromycota fungi are crucial for crop production, fruit quality, plant health and soil fertility, but we know almost nothing about the biomass and nutrient demand of this agriculturally crucial taxon. It is essential and urgent to develop new methodologies

to quantify the dynamics of biomass of the fungi and the content of C, N, P, etc. in their biomass. Such information will help design improved fertilizer management regimes to feed not only the crop but the fungi first.

Finally, one of the most prominent methodology innovations in the last four decades of studying mycorrhizal hyphospheric effects is the mesh-based compartmented rhizobox. By using meshes with pore diameter of 30 μm or 0.45 μm , this approach has successfully separated the zones that are influenced by mycorrhizal roots or only by mycorrhizal hyphae. In vitro culture of AM fungi together with roots or whole plant is another prominent approach that provides a way to test the effects of hyphal exudates on nutrient mobilization or microbiome in the hyphosphere. New in situ analysis methods need to be developed that can facilitate sampling, measuring or imaging at the nano- to micrometer scale. For example, integration of microfluid chip system, nanoSIMS, nanoDESI and single cell transcriptomic methods may uncover new mechanisms governing the interactions between AM hyphae and hyphosphere microbiota.

We think that the application of modern scientific techniques to these key knowledge gaps regarding the function of the hyphosphere will underpin a better understanding of the role of AM fungi in solving some of the most pressing issues that the human race currently faces, such as agricultural sustainability, climate change mitigation and adaptation, and reversing biodiversity loss.

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Declarations

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