nature reviews microbiology

https://doi.org/10.1038/s41579-024-01073-7

Review article

Check for updates

Cross-kingdom nutrient exchange in the plant–arbuscular mycorrhizal fungus–bacterium continuum

Shilong Duan[1], Gu Feng[1], Erik Limpens[2], Paola Bonfante[3] ✉, Xianan Xie[4] ✉ & Lin Zhang[1] ✉

Abstract

The association between plants and arbuscular mycorrhizal fungi (AMF) affects plant performance and ecosystem functioning. Recent studies have identified AMF-associated bacteria as cooperative partners that participate in AMF–plant symbiosis: specific endobacteria live inside AMF, and hyphospheric bacteria colonize the soil that surrounds the extraradical hyphae. In this Review, we describe the concept of a plant–AMF–bacterium continuum, summarize current advances and provide perspectives on soil microbiology. First, we review the top-down carbon flow and the bottom-up mineral flow (especially phosphorus and nitrogen) in this continuum, as well as how AMF–bacteria interactions influence the biogeochemical cycling of nutrients (for example, carbon, phosphorus and nitrogen). Second, we discuss how AMF interact with hyphospheric bacteria or endobacteria to regulate nutrient exchange between plants and AMF, and the possible molecular mechanisms that underpin this continuum. Finally, we explore future prospects for studies on the hyphosphere to facilitate the utilization of AMF and hyphospheric bacteria in sustainable agriculture.

Sections

[1]State Key Laboratory of Nutrient Use and Management, College of Resources and Environmental Sciences, Key Laboratory of Plant–Soil Interactions, Ministry of Education, China Agricultural University, Beijing, China. [2]Laboratory of Molecular Biology, Wageningen University and Research, Wageningen, The Netherlands. [3]Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy. [4]State Key Laboratory of Conservation and Utilization of Subtropical Agro-Bioresources, Guangdong Laboratory for Lingnan Modern Agriculture, Guangdong Key Laboratory for Innovative Development and Utilization of Forest Plant Germplasm, College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou, China.
✉e-mail: paola.bonfante@unito.it; xiexianan8834203@126.com; linzhang@cau.edu.cn

Introduction

Mycorrhizal fungi are crucial components of the plant microbiota[1] and are key actors in terrestrial ecosystems, where they facilitate the exchange of carbon (C) and minerals[2]. Interacting with various plants[3,4], mycorrhizal fungi provide multiple ecological services in natural and agricultural environments. Arbuscular mycorrhizal fungi (AMF) have co-evolved with plants for 400 million years and form symbioses with 72% of land plants. They affect plant performance and ecosystem functioning[5], such as plant health, nutrient cycling and soil structure (Box 1). In these AMF symbioses, plants provide photosynthetically fixed C (including lipids and sugars) in exchange for minerals, especially phosphorus (P) and nitrogen (N)[6].

Bacteria also form part of the plant microbiota and take part in AMF–plant symbiosis; for example, some AMF host endobacteria within their cytoplasm. Moreover, AMF hyphae grow within and extend outside the plant root; the thin soil layer that surrounds these extraradical hyphae (ERH) forms a specialized niche called the 'hyphosphere' (Fig. 1). This niche hosts a plethora of hyphospheric bacteria that feed on fungal metabolites that contain plant-derived C (refs. 7,8). In return, the bacteria support the mineral nutrition of AMF by secreting extracellular enzymes (Supplementary Table 1), as AMF cannot decompose polymer nutrients owing to their limited saprotrophic capability[9,10].

In this Review, we present the most recent advances in our understanding of how plants, AMF and their associated bacteria exchange host plant-derived C and soil-derived minerals. These exchanges provide energy to the microbial partners and economically convenient nutrients to plants. We analyse the potential mechanisms by which the partners orchestrate their three-kingdom interactions. Finally, we outline the potential use of AMF and hyphospheric bacteria as biostimulants for sustainable agriculture.

The hyphosphere and hyphospheric bacteria

AMF form ERH networks in soil[11], expanding their contact area with the soil and releasing various compounds (Box 2). These compounds affect the hyphal surface and the thin layer of soil (approximately 2 mm) adjacent to the ERH. Similar to the rhizosphere, which surrounds plant roots, the thin soil layer that surrounds the ERH is defined as the hyphosphere, which differs from bulk soil because hyphal exudates influence its physical, chemical and biochemical properties[12,13] (Fig. 1).

The hyphosphere provides a high-energy microhabitat for multivarious bacteria and facilitates their dispersal in the soil along the ERH[14,15]. The bacteria that live in the hyphosphere and feed on hyphal exudates are called 'hyphospheric bacteria'[13]. Recent studies have examined their identities and functions[13,16,17] and demonstrated that they include members of 26 phyla. The most abundant hypospheric bacteria belong mostly to Pseudomonadota, Actinomycetota, Gemmatimonadota, Bacteroidota, Chloroflexota, Acidobacteriota and Bacillota, but also include some bacterial taxa that were observed only in specific studies, such as Verrucomicrobiota[7]. At the genus level, the most frequent bacterial taxa belong to *Pseudomonas*, *Streptomyces*, *Paenibacillus*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Massilia* and *Stenotrophomonas*. Different AMF can co-colonize the roots of individual plants and recruit their own specific microbiota by varying the composition of their hyphal exudates[18]. Although different AMF may affect the composition of the hyphospheric bacterial community, some members seem to be stable and not affected by external factors. At the order level, some bacterial groups (for example, Myxococcales, Betaproteobacteriales, Cytophagales and Chloroflexales) were consistently enriched in the hyphosphere across soil types and AMF species, thus aggregating into stable guilds[7,19].

Hyphospheric bacteria have multiple effects on AMF and their host plants. First, some genera of hyphospheric bacteria, such as *Pseudomonas*, *Bacillus* and *Streptomyces*, promote AMF hyphal elongation and branching and improve AMF colonization efficiency within roots. Their functions are similar to those of the mycorrhizal helper bacteria, which were identified for their ecological and evolutionary significance in ectomycorrhizae[20]. Nowadays, the term 'mycorrhizal helper bacteria' has been extended to include all the bacteria that interact with mycorrhizal fungi and plants to promote the establishment of their symbiosis, which depends neither on the type of mycorrhizal symbiosis nor on the taxa of the bacterial strains and the ecological niche in which they dwell[21,22]. Second, hyphospheric bacteria can provide AMF and host plants with otherwise inaccessible P, N and micronutrients that are far from the roots[23,24]. Furthermore, the close interactions of bacteria and AMF in the hyphosphere can influence the biogeochemical cycling of nutrients in terrestrial ecosystems (Box 2), providing benefits for agriculture in a changing climate.

The plant–AMF–bacterium continuum

Plants fix atmospheric C and transfer it to below-ground plant parts to fuel root growth and metabolism[25]. During nutrient acquisition, plants are often helped by their complex microbiota, which thrives within the root or at the root surface by taking advantage of the C-rich environment[2]. AMF connect plants and soil; their intraradical hyphae (IRH) penetrate outer root cortical cells, producing fungal hyphal coils that are surrounded by a plant-derived plasma membrane[3]. Such perifungal membranes also surround the fungal tree-like structures called arbuscules in inner cortical cells, thus delimiting an apoplastic compartment termed the peri-arbuscular space (PAS); the PAS forms the interface for exchange of C and nutrients between plants and AMF[11,26,27]. In contrast to IRH, ERH extend beyond the rhizosphere and grow into soil micropores, where they interact with the microbiota dwelling in the hyphosphere. This forms the interface for exchanging C and nutrients between AMF and soil bacteria[9,28].

These observations suggest the existence of a biological continuum between plants, AMF and soil bacteria. In this plant–AMF–bacterium (PFB) continuum, the peri-arbuscular and hyphosphere interfaces have key roles in the exchange of C and minerals (Fig. 1).

Three-kingdom carbon and mineral flows

In the PFB continuum, top-down C flows and bottom-up nutrient flows contribute to the establishment of a metabolic network between plants, AMF and bacteria (Fig. 2). The arbuscular mycorrhizal fungus functions as the central player that coordinates the stability of the entire continuum in the ecosystem: plants acquire mineral nutrients necessary for growth, and AMF, as well as its associated bacteria, obtain C for metabolism, thus improving the fitness of all members.

Carbon metabolism in mycorrhizal roots and carbon flow towards AMF

A recent study estimated that plants provide approximately 6% of their net photosynthates (particularly fatty acids and sugars) to AMF, depending on the habitat and plant growth type[29].

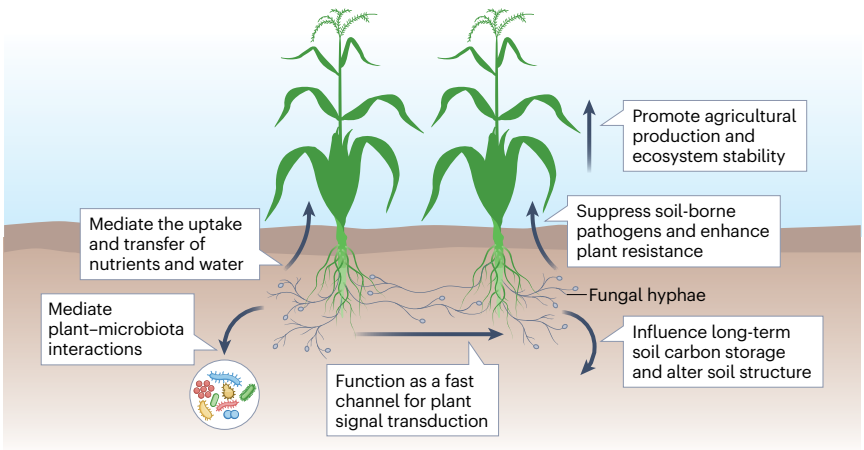
Sugars can be exported extracellularly via members of the SWEET (sugar will eventually be exported transporter) family localized in the peri-arbuscular membrane (PAM)[30,31] (Fig. 2). Physiological and genomic evidence indicates that AMF do not possess reversible

Box 1 | Major features of AMF and their implications for ecosystems

Together with *Geosiphon pyriforme*, arbuscular mycorrhizal fungi (AMF) are the only members of subphylum Glomeromycotina (in Mucoromycota). This ancient lineage of obligate biotrophs lives in soils and establishes a hyphal network that depends on the host plant-delivered carbon source[154,164]. Unlike ectomycorrhizal and ericoid mycorrhizal fungi, which mostly evolved from saprotrophic ancestors[165], AMF are ancient symbiotic fungi that evolved more than 400 million years ago, before fungi developed their enzymatic arsenal for the degradation of organic matter. Owing to their limited saprotrophic capacities, AMF depend on their host plants, with whom they have associated since the emergence of the common ancestor of land plants[3,166].

In nature, multiple AMF can coexist in a single host plant, whereas an individual arbuscular mycorrhizal fungus can also colonize distinct host plants, resulting in an underground 'common hyphal network' that connects different plant species[3]. Despite their ecological importance, the global distribution of AMF taxa has only been partially described. Although initial analyses suggested the 'everything is everywhere' hypothesis, researchers[167] described how the distribution of arbuscular mycorrhizal families differs between climatic zones and continents, even if they still seem to be cosmopolitan. Species distribution shows a slight decrease from low to high latitudes, but this decrease is steeper in the southern than in the northern hemisphere. These findings suggest a 'moderate endemicity model', but the biogeographical patterns of Glomeromycotina remain to be resolved. This probably mirrors a large sampling bias towards heavily studied regions in Europe, North America and China, whereas many tropical regions or large parts of Asia remain understudied[168].

In this ecological context, to further our understanding of AMF, other parameters must also be considered. AMF mediate the uptake of nutrients and water in natural and agricultural ecosystems, affecting primary productivity and long-term soil C storage, owing to their spores and extraradical hyphae[169,170]. Considering current climate change, the biodiversity of AMF must be protected, as its loss can negatively affect C storage and lead to other dangerous scenarios[171,172]. As AMF activate priming responses in plants via plant immunity mechanisms, their decreased presence can limit their suppression of soil-borne pathogens[173]. The underground hyphal network of AMF also serves as a rapid channel for plant signal transduction to anticipate danger[174]. The exploration of AMF genomes has provided clues to help answer some major questions about their biology, evolution and ecology. Genomic data are available for representatives of various AMF orders, from Glomerales to Diversisporales and Archaeosporales[175], revealing that these fungi have unusually large genomes (from approximately 150 Mb in *Rhizophagus* species to 784 Mb in *Gigaspora margarita*), with the exception of the ancestral sister lineage *Paraglomus occultum*, which contains a relatively small genome (39.6 Mb). The nuclear dynamics, the abundance of transposable elements and the epigenomic landscape are emerging parameters that can explain the genetic variability of AMF, in addition to sexuality events, which have recently been demonstrated[176]. These features can explain the adaptability of AMF to a wide host range and to environmental changes. Collectively, all of this evidence highlights the increasingly prominent roles of AMF in terrestrial ecosystems, which are acknowledged as one of the potential beneficial factors promoting agricultural production and ecosystem stability (see the figure).



sucrose synthases and invertases to cleave sucrose and can only utilize monosaccharides, especially glucose and fructose[32–34]. In roots, plant invertases and reversible sucrose synthases facilitate sugar acquisition by AMF[35]. These enzymes process sucrose into monosaccharides to be taken up via the plasma membrane-localized monosaccharide transporters (MSTs) in AMF[36–38]. For example, *Rhizophagus irregularis* (formerly *Glomus* sp.) RiMST2 is a high-affinity MST that scavenges monosaccharides and is crucial for the symbiotic relationship with the host plant[37].

In addition to sugars, AMF obtain host-synthesized fatty acids to facilitate the development of symbiosis[39–42] (Fig. 2). Lipids such as triacylglycerol (TAG) are the main forms of C stored in AMF[32,33]. Notably, no cytoplasmic type I fatty acid de novo synthase-encoding genes were found in the published genomes or transcriptomes of AMF[34,43–45].

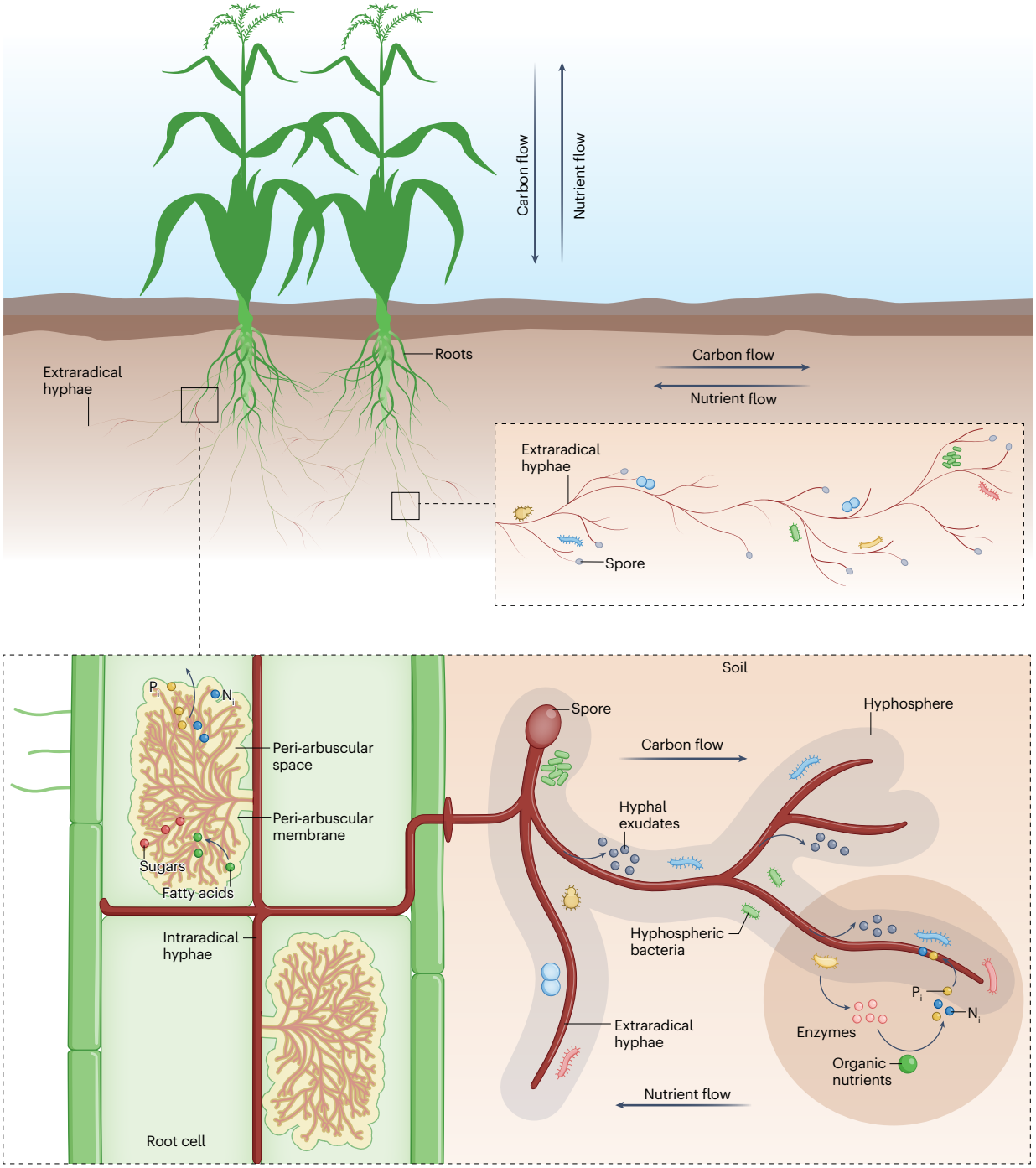
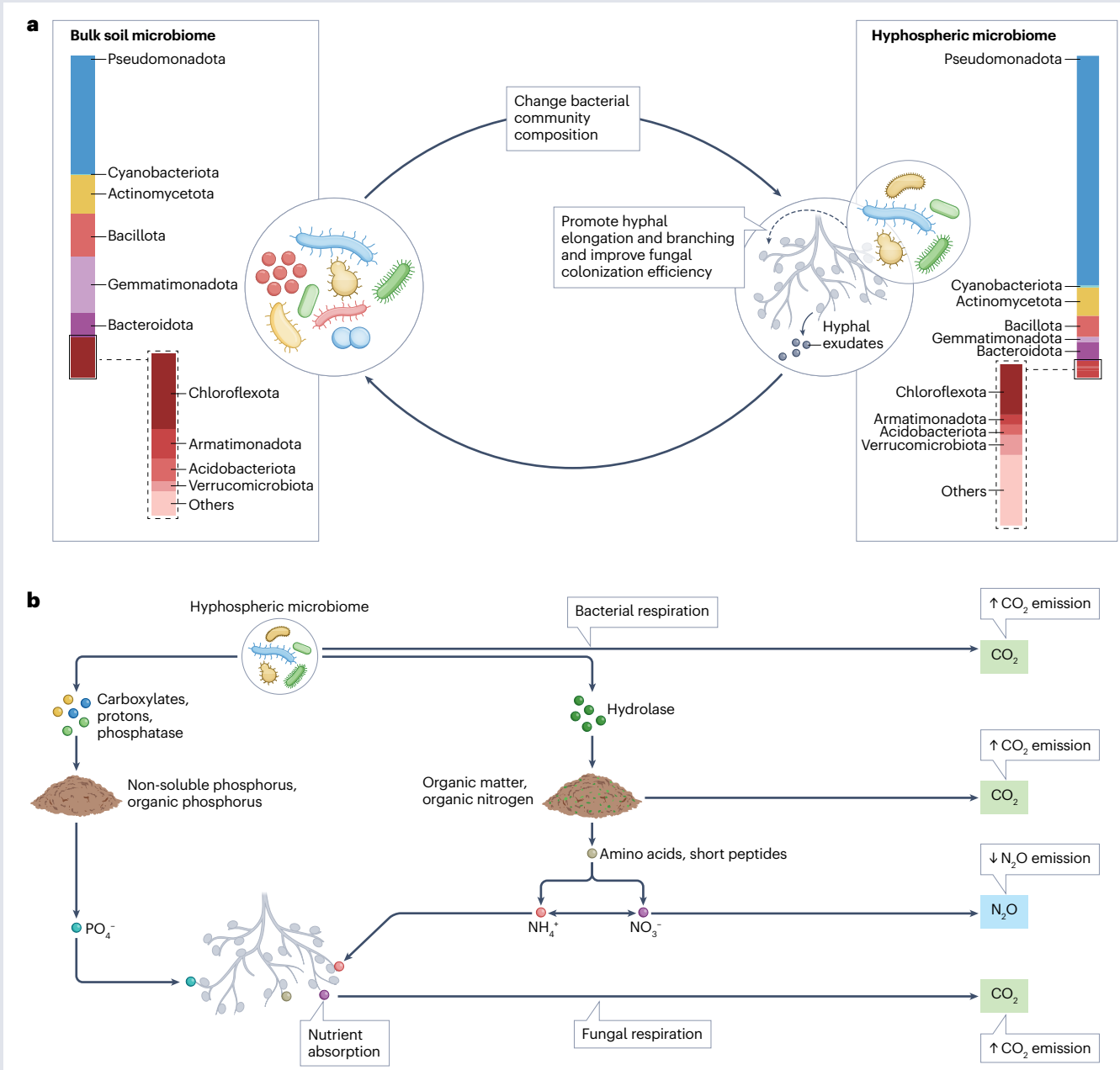


Fig. 1 | An overview of the plant–arbuscular mycorrhizal fungus–bacterium continuum and the two key interfaces involved in nutrient exchange. In the 72% of land plants that establish symbiosis with arbuscular mycorrhizal fungi (AMF), carbon (C) flows from the atmosphere towards the roots, AMF and hyphospheric bacteria, whereas mineral nutrients flow from hyphospheric bacteria and AMF towards the roots and up to the shoots. The intraradical hyphae of AMF invade plant cortical cells and form arbuscules, where each fungal branch is surrounded by the plant peri-arbuscular membrane (see the inset). The area between the peri-arbuscular membrane and the fungal wall is called the 'peri-arbuscular space', where nutrient exchange occurs bidirectionally. The extraradical hyphae of AMF rapidly extend into the soil and release C-containing compounds in the form of hyphal exudates, which attract various bacteria to colonize the hyphosphere. Here, the exchange of C (hyphal exudates) and mineral nutrients (inorganic phosphate (P_i) and inorganic nitrogen (N_i)) between AMF and bacteria takes place. Therefore, plants, AMF and hyphospheric bacteria constitute a biological continuum in which top-down C flow and bottom-up mineral nutrient flow occur continuously via two active interfaces (plant–fungus and fungus–bacterium).

Box 2 | Bacteria–AMF interactions and biogeochemical cycling of nutrients

Soil harbours abundant microorganisms that drive the biogeochemical cycles of nutrients from the local to the global scale[177]. Among the most important microorganisms, photoautotrophic microorganisms are responsible for approximately half of carbon dioxide (CO_2) fixation and oxygen (O_2) production on Earth, whereas heterotrophic microorganisms are mostly responsible for the opposite reaction: the oxidation of organic matter back into CO_2. These two processes drive the biological sequestration of carbon (C), the reduction of atmospheric CO_2 and the maintenance of increased atmospheric O_2 levels. Other microorganisms carry out chemical transformations, including nitrogen (N) and phosphorus (P) flux to and from biologically available states: they fix atmospheric N_2 and form the potent greenhouse gas nitrous oxide (N_2O), whereas others alter the storage form of P to support soil fertility and plant nutrition[178,179]. Thus, microorganisms are a major engine of terrestrial biogeochemistry, driving the turnover of nutrients (for example, C, N and P) by releasing metabolic products, mediating intracellular and extracellular electron transport and releasing cell contents upon



(continued from previous page)
death[60]. All these transformations respond to changes in climate. As discussed previously[180], microorganisms produce and consume the three dominant gases that are responsible for 98% of increased global warming: CO_2, methane (CH_4) and N_2O. How arbuscular mycorrhizal fungi (AMF) and their associated bacteria function in this large-scale scenario is still largely unknown.

AMF can mediate soil C fluxes by affecting the entry of C into the soil to protect soil organic matter from decomposition, releasing CO_2 and producing exudates through metabolism[170,181]. A study[29] estimated that global plant communities allocate 3.93 Gt of CO_2 per year to AMF, but the information on C flux is much more limited when their associated bacteria are considered. As recently demonstrated, bacteria are the main actors in the hyphosphere, as they accelerate the decomposition of complex organic materials, making mineral nutrients more readily available for AMF and plants[23,24]. The hyphosphere microbiome affected by hyphal exudates is substantially different from the bulk soil microbiome (see the figure, part **a**). Phosphorus-solubilizing bacteria are ubiquitous in the hyphosphere, producing carboxylates and protons to solubilize non-soluble P by lowering the pH, chelating cations and competing with phosphate for adsorption sites in soil[182,183]. These bacteria release phosphatases that also hydrolyse organic forms of P in the hyphosphere[72,184]. Under aerobic conditions, ammonia-oxidizing bacteria can oxidize NH_4^+ to NO_3^-, whereas under anaerobic conditions, NO_3^- is used as a respiratory electron acceptor by bacteria and is converted into gaseous N during denitrification[185]. The hyphospheric bacteria can also promote the reduction of N_2O to N_2 to reduce N_2O emissions[62] (see the figure, part **b**). These findings provide insights into the interactions between hyphospheric bacteria and AMF and how they may influence the biochemical cycling of nutrients in terrestrial ecosystems. The figure part **a** is adapted with permission from ref. 13, Elsevier.

The list of molecular components detected in hyphal exudates:

1. Sugars and polyols: fructose, glucose, galactose, trehalose, inositol, raffinose, starch-like compound, putative di- and oligosaccharides, fructose-Leu, methylneuraminate, glycerate, GlcNAc6P
2. Amino acids and amines: asparagine, glutamine, pyroglutamic acid, leucine, proline, tryptophan, tyrosine, 5-oxoproline, theophylline, methionine sulfoxide, prostaglandin E_2, *N*-acetyl-β-alanine, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranosyl 5′-monophosphate, pipecolate, arginine, riboflavin, carnitine, melilotate
3. Carboxylates: formiate, acetate, citric acid; succinic acid, aconitic acid, tartrate, *N*-acetylneuraminic acid
4. Nucleic acids: deoxyadenosine, deoxycytidine, deoxyguanosine
5. Proteins and peptides: secreted proteins
6. Potential signals: catalpol, methyl salicylate

Furthermore, stable isotope labelling experiments provided solid evidence that AMF cannot use monosaccharides to synthesize fatty acids de novo[40]. In host roots, de novo fatty acid biosynthesis first occurs in the plastid stroma, where acetyl-CoA undergoes a multistep conversion to generate fatty acids (C16:0), a process catalysed by the mycorrhizal-plant-specific enzymes β-keto-acyl carrier protein synthase I (DIS) and acyl-acyl carrier protein thioesterase (FatM)[39,42,46]. In the endoplasmic reticulum, fatty acids (C16:0) are then converted into β-monoacylglycerols (β-MAGs), a process that is regulated by another mycorrhizal-plant-specific enzyme, glycerol-3-phosphate acyltransferase (RAM2)[39,42]. Finally, these β-MAGs (C14:0 and C16:0) are thought to be transferred from host plants to the PAS via the specific PAM-localized heterodimeric half-size ATP-binding cassette G-type (ABCG) transporters STR and STR2 (STR represents stunted arbuscule)[39,47,48]. The β-MAGs (C14:0 and C16:0) in the PAS might be taken up by putative ABCG transporters on the arbuscular membranes of AMF[49,50]. Alternatively, the AMP-binding domain fatty acid transporters (RiFAT1 and RiFAT2) from the arbuscular mycorrhizal fungus *R. irregularis* participate in the import of myristic acid (C14:0) and palmitic acid (C16:0) into the arbuscules[51]. Intriguingly, myristate (C14:0) treatment stimulated the asymbiotic growth and sporulation of AMF[52,53]. Despite this considerable progress, how C is allocated and the relative contributions of fatty acid and sugar transport from host plants to AMF (in terms of AMF colonization and development) remain elusive.

Carbon metabolism in AMF and carbon flow towards hyphospheric bacteria

In IRH, the fatty acids acquired by AMF from the host plant can be elongated and desaturated to form TAGs, which are transferred to ERH as lipid droplets[33]. There, the lipids can be converted into sugars via the gluconeogenesis and glyoxylate cycle pathways. Plant-derived glucose and fructose can enter the oxidative pentose phosphate pathway, glycolysis pathway and tricarboxylic acid (TCA) cycle to participate in the C metabolism of IRH[32]. A portion of the glucose is converted into glycogen, which acts as the main transport form for sugars from IRH to ERH, where it can be stored long term within spores or used to buffer intracellular monosaccharide levels throughout the life cycle[54–56]. Moreover, lipids in spores are converted into sugars as an energy source for asymbiotic growth. For example, the biosynthesis of storage and structural polymers such as chitin requires abundant carbohydrates during spore germination[45], and the gluconeogenesis and glyoxylate cycles exhibit substantial metabolic fluxes at this asymbiotic stage[57]. In addition, biochemical and omics evidence revealed that the glycogen biosynthesis and chitin metabolism pathways are activated in AMF spores[45,56,58].

Carbon metabolism in AMF underlies C flow towards bacteria at the hyphosphere interface. Only a small proportion of bacteria (less than 5%) in the soil are known to remain active without easily available C inputs, and a large proportion of bacteria maintain a viable but non-active, dormant state[59,60]. These bacteria can transition from the dormant to the active state within minutes to hours[59]. In the case of the hyphosphere of AMF, the signal for bacterial activation may be given by the release into the soil of C-rich compounds produced via sugar and lipid metabolism in ERH of AMF. The hyphal exudates contain sugars (for example, fructose, glucose and trehalose), amino acids (for example, proline, leucine and tryptophan), carboxylates (for example, citric acid, succinic acid and uric acid), nucleic acids (for example, deoxyadenosine, deoxycytidine and deoxyguanosine), proteins and peptides[13,61]. The concentration of C released in hyphal exudate per gram of dry hyphae within 4 weeks was approximately 30 mM[28], among which the concentrations of fructose, glucose and trehalose were 20.1, 15.7 and 0.17 μM, respectively[9].

As hyphal exudates function as C-rich and energy-rich sources, they can substantially stimulate the growth and development of hyphospheric bacteria[15,28,62]. Indeed, stable isotope labelling experiments with $^{13}CO_2$ showed that AMF can function as a rapid hub for translocating C-photosynthates to soil, releasing hyphal exudates to feed the hyphospheric bacteria[63–65]. At least 92 amplicon sequence variants in the hyphosphere were substantially enriched with ^{13}C and at the order level, the taxa that assimilated the most plant-derived $^{13}CO_2$ were Solibacterales, Sphingobacteriales, Myxococcales and Nitrososphaerales[65]. Metagenomic analysis indicated that most highly ^{13}C-enriched bacteria have the potential to secrete enzymes to degrade soil organic C (ref. 64). Several of these soil bacteria (for example, *Saccharibacteria* sp. and *Rahnella aquatilis*) preferentially used fructose for their metabolism when they were exposed to mixed C sources[9,66]. In *R. aquatilis*, the sugars enter the glycolysis pathway to produce pyruvate, which leads to an increase in the acetyl-CoA concentration. Acetyl-CoA then functions as the substrate of the TCA cycle to produce ATP, further enhancing the metabolic activity of *R. aquatilis*[67].

Strikingly, the fructose and carboxylates present in hyphal exudates were shown to function as signalling molecules and/or attractants that induce the expression of *R. aquatilis* phosphatase genes and the *Pseudomonas fluorescens nosZ* gene, triggering *R. aquatilis*-mediated organic phosphorus (P_o) mineralization and *P. fluorescens*-mediated N_2O emission, respectively[9,62]. However, our knowledge about C flow from AMF towards hyphospheric bacteria remains limited. Future research should focus on the identity of hyphal exudate components that induce specific processes in hyphospheric bacteria.

Mineral transfer from the hyphosphere to mycorrhizal plants

The hyphosphere and peri-arbuscular interfaces have key roles in nutrient acquisition and transfer across the soil bacteria–AMF–roots continuum. Given that most plants in natural environments experience low inorganic phosphate (P_i) and low N conditions, the uptake and transfer of P_i and N in AMF symbionts are described in detail (Fig. 2).

Transfer of phosphate and nitrogen from the hyphosphere to AMF. In the PFB continuum, the hyphospheric bacteria actively assist the AMF in acquiring mineral nutrients (particularly P and N) from the soil and transporting them to plant cells via the tightly coordinated activities of the P_i and N transport systems[11,68–71]. Specifically, hyphospheric bacteria produce and secrete phosphatases and carbohydrate-degrading enzymes; indeed, the genes that encode these enzymes are ubiquitous in hyphospheric bacteria[72,73]. These enzymes can hydrolyse complex organically bound mineral nutrients into inorganic forms such as phosphate (PO_4^-), ammonium (NH_4^+) and nitrate (NO_3^-), and even organic forms such as amino acids and short peptides for direct absorption and utilization by AMF (Fig. 2). Moreover, the expression of mineral nutrient transporter genes in IRH and ERH increases in the presence of a hyphospheric bacterium[24].

Transfer of phosphate in arbuscular mycorrhizal symbiosis. The ERH of AMF take up P_i through high-affinity P_i transporters (for example, GvPT from *Glomus versiforme*, GintPT from *Glomus intraradices* and GigmPT from *Gigaspora margarita*) that belong to the phosphate transporter 1 (PHT1) family localized on the plasma membranes of ERH[74–76]. Once in the cytosol, P_i is rapidly polymerized into polyphosphate (Poly-P) by the vacuolar transporter chaperon (VTC) complex on the vacuole membrane, and Poly-P is released into the tubular vacuoles[77,78]. During its long-distance translocation to the host plant, Poly-P transport may be driven by water flow mediated by plant transpiration[79]. When the Poly-P flux reaches IRH and the thin arbuscule branches, the fungal exopolyphosphatases PPX1 and PPN1 hydrolyse the Poly-P into free P_i, which is thought to be exported to the cytosol through the putative vacuolar P_i exporter PHO91 (ref. 80). The P_i not used by the fungus is then released into the PAS through P_i transporters located in the arbuscules and IRH.

A recent study provided the first insights into P_i release from the fungus to the PAS: the authors identified the SYG1–Pho81–XPR1 (SPX) domain-containing P_i transporter RiPT7 located on the membranes of *R. irregularis*[81]. RiPT7 is responsible for arbuscule development and symbiotic P_i delivery under moderate to low P_i conditions, as silencing the underlying gene impaired arbuscule morphogenesis, which led to smaller and early-degrading arbuscules and the accumulation of Poly-P in the fungus[81]. P_i present in arbuscules may also be exported to the PAS by the AMF PHO1-type P_i transporters RiPHO1, RcSYG1 (from *Rhizophagus clarus*) and GmPHO1 (from *G. margarita*) via an exocytotic pathway[45,70,81,82]. Under conditions of extremely low P_i, AMF PHT1-type P_i transporters that are expressed in arbuscule-containing cells may compete with P_i transporters of the host plant to retrieve P_i from the PAS[75,76,81,83]. An additional route was recently proposed in which Poly-P is unloaded through the VTC complex to the PAS, where an apoplastic acid phosphatase hydrolyses Poly-P into P_i (refs. 70,83,84). However, this potential pathway needs to be confirmed by further experiments.

Finally, P_i in the PAS is acquired by the mycorrhizal-plant-specific PHT1 family of P_i transporters localized on the PAMs of arbuscule-containing cells, such as *Medicago truncatula* MtPT4, *Lotus japonicus* LjPT4 and rice (*Oryza sativa*) OsPT11 (refs. 85–90). As these mycorrhizal-plant-specific P_i transporters belong to the P_i–H^+ group of symporters, this transport process also requires H^+-ATPases (such as MtHA1, OsHA1 and tomato (*Solanum lycopersicum*) SlHA8) to energize a proton gradient across the PAM[91–93]. These discoveries imply that the uptake and transport of P_i through the mycorrhizal uptake pathway is rigorously controlled, but the intricate regulation of these systems remains to be unravelled, as discussed below.

Transfer of nitrogen in arbuscular mycorrhizal symbiosis. Using physiological, molecular and omics approaches, various studies have identified at least three mycorrhizal N-uptake pathways in the soil–AMF–roots continuum. First, the AMF NH_4^+ transporters (GintAMT1 and GintAMT3 from *G. intraradices*) on ERH can take up NH_4^+ from the soil[94,95]. The NH_4^+ is then assimilated into arginine through the glutamine synthetase–glutamate synthase (GS–GOGAT) pathway and transferred to IRH coupled with Poly-P (ref. 96). In arbuscules, NH_4^+ is hydrolysed from arginine, exported from arbuscules to the PAS and imported into root cells via mycorrhizal-plant-inducible NH_4^+ transporters (for example, LjAMT2;2, MtAMT2;4, maize (*Zea mays*) ZmAMT3;1 and *Sorghum bicolor* SbAMT3;1) that are localized on the PAMs[97–100]. Second, the NO_3^- in soils might be acquired by the potential NO_3^- transporter GiNT in *G. intraradices*[101]. A fraction of the NO_3^- in ERH might be converted into NH_4^+ by nitrate reductase and nitrite reductase[102] and assimilated into glutamine via the GS–GOGAT pathway. NO_3^- absorbed by ERH may also be directly translocated into IRH and arbuscules to be unloaded into the interfacial apoplasts. Subsequently, the PAM-localized NO_3^- transporter OsNPF4.5 facilitates NO_3^- transport into root cells[103]. Third, organic nitrogen in soils, such as amino acids and short peptides, might be acquired by *R. irregularis* RiPTR2 and

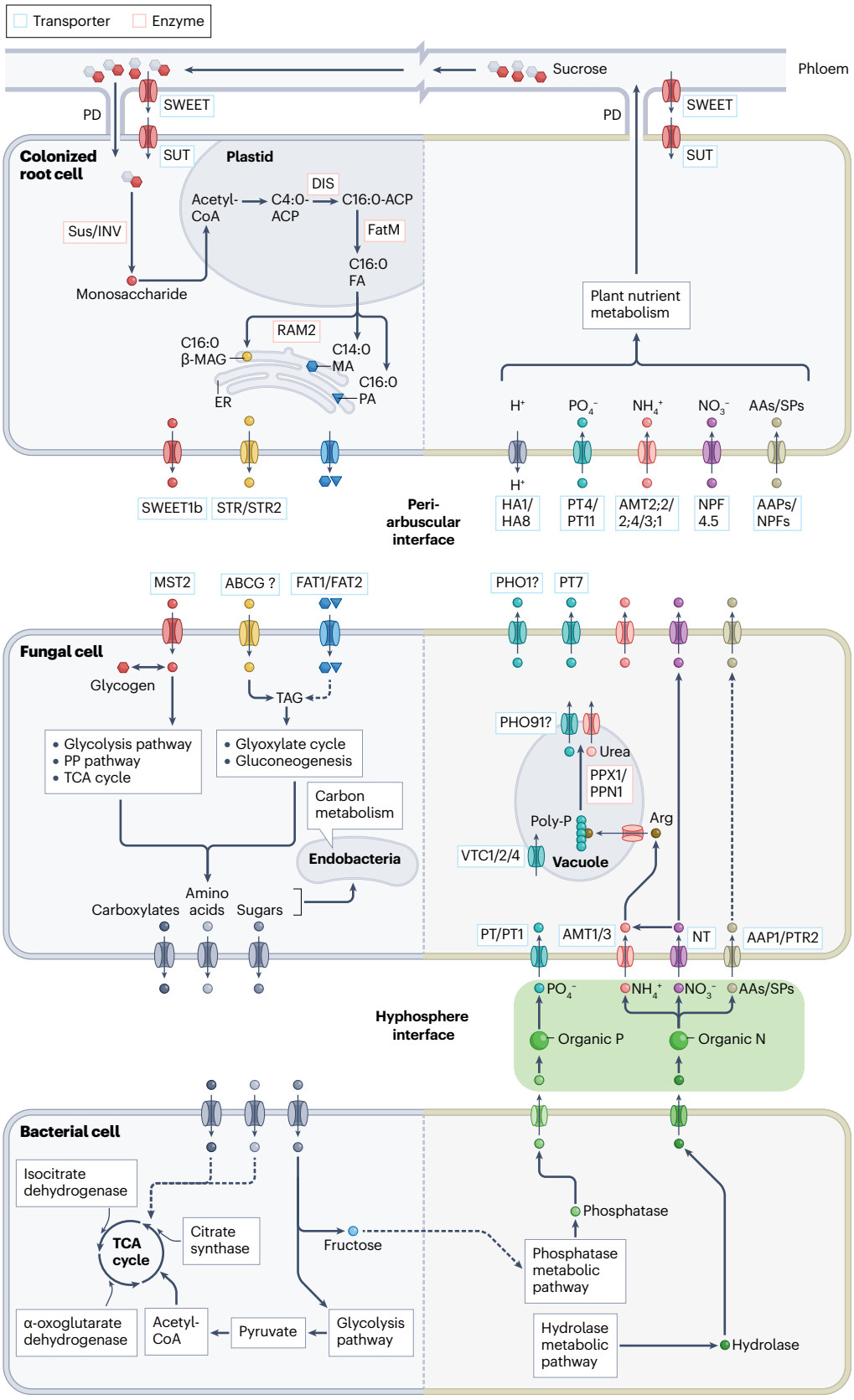

Transporter
Enzyme
Sucrose
Phloem
PD
SWEET
SUT
Colonized root cell
Plastid
Acetyl-CoA
DIS
C4:0-ACP
C16:0-ACP
FatM
C16:0 FA
Sus/INV
Monosaccharide
RAM2
C16:0 β-MAG
C14:0 MA
C16:0 PA
ER
Plant nutrient metabolism
H+
PO4-
NH4+
NO3-
AAs/SPs
SWEET1b
STR/STR2
Peri-arbuscular interface
HA1/HA8
PT4/PT11
AMT2;2/2;4/3;1
NPF 4.5
AAPs/NPFs
MST2
ABCG ?
FAT1/FAT2
PHO1?
PT7
Fungal cell
Glycogen
TAG
Glycolysis pathway
PP pathway
TCA cycle
Glyoxylate cycle
Gluconeogenesis
Carbon metabolism
Endobacteria
Amino acids
Carboxylates
Sugars
PHO91?
Urea
PPX1/PPN1
Poly-P
Arg
VTC1/2/4
Vacuole
PT/PT1
AMT1/3
NT
AAP1/PTR2
Hyphosphere interface
Organic P
Organic N
Bacterial cell
Isocitrate dehydrogenase
TCA cycle
Citrate synthase
Fructose
α-oxoglutarate dehydrogenase
Acetyl-CoA
Pyruvate
Glycolysis pathway
Phosphatase
Phosphatase metabolic pathway
Hydrolase metabolic pathway
Hydrolase

Fig. 2 | Carbon and mineral flows in the plant–arbuscular mycorrhizal fungus–bacterium continuum. Sucrose biosynthesized by plant mesophyll cells is unloaded from the phloem into root cells through the symplastic and apoplastic pathways. Here, sucrose is hydrolysed to monosaccharides for the biosynthesis of long-chain fatty acids (myristic acid (MA), palmitic acid (PA) and β-monoacylglycerols (β-MAGs)). Subsequently, plant-derived fatty acids (FAs) and monosaccharides are transported to arbuscular mycorrhizal fungi (AMF) through the two membranes (the plant peri-arbuscular membrane and the fungal arbuscular membrane) for multiple types of carbon (C) metabolism. Fatty acids are first converted into triacylglycerols (TAGs) and then enter the glyoxylate cycle and gluconeogenesis pathway to be degraded into small molecules. The resulting sugars, amino acids and carboxylates can be used as C and energy sources for the growth and development of endobacteria and hyphospheric bacteria. Fructose functions as a signal to stimulate the hyphospheric bacterial phosphatase metabolic pathway, accelerating the mineralization of organic phosphorus. The hydrolase produced by the hydrolase metabolic pathway can promote the conversion of organic nitrogen into inorganic nitrogen. The nutrients can be absorbed by AMF and transported to the arbuscules over long distances, where they will then enter plant root cells through the two membranes for multiple types of nutrient metabolism. The following transporters and enzymes participate in the transport of C and nutrients in the continuum: amino acid (AA) transporters (AAP1 and AAPs); ATP-binding cassette G-type transporter (ABCG); acyl carrier protein (ACP); ammonium transporters (AMT1, AMT3, AMT2;2, AMT2;4 and AMT3;1); β-keto-acyl carrier protein synthase I (DIS); acyl-acyl carrier protein thioesterase (FatM); fatty acid transporters (FAT1 and FAT2); H^+-ATPases (HA1 and HA8); invertase (INV); monosaccharide transporter MST2; nitrate transporters (NT and NPF4.5); SYG1–Pho81–XPR1 (SPX) domain-containing phosphate transporters (PHO1 and PHO91); AMF exopolyphosphatases (PPX1 and PPN1); phosphate transporters (PT, PT1, PT4, PT7 and PT11); short peptide transporters (PTR2 and NPFs); glycerol-3-phosphate acyltransferase (RAM2); heterodimeric half-size ABCG transporters (STR and STR2); reversible sucrose synthase (Sus); sucrose uptake transporter (SUT); sugars will eventually be exported transporters (SWEET and SWEET1b); TAGs; vacuolar transporter chaperon (VTC) complex (VTC1, VTC2 and VTC4). The solid line represents the currently identified metabolic pathway, and the dotted line needs further verification. Transporters marked with question marks have not been functionally verified, and unmarked transporters are unknown. ER, endoplasmic reticulum; PD, plasmodesmata; PP pathway, pentose phosphate pathway; Poly-P, polyphosphate; SP, short peptide; TCA cycle, tricarboxylic acid cycle.

Funneliformis mosseae (formerly known as *Glomus mosseae*) transporters located in ERH[104,105], transported into IRH and released into the PAS by yet-unidentified amino acid or short peptide transporters in arbuscules. Subsequently, the putative PAM-localized amino acid transporters and nitrate/peptide transporters mediate the import of amino acids or short peptides into cortical cells[103,106]. These findings provide important insights into the transport mechanisms that underlie N acquisition via the mycorrhizal uptake pathway and reveal that the ability of AMF to act as a biofertilizer is not limited to the largely acknowledged process of P_i uptake.

Nutrient exchange in arbuscular mycorrhizae

A popular notion is that plants can reward the best AMF partners by providing them with more C (ref. 107); in return, their fungal partners enhance cooperation by increasing the P supply to host plants that provide more C, indicating that resource exchange by plant and AMF partners ensures a 'fair trade' between partners[108]. A similar trade mechanism also regulates N flux during arbuscular mycorrhizal (AM) symbiosis[109]. The resource exchange also requires broader consideration of the specific traits of AM symbiosis such as plant sink strength, resource exchange surpluses and environmental conditions[110]. These phenomena are explained by the biological market theory, which states that this trade enables individuals to provide what they can at lower cost in exchange for resources that would otherwise be impossible or more difficult to obtain[111]. Although the ecological impact of this metaphorical 'biological market' has been largely acknowledged, the molecular basis that underlies C–P exchange is less defined, as discussed below.

The regulation of carbon–phosphorus exchange at the peri-arbuscular interface

Phosphate starvation response (PHR) transcription factors are major regulators of phosphate starvation-induced (PSI) gene expression to increase P_i uptake via the root uptake pathway[112]. Two pioneering studies demonstrated that AM symbiosis is also regulated by the PHR2-centred network[113,114] (Fig. 3). In *O. sativa*, the transcriptional regulatory network of AM symbiosis consists of 266 transcription factors and 47 promoters of mycorrhiza-related genes that control P_i uptake by the root uptake pathway and/or mycorrhizal uptake pathway under different P_i levels[113]. Low P_i levels trigger OsPHR2 activity to directly bind to the P1BS (GNATATNC) elements in the promoter regions of PSI genes and symbiosis-related genes involved in AMF colonization and development as well as nutrient exchange during symbiosis. Under high P_i conditions, P_i-sensing SPX domain-containing proteins inhibit the OsPHR2-mediated induction of symbiosis-related genes by binding to OsPHR2 and thus preventing its binding to the P1BS element[115,116], resulting in the suppression of PSI gene expression as well as mycorrhizal colonization[113,114]. A similar SPX1–PHR module mediates the suppression of AM symbiosis in *S. lycopersicum* under high P_i conditions[117].

Other recent studies have shown that the plant SPX–PHR module may be wired differently in different plants[118]. For example, overexpression of *SPX* genes in *O. sativa* reduced mycorrhizal colonization but did not influence arbuscule development[113]. This is consistent with the finding that P_i treatment inhibited new arbuscule development but not arbuscule maintenance in mycorrhizal *O. sativa* roots[119]. However, overexpression of Mt*SPX1* and Mt*SPX3* in *M. truncatula* induced the premature degeneration of arbuscules, whereas knockout of Mt*SPX1* and Mt*SPX3* decreased the level of mycorrhizal colonization but increased the abundance of mature arbuscules[118]. Therefore, in contrast to *O. sativa*, MtSPX1 and MtSPX3 in *M. truncatula* positively affect the early stages of mycorrhizal colonization. Other studies revealed distinct patterns of PHR2-mediated regulation of gene expression in mycorrhizal roots of *M. truncatula* and *O. sativa*: Mt*PHR2* expression was weakly detected in mycorrhizal roots during symbiosis, whereas Os*PHR2* was highly induced in the arbuscule-containing cells of roots; in addition, Mt*SPX1* and Mt*SPX3* were highly expressed in arbuscule-containing cells, whereas SPX induction was not detected in mycorrhizal *O. sativa* roots[113,118].

How do we explain these differences in the SPX–PHR2 module? Besides containing P1BS elements, many symbiosis-induced genes also harbour a MYC element (CTTC motif, NTTCTTGTTN) and/or AW boxes (CG(N)7CNANG) in their promoter regions that are bound by WRINKLED1-like (WRI) transcription factors[120–123]. Genomic analysis suggested a strong correlation between the presence of the P1BS and MYC *cis*-acting elements in the promoters of mycorrhiza-inducible genes

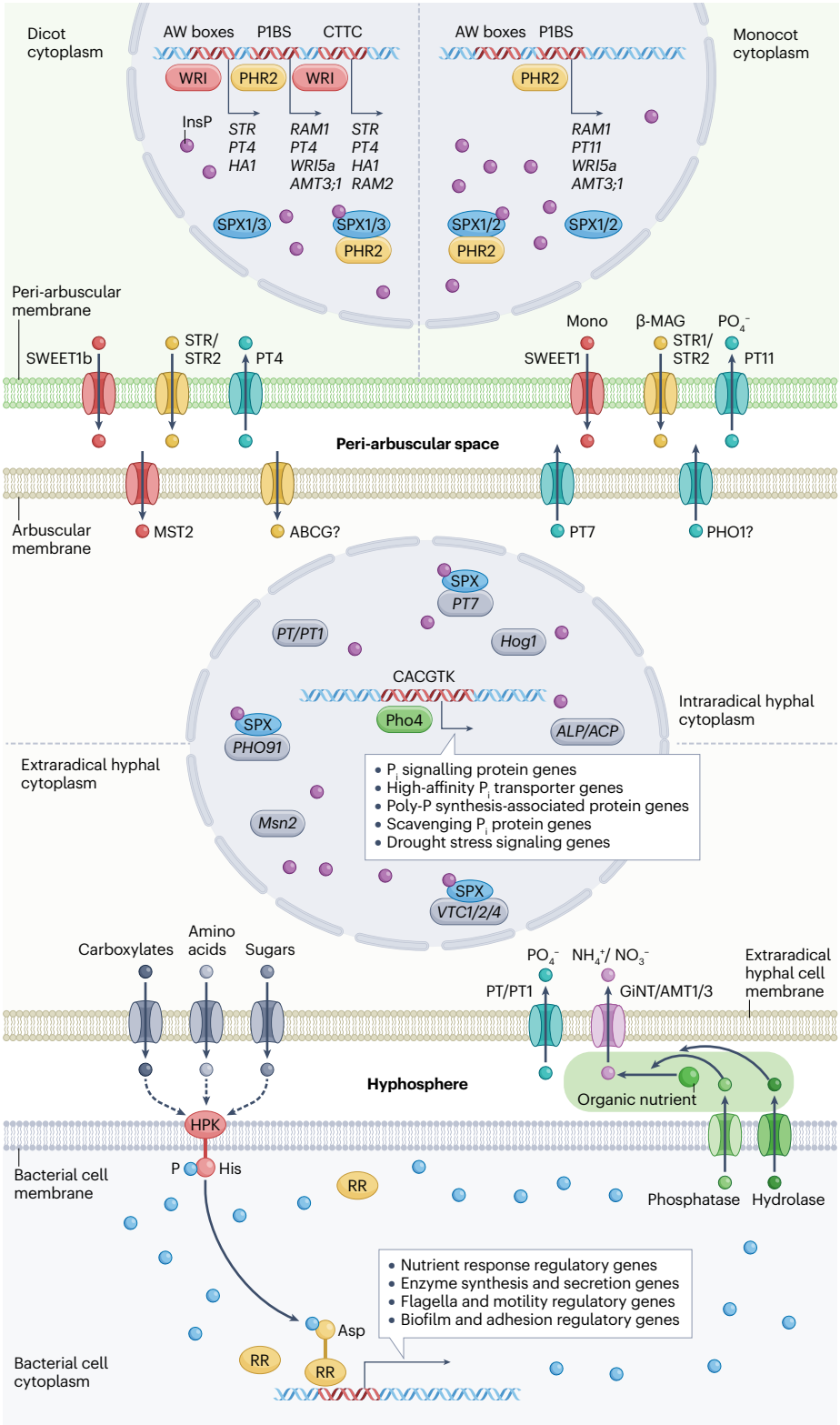


Fig. 3 | Molecular mechanisms regulating carbon and mineral exchange in the plant–arbuscular mycorrhizal fungus–bacterium continuum. In mycorrhizal plants, monocots and dicots have different regulatory models for carbon (C) and mineral exchange. The regulatory network centred on the SYG1–Pho81–XPR1 domain (SPX) domain–PHR2 transcription factor module exists in both monocots and dicots. PHR2 directly binds to the P1BS elements in the promoter regions of phosphate starvation-induced genes and symbiosis-related genes, such as *RAM1*, *PT11*, *WRI5a* and *AMT3;1*. In dicots, the WRINKLED1-like (WRI) regulatory pathway can induce the expression of genes involved in bidirectional C and inorganic phosphate (P_i) exchange and symbiosis-related genes, such as *STR*, *PT4*, *HA1* and *RAM2*, by binding to the CTTC elements and/or AW boxes in their promoters. In arbuscular mycorrhizal fungi (AMF), SPX domain-containing proteins in the PHO pathway are master regulators of P_i homeostasis, and the PHO4-centred regulatory hub binds to the CACGTK *cis*-element to regulate the expression of fungal genes involved in P_i homeostasis. The bacterial two-component signal transduction system, which is used to sense external stimuli and environmental changes, consists of a sensor histidine protein kinase (HPK) and a cognate response regulator (RR). When bacteria sense external stimuli, the HPK catalyses an autophosphorylation reaction on a histidine residue (His), producing a high-energy phosphoryl group (P), which is then transferred to the RR aspartic acid residue (Asp). Phosphorylated RR recognizes and specifically binds to its target DNA sites, activating or repressing the transcription of genes, thereby leading to a specific metabolic response. InsP, inositol polyphosphate; β-MAG, β-monoacylglycerol; mono, monosaccharide.

from dicots. Moreover, promoter deletion revealed that both motifs were required for the expression of mycorrhiza-activated P_i transporter genes in dicot species[120]. Surprisingly, Os*PT11* in *O. sativa* does not contain the canonical MYC element in its promoter, whereas in potato

(*Solanum tuberosum*), *L. japonicus* and *M. truncatula*, both P1BS and MYC elements occur in the mycorrhiza-inducible P_i transporter genes St*PT3*, Lj*PT3*, Lj*PT4* and Mt*PT4* (refs. 120,121,124). Nevertheless, only the MYC element was found to be essential for the expression of these genes[121].

P1BS elements located next to MYC or AW elements are conserved in genes that are induced in arbuscule-containing cells. In *Arabidopsis* (*Arabidopsis thaliana*), PHR transcription factors enhance the chromatin accessibility of the target genes[125]. Therefore, PHRs may potentiate the symbiotic activation of these genes by WRI transcription factors. Notably, WRI transcription factors also directly regulate lipid exchange from the liverwort *Marchantia paleacea* to AMF, pointing to an evolutionarily conserved role of these transcription factors[126]. AW boxes also exist in the promoters of PHR and P_i transporter genes in *O. sativa*, suggesting that OsPHRs may also be directly regulated by WRI transcription factors. However, their contribution to the regulation of genes involved in P_i signalling and transport in mycorrhizal roots remains to be further studied.

These studies raise the question of whether the master regulators WRI transcription factors (*M. paleacea* MpaWRI, MtWRI5a, LjCBX1, OsWRI5a) also act as hubs to control the bidirectional exchange of C and P_i at the symbiotic interface. There is good evidence that the WRI transcription factors in *L. japonicus* and *M. truncatula* function as master regulators of mycorrhiza-inducible genes involved in fatty acid biosynthesis and P_i uptake in arbuscule-containing cells. For example, MtWRI5a in *M. truncatula* directly induces the expression of *STR* and Mt*PT4* by binding to the AW boxes in their promoters[122], suggesting that a WRI transcription factor in dicots regulates the bidirectional exchange of C and P_i during AM symbiosis[123]. WRI gene expression is thought to be controlled via the common symbiotic signalling pathway. However, whether the WRI transcription factors can also regulate the nutrient exchange and arbuscule development in response to host-derived C signals remains to be studied in future.

Phosphate homeostasis inside the hyphae of AMF regulates carbon–phosphorus exchange

Although the host plant regulates C and P exchange, AMF can also orchestrate P_i acquisition and transport during symbiosis. A method to visualize P_i using fluorescent quantum-dot nanoparticles of different colours was used to examine this process based on the resource exchange strategy[127–129]. Using this method, AMF were shown to control P_i transfer during trade with the host plant when P_i availability in the hyphal network suddenly changed. If the hyphal network was exposed to a P_i surge, AMF would store the surplus P_i instead of immediately trading it, until the plant demand for P_i increased, thereby increasing the 'value' of the P_i. If P_i availability plummeted, AMF redistributed the P_i in the hyphal network, transferring it from alternative pools closer to the roots for trade with P_i-starved plants[128]. As the hyphal network can link different host plants within its growth zone, this trade preferentially occurred with host plants that showed a higher demand for P_i (ref. 129). These descriptions of the biological market in the hyphal network once again open the question of the molecular basis that underlies such fungal–plant trade networks.

Many mechanisms that collectively maintain P_i homeostasis have been put forward to explain P_i transport and redistribution inside the hyphae of AMF (Fig. 3). Among these, SPX domain-containing proteins in the phosphate-responsive signalling (PHO) pathway are thought to be master regulators of P_i homeostasis in AMF. These proteins include P_i signalling proteins, low-affinity P_i transporters, Poly-P synthesis-associated proteins and proteins involved in scavenging P_i (refs. 70,81). The genes that encode these proteins are actively regulated by the key transcription factor Pho4 and its cofactor Pho2 (refs. 76,130,131). At least 12 core components involved in the PHO pathway are likely evolutionarily conserved[132]. In *R. irregularis*, Ri*Pho4* is substantially upregulated by P starvation. RiPho4 coordinates P_i acquisition and homeostasis in AMF by regulating the downstream components (RiPTs, Ri*ALP*, Ri*ACP* and Ri*PPX1*) of the PHO pathway and orchestrates arbuscule development during AM symbiosis[131]. In recent years, the crystal structures of SPX domains have been characterized. The core of the SPX domain consists of helical bundles that provide a positively charged ligand-binding surface for inositol polyphosphates (InsPs)[133,134]. InsPs can communicate cytosolic P_i levels to the SPX domains and induce them to interact with a multitude of proteins to perform their roles in regulating P_i levels[135–137]. However, whether the SPX domain-containing proteins in AMF also possess similar binding affinities for InsPs to sense hyphal P_i concentrations remains unclear.

Various hypotheses have been raised to explain how land plants and the Glomeromycotina have maintained their interaction, which occurs in 72% of land plants and dates back to 470 million years ago[138]. According to the luxury resource exchange hypothesis, C–P exchanges could be regulated by the amount of available resources in the two partners: plants do not allow AMF colonization when the environmental P_i is high[113,114], whereas the AMF does not release P_i to the plant when it needs the P that it has taken up[76,81]. Some lines of evidence suggest a regulatory role for the Pho4-CACGTK *cis*-acting element module in AMF[76,131,132]. Under P_i-limited conditions, the transcription factor Pho4 is transported from the cytoplasm into the nucleus where it binds to the CACGTK elements in its target genes[131]. Arbuscule development in Ri*Pho4*-RNA interference lines was substantially inhibited under low P_i conditions, and RiPho4 was shown to positively regulate P_i transporter genes of the PHO pathway from *R. irregularis*[131]. Genome-wide analysis revealed CACGTK elements in the promoters of 106 genes from *R. irregularis* with predicted functions in the transport and metabolism of P_i, sugars and lipids[132], such as genes that might be related to β-MAGs (C14:0 and C16:0) and monosaccharide transport, including Ri*MST2* and Ri*ABCG*[37,49]. Furthermore, in silico analysis predicted that 67% of AMF transcription factor-encoding genes in *R. irregularis* could be directly regulated by RiPho4, suggesting that RiPho4 functions as a central regulatory hub[131]. Several key node genes directly controlled by RiPho4 were also identified in *R. irregularis*, such as genes that function in response to drought-stress signalling, including Ri*Msn2* (encoding a C_2H_2 zinc finger transcriptional activator) and Ri*Hog1* (encoding a high-osmolarity glycerol 1 MAPK)[139,140]. Despite these findings, we lack conclusive evidence that can clarify whether Pho4 functions as a hub for integration of C and P_i nutrient exchange during symbiosis in AMF under P_i starvation. However, this molecular context recalls the PHR regulatory system identified in the host plant. These recent findings suggest a possible scenario in which both plants and AMF perceive environmental factors (such as P_i availability) through integrated dual regulatory systems that enable the success of AM symbiosis.

The effects of AMF-associated bacteria on carbon–phosphorus exchange in arbuscular mycorrhizae

In the PFB continuum, hyphospheric bacteria and endobacteria consume plant-derived C for their growth and metabolism, and in return, these bacteria provide benefits to the AMF. However, our knowledge of the direct or indirect effects of AMF-associated bacteria on the host plant is scant, and experimental evidence of how AMF actively regulate the metabolic profiles of their host plants, depending on the

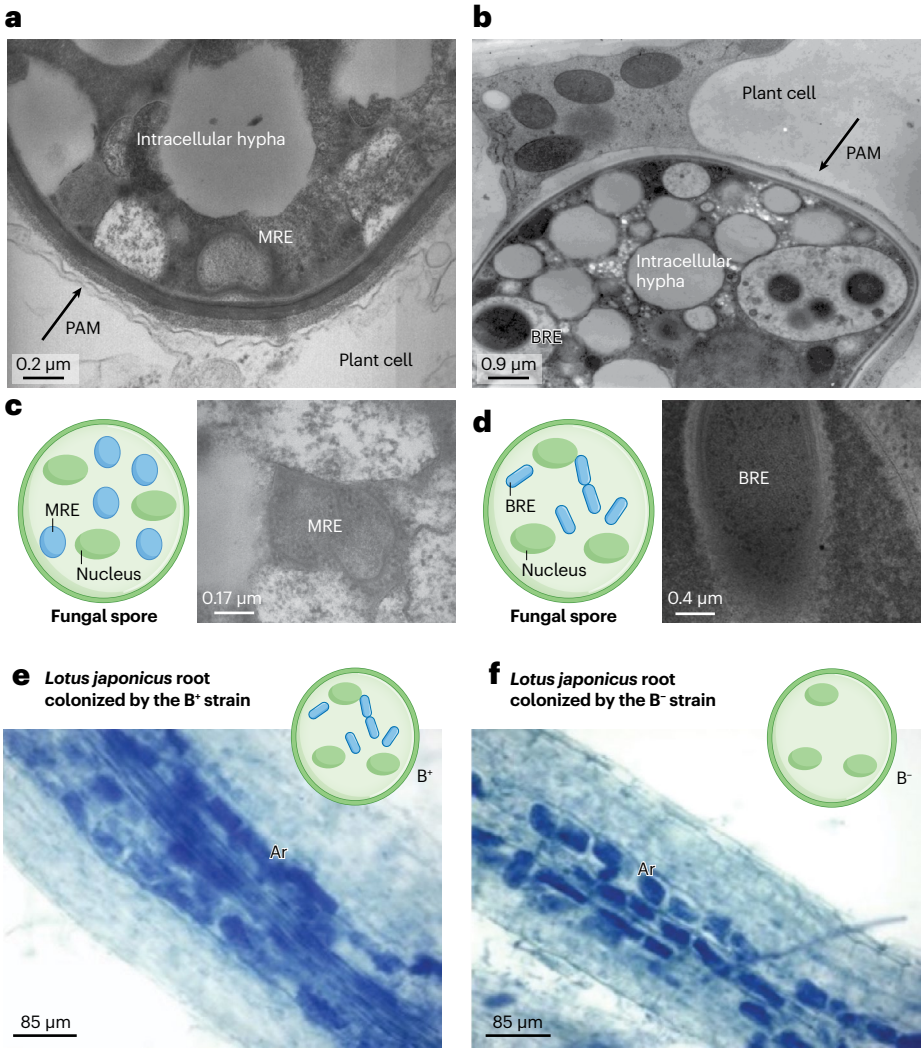


Fig. 4 | The bacteria living inside the AMF cytoplasm. a,**b**, The endobacteria detected in arbuscular mycorrhizal fungi (AMF) of the Glomeromycotina belong to two typologies with a coccoid and rod shape, respectively. The first group comprises *Mycoplasma*-related endobacteria (MRE), and the second group comprises *Burkholderia*-related endobacteria (BRE). **a**, Transmission electron microscopy of an MRE-type endobacterium detected in the cytoplasm of an arbuscular mycorrhizal fungus that colonizes the liverwort *Conocephalum*. **b**, An intracellular hypha of *Gigaspora margarita* colonizing a clover root, which contains many *Candidatus* Glomeribacter gigasporarum bacteria living inside vacuole-like structures. The rod-shaped BREs were cut longitudinally. **c**,**d**, Illustrations of the two spore typologies; electron microscopy reveals a dividing MRE (part **c**) and details of *Candidatus* Glomeribacter gigasporarum, with its rod shape and Gram-negative cell wall (part **d**). **e**,**f**, The development of a cured line (lacking endobacteria, B^-) enables direct comparison with the line containing the endobacteria (B^+). Despite their morphological and relevant molecular differences[141], both fungal isogenic lines efficiently colonize *Lotus japonicus* roots. PAM, peri-arbuscular membrane, which surrounds the fungal branch. Ar, arbuscule. Bars indicate the magnification of the electron micrographs. Part **a** adapted with permission from ref. 163, Wiley. Parts **b**, **c** and **d** provided by P. Bonfante. Parts **e** and **f** are adapted from ref. 141, CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

presence or absence of their associated bacteria, is limited to a few examples[24,141]. One such example showed that the presence of *R. aquatilis* in the hyphosphere of *R. irregularis* may enhance C and P exchange between the plant and its mycorrhizal partner. Specifically, the expression of genes encoding AMF P_i transporters (for example, Ri*PT7* and Ri*PHO91*), as well as plant genes involved in lipid biosynthesis and transport (for example, *FatM*, *RAM2* and *STR*), substantially increased in the presence of hyphospheric *R. aquatilis*, which ultimately promoted the formation and development of AM symbiosis[24]. In another example, the availability of two isogenic lines of *G. margarita* (one with an endobacterium and the other without) enabled researchers to compare the metabolism of *L. japonicus* upon mycorrhization with the two fungal isogenic lines. This comparison revealed that the presence of the endobacterium, *Candidatus* Glomeribacter gigasporarum (*Ca*Gg), has a strong effect on mycorrhizal plants (Fig. 4). Plant respiration and lipid biosynthesis were negatively affected by the absence of the endobacterium. These included proteins involved in the metabolic pathway that produces β-MAGs, such as FabZ, which functions upstream of the key lipid biosynthetic enzyme FatM. More importantly, the activity of the arbuscular mycorrhizal-inducible gene Lj*PT4*, a marker of mycorrhizal functionality in plants that enables the transfer of P_i from the fungus to the plant cell, was upregulated in the B^+ line, that is, in the presence of the bacterium[141]. Taken together, these findings demonstrate that the presence of the hyphospheric *R. irregularis* or endobacterium *Ca*Gg has a positive impact on the exchange of nutrients between the fungus and the host plant. It seems that the market theory also helps to explain the interactions of AMF with their hyphospheric bacteria and endobacteria, prompting the question of whether this can be confirmed for other tri-kingdom interactions.

The molecular basis of nutrient trade between AMF and associated bacteria

Even though AMF expand the range of nutrient uptake for plants, owing to the limited saprotrophic capability of AMF, they cannot exploit many mineral nutrients that occur in organic form or are complexed with metal ions. AMF have to rely on soil bacteria that can exploit many different substrates[142,143]. Many recent studies have demonstrated that, indeed, AMF may interact with soil bacteria to obtain nutrients that ultimately are exchanged for C with the host plant.

Carbon and mineral exchange between AMF and hyphospheric bacteria

AMF release C-rich compounds to the soil in the form of hyphal exudates[9,13,28], such as sugars, amino acids and carboxylates. However, a deeper understanding of the trade-off between AMF and hyphospheric bacteria will require us to determine whether these hyphal exudates are released to attract beneficial bacteria. Intriguingly, some data have suggested that AMF rapidly distribute newly assimilated plant C to hyphospheric bacteria and that hyphal 'exudation' is not a purely passive-loss process[144].

In this context, the cooperation between AMF and phosphate-solubilizing bacteria has become a hot topic. Hyphal exudates can induce phosphate-solubilizing bacteria to mineralize P_o in the soil[9,28]. In the phosphate-solubilizing bacterium *R. aquatilis*, the expression of phosphatase- and glucose dehydrogenase-encoding genes was shown to be regulated by two-component signalling systems that recognize and respond to hyphal exudates[145] (Fig. 3). The increased expression of these genes leads to the secretion of more phosphatases and gluconic acids into the hyphosphere, which in turn enhance the solubilization of non-soluble P_i and the mineralization of P_o, ultimately improving P-utilization efficiency in the AMF[24]. However, the C- and P-dominated interactions between AMF and soil bacteria depend on background P_i availability. Upon extremely low soil P_i availability, the bacteria compete with AMF for P_i nutrients and can inhibit hyphal growth; when the level of available P_i in the soil gradually increases (from 5 to 10 mg P per kilogram), the bacteria facilitate P_i acquisition by AMF and increase hyphal length density[28], indicating that they improve the benefits of AMF under such relatively low P_i conditions. In fact, the stoichiometric ratio of soil minerals can profoundly affect C and mineral exchange between the AMF and hyphospheric bacteria, as well as between the AMF and the plant host. However, how C:P ratios, or even C:N and N:P ratios, in the hyphosphere or rhizosphere influence nutrient exchange and regulation in all members of the PFB continuum remains unclear, and these limitations are open avenues for future research.

AMF hyphae do not benefit all bacteria in the hyphosphere; indeed, they can inhibit the growth of some bacterial species, such as certain Betaproteobacteria[64,146]. By contrast, beneficial bacteria usually have positive effects on AMF fitness[64,147]. These observations suggest that AMF may select specific bacteria with which to cooperate. In other words, AMF can efficiently utilize plant-derived C to shape the hyphospheric bacterial community and select specific functional bacteria to compensate for their limited saprotrophic capability, thus forming a core microbiome[19]. The P turnover functional profiles of these core bacterial community members are regulated by hyphal exudates and significantly increase soil phosphatase activity, accelerating in situ mineralization of soil P_o in the field[19,72,148]. *Paenibacillus* sp. that attach to

Box 3 | Potential applications of AMF in agriculture

To be sustainable, agriculture must produce sufficient amounts of high-quality food globally while using environmentally friendly technologies to reduce the input of chemical fertilizers and pesticides. Arbuscular mycorrhizal fungi (AMF) have been shown to increase yields in many cereal and tuber crops, such as wheat (*Triticum aestivum*), maize (*Zea mays*), rice (*Oryza sativa*), potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*), while reducing the need for chemical fertilization and pesticides[186,187].

AMF–plant symbiosis might also be used as an eco-friendly approach to increase the quality of medicinal and aromatic plants in sustainable agriculture. Indeed, inoculating medicinal and aromatic plants with AMF directly enhanced plant secondary metabolite production by improving photosynthetic capacity[188,189]. In addition, AMF may be useful in mitigation of heavy metal phytotoxicity; AMF-assisted phytoremediation might represent an environmentally friendly, cost-effective strategy to remove heavy metals from the environment[190].

Factors such as the intensification of agriculture, low crop diversity, high nutrient inputs and high tillage frequency limit the establishment of AM symbiosis and the successful application of AMF in agricultural ecosystems[191,192]. The host genotype can also directly influence AM symbiosis[193], and crop plants vary in their responsiveness to different AMF owing to their physiology and morphology[194]. Polyculture fields have richer and more diverse AMF communities than monoculture fields[195]. Indeed, in agricultural ecosystems in which crops are subjected to intense P fertilization, mycorrhizal colonization is often inhibited, reducing the nutritional benefits of the symbiosis[196]. As domestication could have altered the regulation of nutrient trafficking in mycorrhizal crops[197], perhaps wild relatives could function as genetic resources to overcome such an obstacle.

In addition to the challenges posed by standard agricultural practices, the successful application of AMF in agriculture still faces technical challenges. First, it is difficult to produce AMF inoculum on a large scale owing to the obligate biotrophic nature of the AMF. Based on the fatty acid theory[40,41], several pure-culture approaches have recently been reported[52,53,198]. For example, supplying AMF with myristate, plant hormones and organic nitrogen induced the production of colonization-competent pure spores in asymbiotic cultures[53]. Second, different strains may be required to fully realize the potential of AMF in different crop systems based on plant and AMF genotypes, colonization ability, inter- and intra-species diversity, and symbiotic efficiency. Third, understanding the factors that influence the function of AMF and AMF-associated bacteria and the measures that may enhance their interactions is crucial for the successful deployment of AMF bio-inoculants in agricultural ecosystems[17]. Some stable bacterial types are shared by different AMF taxa and in different soil types. These stable bacterial types may support AMF colonization by promoting pre-symbiotic processes such as sporulation, germination, hyphal growth and branching, thereby improving the effectiveness of bio-inoculants in soil[17]. In addition, the application of biostimulants (for example, chito-oligosaccharides) can also promote symbiotic development, increase AMF colonization efficiency and improve plant species evenness and productivity[199]. To date, there are only a few commercially available and useful AMF bio-inoculants and no quality management framework and global quality standards. Understanding plant–AMF–bacterial interactions and developing effective protocols and standards are expected to make AMF-based bio-inoculants a reliable part of sustainable agriculture in the future[200].

the surfaces of AMF spore cell walls are capable of degrading cellulose and chitin by producing the corresponding degradative enzymes[149], enhancing the acquisition of soil organic nitrogen (N_o). The presence of the protist *Polysphondylium pallidum* along with *Paenibacillus* sp. in the N_o zone was found to accelerate chitin decomposition[10]. In addition to mineralizing organic nutrients, some hyphospheric bacteria also contain *nifH* genes to help AMF benefit from biological N fixation[150]. Although the number of studies exploring the hyphospheric microbiota in diverse environments is increasing, the molecular dialogue between AMF and their hyphospheric bacteria is still largely unknown and will require novel, refined metabolomic analyses.

Crosstalk between endobacteria and AMF

Starting in the 1970s, microscopic observations revealed the presence of endobacteria living inside the cytoplasm of many AMF isolates[151]. Molecular tools, including 16S rDNA sequencing, identified two endobacterial groups. The first group, comprising rod-shaped bacteria located inside fungal vacuoles, is related to *Burkholderia* (*Burkholderia*-related endobacteria, BRE) and one of the strains has been identified as *Ca*Gg[152]. The second group, which is related to Mycoplasmataceae (*Mycoplasma*-related endobacteria, MRE), reside in the cytoplasm of AMF and have a coccoid structure[153]; one isolate has been named *Candidatus* Moeniiplasma glomeromycotorum (*Ca*Mg) (Fig. 4). Whereas BRE seem to be restricted to Gigasporales, MRE have been detected in phylogenetically distinct AMF lineages, revealing a wider diversity. Both endobacterial groups are presumed to be transmitted vertically from spores to progeny[154] and can coexist, forming an intracellular microbiome of AMF. BRE and MRE have smaller genomes (approximately 2 Mb and 0.7–1.3 Mb, respectively) and substantially lower metabolic capacities than their free-living relatives[155–157]. Notably, closely related BRE and MRE have been found in other Mucoromycota (Mortierellomycotina and Mucoromycotina), suggesting that they might represent a signature for the evolution of this ancient fungal phylum[8,158].

The development of a *G. margarita* line devoid of endobacteria (that is, cured line[159]) enabled functional studies on the fungal–endobacterial association by comparing fungal behaviour in an isogenic background with and without endosymbionts (Fig. 4). This approach demonstrated that endobacteria depend on C and energy provided by AMF, which are also a source of amino acids and mineral nutrients. Evidence suggests that *Ca*Gg also utilizes mineral nutrients from the host fungus, as a large set of proteins involved in P_i and zinc uptake was identified in its genome[155]. By contrast, endobacteria affect the development and metabolism of their fungal hosts[45,160]. *Ca*Gg promoted hyphal elongation and branching, and its presence substantially affected the expression of approximately 25% of *G. margarita* genes[45], strongly impacting its lipid profile[161]. A study that combined transcriptomics with cell biological and physiological analyses revealed that *Ca*Gg improves the ecological fitness of its fungal host, increasing ATP production and eliciting mechanisms to detoxify reactive oxygen species[160]. A study using high-resolution secondary ion mass spectrometry (coupled to electron microscopy) revealed the C flow along the plant–AMF–endobacterium continuum: C flows from the plant to *G. margarita* strain MAFF520054, which hosts both BRE and MRE[162]. The AMF seemed to provide more C to MRE than to BRE[162]. This observation is in line with the suggestion that MRE act as weak parasites that are more opportunistic than BRE. However, as attempts to clear MRE in AMF have been unsuccessful, the interaction of MRE with their hosts cannot yet be identified as mutualistic or parasitic[158].

Conclusions and future prospects

The PFB continuum represents a complex, multi-layered and still enigmatic type of tri-kingdom interaction. The exchange and regulation of C and minerals is at the heart of this continuum, which, in addition to the dynamics of the three partners, is also continuously subjected to other abiotic and biotic environmental factors. AMF and their associated bacteria have great potential for use in maintaining soil fertility, plant productivity and health; they may replace chemical fertilizers to some extent and become a key factor in sustainable agriculture (Box 3). More studies of these complex below-ground interactions are needed, as the role of bacteria as a relevant factor for successful mycorrhization has rarely been considered. The impact of C flow from plants to mycorrhizal fungi on the global C pool has recently been demonstrated[29], but the reported results can change when the associated bacterial communities are considered. Below, we propose some questions to be addressed to better understand the communications across the three kingdoms.

1. To what extent do AMF-associated bacteria reinforce the root sink demand for plant C, and how does the tripartite interaction affect the C and nutrient fluxes in the whole system?
2. Are hyphosphere bacteria or endobacteria capable of directly interacting with plants, or are their responses solely mediated by the arbuscular mycorrhizal fungus? What are the mechanisms behind this? How can we discriminate between a direct and an indirect response?
3. In the case of the *L. japonicus*, *G. margarita* and *Ca*Gg interaction, the main fungal responses to the endobacterium (mitochondrial activity, respiration, antioxidant activity) were similar to those found in the plant. This raises the question of whether (and which) signalling molecules released by bacteria and AMF elicit common responses in their respective hosts, AMF and plants, respectively.
4. Does Pho4 in AMF serve as a signalling hub for the exchange of C (such as soluble sugars and fatty acids), mineral nutrients (such as P and N) and water in the bacteria–AMF–roots continuum during P starvation? Is C and nutrient exchange between ERH and soil bacteria that occurs in the hyphosphere interface regulated by other transcription factors in AMF?
5. Is there a dual regulatory system (the host-derived PHR2 versus the arbuscular mycorrhizal fungus-derived Pho4) to simultaneously coordinate the exchange of C and mineral nutrients across the three kingdoms?
6. In the PFB continuum, are there signalling molecules that enhance the exchange of C for minerals and trigger regulatory mechanisms?
7. Can plant-derived fatty acids as 'hard currency' be directly exuded by ERH and then absorbed by hyphospheric bacteria? Are there other fates (secondary metabolism) for fatty acids in AMF, in addition to participating in C storage and metabolism?
8. How do soil bacteria recognize AMF signals and successfully colonize the hyphosphere? Does a specific molecular dialogue between AMF and hyphospheric bacteria elicit mycorrhization?
9. Can new technologies and approaches (for example, single-cell sequencing and spatial transcriptomics) shed light on the processes by which the tri-kingdom symbiosis is established and the mechanisms of dynamic interactions in the PFB continuum?

The answers to these questions can help to reveal the mechanisms of cross-kingdom interactions and their profound impact on terrestrial ecosystems.

Published online: 16 July 2024

References

1. Chialva, M., Lanfranco, L. & Bonfante, P. The plant microbiota: composition, functions, and engineering. *Curr. Opin. Biotechnol.* **73**, 135–142 (2022).
2. Fitzpatrick, C. R. et al. The plant microbiome: from ecology to reductionism and beyond. *Annu. Rev. Microbiol.* **74**, 81–100 (2020).
   This review integrates ecological and reductionism approaches for a more comprehensive understanding of plant microbiomes.
3. Genre, A., Lanfranco, L., Perotto, S. & Bonfante, P. Unique and common traits in mycorrhizal symbioses. *Nat. Rev. Microbiol.* **18**, 649–660 (2020).
   The review summarizes the origin and evolutionary history of all mycorrhizal fungal typologies and their relationships with land plants from the aspects of phylogenomics and molecular and cell biology.
4. Averill, C. et al. Alternative stable states of the forest mycobiome are maintained through positive feedbacks. *Nat. Ecol. Evol.* **6**, 375–382 (2022).
5. Choi, J., Summers, W. & Paszkowski, U. Mechanisms underlying establishment of arbuscular mycorrhizal symbioses. *Annu. Rev. Phytopathol.* **56**, 135–160 (2018).
6. Shi, J., Wang, X. & Wang, E. Mycorrhizal symbiosis in plant growth and stress adaptation: from genes to ecosystems. *Annu. Rev. Plant Biol.* **74**, 569–607 (2023).
7. Emmett, B. D., Lévesque-Tremblay, V. & Harrison, M. J. Conserved and reproducible bacterial communities associate with extraradical hyphae of arbuscular mycorrhizal fungi. *ISME J.* **15**, 2276–2288 (2021).
   This work identifies a conserved bacterial community associated with the extraradical hyphae of AMF, supporting the existence of a core microbiome in the hyphosphere.
8. Bonfante, P. & Desirò, A. Who lives in a fungus? The diversity, origins and functions of fungal endobacteria living in Mucoromycota. *ISME J.* **11**, 1727–1735 (2017).
   This is a contribution to the message that fungal endobacteria are probably active tenants of their fungal homes and may propagate benefits to the interacting plant, leading to a three-level inter-domain interaction.
9. Zhang, L., Feng, G. & Declerck, S. Signal beyond nutrient, fructose, exuded by an arbuscular mycorrhizal fungus triggers phytate mineralization by a phosphate solubilizing bacterium. *ISME J.* **12**, 2339–2351 (2018).
   This work reveals that fructose in hyphal exudates acts as signal to induce phosphatase gene expression in bacteria.
10. Rozmoš, M. et al. Organic nitrogen utilisation by an arbuscular mycorrhizal fungus is mediated by specific soil bacteria and a protist. *ISME J.* **16**, 676–685 (2022).
11. Wipf, D., Krajinski, F., Van Tuinen, D., Recorbet, G. & Courty, P. Trading on the arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks. *New Phytol.* **223**, 1127–1142 (2019).
12. Johansson, J. F., Paul, L. R. & Finlay, R. D. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiol. Ecol.* **48**, 1–13 (2004).
13. Zhang, L., Zhou, J., George, T. S., Limpens, E. & Feng, G. Arbuscular mycorrhizal fungi conducting the hyphosphere bacterial orchestra. *Trends Plant Sci.* **27**, 402–411 (2022).
14. Jansa, J. & Hodge, A. Swimming, gliding, or hyphal riding? On microbial migration along the arbuscular mycorrhizal hyphal highway and functional consequences thereof. *New Phytol.* **230**, 14–16 (2021).
15. Jiang, F., Zhang, L., Zhou, J., George, T. S. & Feng, G. Arbuscular mycorrhizal fungi enhance mineralisation of organic phosphorus by carrying bacteria along their extraradical hyphae. *New Phytol.* **230**, 304–315 (2021).
16. Faghihinia, M., Jansa, J., Halverson, L. J. & Staddon, P. L. Hyphosphere microbiome of arbuscular mycorrhizal fungi: a realm of unknowns. *Biol. Fertil. Soils* **59**, 17–34 (2023).
17. Basiru, S., Ait Si Mhand, K. & Hijri, M. Disentangling arbuscular mycorrhizal fungi and bacteria at the soil-root interface. *Mycorrhiza* **33**, 119–137 (2023).
18. Zhou, J. et al. Different arbuscular mycorrhizal fungi cocolonizing on a single plant root system recruit distinct microbiomes. *mSystems* **5**, e00929-20 (2020).
19. Wang, L., Zhang, L., George, T. S. & Feng, G. A core microbiome in the hyphosphere of arbuscular mycorrhizal fungi has functional significance in organic phosphorus mineralization. *New Phytol.* **238**, 859–873 (2023).
20. Garbaye, J. Tansley review no. 76 helper bacteria: a new dimension to the mycorrhizal symbiosis. *New Phytol.* **128**, 197–210 (1994).
21. Frey-Klett, P., Garbaye, J. & Tarkka, M. The mycorrhiza helper bacteria revisited. *New Phytol.* **176**, 22–36 (2007).
22. Berrios, L. et al. Positive interactions between mycorrhizal fungi and bacteria are widespread and benefit plant growth. *Curr. Biol.* **33**, 2878–2887 (2023).
23. Hestrin, R., Hammer, E. C., Mueller, C. W. & Lehmann, J. Synergies between mycorrhizal fungi and soil microbial communities increase plant nitrogen acquisition. *Commun. Biol.* **2**, 233 (2019).
24. Duan, S., Declerck, S., Feng, G. & Zhang, L. Hyphosphere interactions between *Rhizophagus irregularis* and *Rahnella aquatilis* promote carbon–phosphorus exchange at the peri-arbuscular space in *Medicago truncatula*. *Environ. Microbiol.* **25**, 867–879 (2023).
25. Dusenge, M. E., Duarte, A. G. & Way, D. A. Plant carbon metabolism and climate change: elevated CO_2 and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytol.* **221**, 32–49 (2019).
26. Bonfante, P. In *Fungal Associations* (ed. Hock, B.) 45–61 (Springer, 2001).
27. Parniske, M. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat. Rev. Microbiol.* **6**, 763–775 (2008).
28. Zhang, L. et al. Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. *New Phytol.* **210**, 1022–1032 (2016).
    This study reports that AMF and bacteria cooperate with each other based on C and P exchange.
29. Hawkins, H. J. et al. Mycorrhizal mycelium as a global carbon pool. *Curr. Biol.* **33**, R560–R573 (2023).
    This work provides quantitative estimates of the contribution of main functional AMF to global soil carbon pools.
30. Manck-Götzenberger, J. & Requena, N. Arbuscular mycorrhiza symbiosis induces a major transcriptional reprogramming of the potato *SWEET* sugar transporter family. *Front. Plant Sci.* **7**, 487 (2016).
31. An, J. et al. A *Medicago truncatula* SWEET transporter implicated in arbuscule maintenance during arbuscular mycorrhizal symbiosis. *New Phytol.* **224**, 396–408 (2019).
32. Pfeffer, P. E., Douds, D. D. Jr, Bécard, G. & Shachar-Hill, Y. Carbon uptake and the metabolism and transport of lipids in an arbuscular mycorrhiza. *Plant Physiol.* **120**, 587–598 (1999).
33. Bago, B., Pfeffer, P. E., Zipfel, W., Lammers, P. & Shachar-Hill, Y. Tracking metabolism and imaging transport in arbuscular mycorrhizal fungi. *Plant Soil.* **244**, 189–197 (2002).
34. Tisserant, E. et al. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. *Proc. Natl Acad. Sci. USA* **110**, 20117–20122 (2013).
35. Blee, K. A. & Anderson, A. J. Transcripts for genes encoding soluble acid invertase and sucrose synthase accumulate in root tip and cortical cells containing mycorrhizal arbuscules. *Plant Mol. Biol.* **50**, 197–211 (2002).
36. Schüßler, A., Martin, H., Cohen, D., Fitz, M. & Wipf, D. Characterization of a carbohydrate transporter from symbiotic glomeromycotan fungi. *Nature* **444**, 933–936 (2006).
37. Helber, N. et al. A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus *Glomus sp* is crucial for the symbiotic relationship with plants. *Plant Cell* **23**, 3812–3823 (2011).
38. Lahmidi, N. A. et al. Sugar exchanges in arbuscular mycorrhiza: RiMST5 and RiMST6, two novel *Rhizophagus irregularis* monosaccharide transporters, are involved in both sugar uptake from the soil and from the plant partner. *Plant Physiol. Biochem.* **107**, 354–363 (2016).
39. Bravo, A., Brands, M., Wewer, V., Dörmann, P. & Harrison, M. J. Arbuscular mycorrhiza-specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of arbuscular mycorrhiza. *New Phytol.* **214**, 1631–1645 (2017).
40. Jiang, Y. et al. Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi. *Science* **356**, 1172–1175 (2017).
    This study introduces the concept that obligate biotrophic fungi depend on their host plants for fatty acids.
41. Luginbuehl, L. H. et al. Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. *Science* **356**, 1175–1178 (2017).
42. Keymer, A. et al. Lipid transfer from plants to arbuscular mycorrhiza fungi. *eLife* **6**, e29107 (2017).
43. Tang, N. et al. A survey of the gene repertoire of *Gigaspora rosea* unravels conserved features among Glomeromycota for obligate biotrophy. *Front. Microbiol.* **7**, 233 (2016).
44. Kobayashi, Y. et al. The genome of *Rhizophagus clarus* HR1 reveals a common genetic basis for auxotrophy among arbuscular mycorrhizal fungi. *BMC Genomics* **19**, 465 (2018).
45. Venice, F. et al. At the nexus of three kingdoms: the genome of the mycorrhizal fungus *Gigaspora margarita* provides insights into plant, endobacterial and fungal interactions. *Environ. Microbiol.* **22**, 122–141 (2020).
46. Brands, M., Wewer, V., Keymer, A., Gutjahr, C. & Dörmann, P. The *Lotus japonicus* acyl-acyl carrier protein thioesterase FatM is required for mycorrhiza formation and lipid accumulation of *Rhizophagus irregularis*. *Plant J.* **95**, 219–232 (2018).
47. Zhang, Q., Blaylock, L. A. & Harrison, M. J. Two *Medicago truncatula* half-ABC transporters are essential for arbuscule development in arbuscular mycorrhizal symbiosis. *Plant Cell* **22**, 1483–1497 (2010).
48. Gutjahr, C. et al. The half-size ABC transporters STR1 and STR2 are indispensable for mycorrhizal arbuscule formation in rice. *Plant J.* **69**, 906–920 (2012).
49. Kovalchuk, A., Kohler, A., Martin, F. & Asiegbu, F. O. Diversity and evolution of ABC proteins in mycorrhiza-forming fungi. *BMC Evol. Biol.* **15**, 249 (2015).
50. Víglaš, J. & Olejníková, P. An update on ABC transporters of filamentous fungi–from physiological substrates to xenobiotics. *Microbiol. Res.* **246**, 126684 (2021).
51. Brands, M. & Dörmann, P. Two AMP-binding domain proteins from *Rhizophagus irregularis* involved in import of exogenous fatty acids. *Mol. Plant-Microbe Interact.* **35**, 464–476 (2022).
52. Sugiura, Y. et al. Myristate can be used as a carbon and energy source for the asymbiotic growth of arbuscular mycorrhizal fungi. *Proc. Natl Acad. Sci. USA* **117**, 25779–25788 (2020).
53. Tanaka, S. et al. Asymbiotic mass production of the arbuscular mycorrhizal fungus *Rhizophagus clarus*. *Commun. Biol.* **5**, 43 (2022).
54. Shachar-Hill, Y. et al. Partitioning of intermediary carbon metabolism in vesicular-arbuscular mycorrhizal leek. *Plant Physiol.* **108**, 7–15 (1995).
55. Bago, B. et al. Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrate as well as lipid. *Plant Physiol.* **131**, 1496–1507 (2003).
56. Bücking, H. et al. Root exudates stimulate the uptake and metabolism of organic carbon in germinating spores of *Glomus intraradices*. *New Phytol.* **180**, 684–695 (2008).

57. Lammers, P. J. et al. The glyoxylate cycle in an arbuscular mycorrhizal fungus. Carbon flux gene expression. *Plant Physiol.* **127**, 1287–1298 (2001).
58. Lin, K. et al. Single nucleus genome sequencing reveals high similarity among nuclei of an endomycorrhizal fungus. *PLoS Genet.* **10**, e1004078 (2014).
59. Blagodatskaya, E. & Kuzyakov, Y. Active microorganisms in soil: critical review of estimation criteria and approaches. *Soil. Biol. Biochem.* **67**, 192–211 (2013).
60. Sokol, N. W. et al. Life and death in the soil microbiome: how ecological processes influence biogeochemistry. *Nat. Rev. Microbiol.* **20**, 415–430 (2022).
61. Luthfiana, N. et al. Metabolite profiling of the hyphal exudates of *Rhizophagus clarus* and *Rhizophagus irregularis* under phosphorus deficiency. *Mycorrhiza* **31**, 403–412 (2021).
62. Li, X. et al. Mycorrhiza-mediated recruitment of complete denitrifying *Pseudomonas* reduces N_2O emissions from soil. *Microbiome* **11**, 45 (2023).
63. Drigo, B. et al. Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2. *Proc. Natl Acad. Sci. USA* **107**, 10938–10942 (2010).
64. Nuccio, E. E. et al. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. *Environ. Microbiol.* **15**, 1870–1881 (2013).
65. Kakouridis, A. et al. Arbuscular mycorrhiza convey significant plant carbon to a diverse hyphosphere microbial food web and mineral-associated organic matter. *New Phytol.* **244**, 1661–1675 (2024).
66. Zhang, L., Peng, Y., Zhou, J., George, T. S. & Feng, G. Addition of fructose to the maize hyphosphere increases phosphatase activity by changing bacterial community structure. *Soil Biol. Biochem.* **142**, 107724 (2020).
67. Zhang, L., Fan, J., Feng, G. & Declerck, S. The arbuscular mycorrhizal fungus *Rhizophagus irregularis* MUCL 43194 induces the gene expression of citrate synthase in the tricarboxylic acid cycle of the phosphate-solubilizing bacterium *Rahnella aquatilis* HX2. *Mycorrhiza* **29**, 69–75 (2019).
68. Calabrese, S. et al. Transcriptome analysis of the *Populus trichocarpa–Rhizophagus irregularis* mycorrhizal symbiosis: regulation of plant and fungal transportomes under nitrogen starvation. *Plant Cell Physiol.* **58**, 1003–1017 (2017).
69. Calabrese, S. et al. Imbalanced regulation of fungal nutrient transports according to phosphate availability in a symbiocosm formed by poplar, sorghum, and *Rhizophagus irregularis*. *Front. Plant Sci.* **10**, 1617 (2019).
70. Ezawa, T. & Saito, K. How do arbuscular mycorrhizal fungi handle phosphate? New insight into fine-tuning of phosphate metabolism. *New Phytol.* **220**, 1116–1121 (2018).
71. Chialva, M. et al. The mycorrhizal root-shoot axis elicits *Coffea arabica* growth under low phosphate conditions. *New Phytol.* **239**, 271–285 (2023).
72. Zhang, L. et al. Arbuscular mycorrhizal fungi stimulate organic phosphate mobilization associated with changing bacterial community structure under field conditions. *Environ. Microbiol.* **20**, 2639–2651 (2018).
73. Nuccio, E. E. et al. HT-SIP: a semi-automated stable isotope probing pipeline identifies cross-kingdom interactions in the hyphosphere of arbuscular mycorrhizal fungi. *Microbiome* **10**, 199 (2022).
74. Harrison, M. J. & van Buuren, M. L. A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. *Nature* **378**, 626–629 (1995).
75. Fiorilli, V., Lanfranco, L. & Bonfante, P. The expression of *GintPT*, the phosphate transporter of *Rhizophagus irregularis*, depends on the symbiotic status and phosphate availability. *Planta* **237**, 1267–1277 (2013).
76. Xie, X. et al. Arbuscular mycorrhizal symbiosis requires a phosphate transceptor in the *Gigaspora margarita* fungal symbiont. *Mol. Plant* **9**, 1583–1608 (2016).
77. Uetake, Y., Kojima, T., Ezawa, T. & Saito, M. Extensive tubular vacuole system in an arbuscular mycorrhizal fungus, *Gigaspora margarita*. *New Phytol.* **154**, 761–768 (2002).
78. Ezawa, T., Cavagnaro, T. R., Smith, S. E., Smith, F. A. & Ohtomo, R. Rapid accumulation of polyphosphate in extraradical hyphae of an arbuscular mycorrhizal fungus as revealed by histochemistry and a polyphosphate kinase/luciferase system. *New Phytol.* **161**, 387–392 (2004).
79. Kikuchi, Y. et al. Aquaporin-mediated long-distance polyphosphate translocation directed towards the host in arbuscular mycorrhizal symbiosis: application of virus-induced gene silencing. *New Phytol.* **211**, 1202–1208 (2016).
80. Kikuchi, Y. et al. Polyphosphate accumulation is driven by transcriptome alterations that lead to near-synchronous and near-equivalent uptake of inorganic cations in an arbuscular mycorrhizal fungus. *New Phytol.* **204**, 638–649 (2014).
81. Xie, X. et al. A SPX domain-containing phosphate transporter from *Rhizophagus irregularis* handles phosphate homeostasis at symbiotic interface of arbuscular mycorrhizas. *New Phytol.* **234**, 650–671 (2022).
A recent study unveils insights into how AMF release phosphate to the symbiotic interface.
82. Chiu, C. H. & Paszkowski, U. Mechanisms and impact of symbiotic phosphate acquisition. *Cold Spring Harb. Perspect. Biol.* **11**, a034603 (2019).
83. Saito, K. & Ezawa, T. In *Molecular Mycorrhizal Symbiosis* (ed. Martin, F.) 197–216 (Wiley Online Library, 2016).
84. Nguyen, C. T. & Saito, K. Role of cell wall polyphosphates in phosphorus transfer at the arbuscular interface in mycorrhizas. *Front. Plant Sci.* **12**, 1980 (2021).
85. Harrison, M. J., Dewbre, G. R. & Liu, J. A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell* **14**, 2413–2429 (2002).
86. Javot, H., Penmetsa, R. V., Terzaghi, N., Cook, D. R. & Harrison, M. J. A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. *Proc. Natl Acad. Sci. USA* **104**, 1720–1725 (2007).
87. Kobae, Y. & Hata, S. Dynamics of periarbuscular membranes visualized with a fluorescent phosphate transporter in arbuscular mycorrhizal roots of rice. *Plant Cell Physiol.* **51**, 341–353 (2010).
88. Pumplin, N., Zhang, X., Noar, R. D. & Harrison, M. J. Polar localization of a symbiosis-specific phosphate transporter is mediated by a transient reorientation of secretion. *Proc. Natl Acad. Sci. USA* **109**, E665–E672 (2012).
89. Yang, S. et al. Nonredundant regulation of rice arbuscular mycorrhizal symbiosis by two members of the *PHOSPHATE TRANSPORTER1* gene family. *Plant Cell* **24**, 4236–4251 (2012).
90. Volpe, V., Giovannetti, M., Sun, X. G., Fiorilli, V. & Bonfante, P. The phosphate transporters LjPT4 and MtPT4 mediate early root responses to phosphate status in non mycorrhizal roots. *Plant Cell Env.* **39**, 660–671 (2016).
91. Krajinski, F. et al. The H^+-ATPase HA1 of *Medicago truncatula* is essential for phosphate transport and plant growth during arbuscular mycorrhizal symbiosis. *Plant Cell* **26**, 1808–1817 (2014).
92. Wang, E. et al. A H^+-ATPase that energizes nutrient uptake during mycorrhizal symbioses in rice and *Medicago truncatula*. *Plant Cell* **26**, 1818–1830 (2014).
93. Liu, J. et al. A mycorrhiza-specific H^+-ATPase is essential for arbuscule development and symbiotic phosphate and nitrogen uptake. *Plant Cell Env.* **43**, 1069–1083 (2020).
94. López-Pedrosa, A., González-Guerrero, M., Valderas, A., Azcón-Aguilar, C. & Ferrol, N. *GintAMT1* encodes a functional high-affinity ammonium transporter that is expressed in the extraradical mycelium of *Glomus intraradices*. *Fungal Genet. Biol.* **43**, 102–110 (2006).
95. Calabrese, S. et al. GintAMT3 – a low-affinity ammonium transporter of the arbuscular mycorrhizal *Rhizophagus irregularis*. *Front. Plant Sci.* **7**, 679 (2016).
96. Johansen, A., Finlay, R. D. & Olsson, P. A. Nitrogen metabolism of external hyphae of the arbuscular mycorrhizal fungus *Glornus intraradices*. *New Phytol.* **133**, 705–712 (1996).
97. Guether, M. et al. A mycorrhizal-specific ammonium transporter from *Lotus japonicus* acquires nitrogen released by arbuscular mycorrhizal fungi. *Plant Physiol.* **150**, 73–83 (2009).
98. Koegel, S. et al. The family of ammonium transporters (AMT) in *Sorghum bicolor*: two AMT members are induced locally, but not systemically in roots colonized by arbuscular mycorrhizal fungi. *New Phytol.* **198**, 853–865 (2013).
99. Breuillin-Sessoms, F. et al. Suppression of arbuscule degeneration in *Medicago truncatula phosphate transporter4* mutants is dependent on the ammonium transporter 2 family protein AMT2;3. *Plant Cell* **27**, 1352–1366 (2015).
100. Hui, J. et al. The mycorrhiza-specific ammonium transporter ZmAMT3;1 mediates mycorrhiza-dependent nitrogen uptake in maize roots. *Plant Cell* **34**, 4066–4087 (2022).
101. Tian, C. et al. Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: gene characterization and the coordination of expression with nitrogen flux. *Plant Physiol.* **153**, 1175–1187 (2010).
102. Govindarajulu, M. et al. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* **435**, 819–823 (2005).
103. Wang, S. et al. Functional analysis of the OsNPF4.5 nitrate transporter reveals a conserved mycorrhizal pathway of nitrogen acquisition in plants. *Proc. Natl Acad. Sci. USA* **117**, 16649–16659 (2020).
This work reveals the presence of a conserved mycorrhizal route for nitrate uptake in plants.
104. Cappellazzo, G., Lanfranco, L., Fitz, M., Wipf, D. & Bonfante, P. Characterization of an amino acid permease from the endomycorrhizal fungus *Glomus mosseae*. *Plant Physiol.* **147**, 429–437 (2008).
105. Belmondo, S. et al. A dipeptide transporter from the arbuscular mycorrhizal fungus *Rhizophagus irregularis* is upregulated in the intraradical phase. *Front. Plant Sci.* **5**, 436 (2014).
106. Drechsler, N., Courty, P.-E., Brulé, D. & Kunze, R. Identification of arbuscular mycorrhiza-inducible *Nitrate Transporter 1/Peptide Transporter Family* (*NPF*) genes in rice. *Mycorrhiza* **28**, 93–100 (2018).
107. Bever, J. D., Richardson, S. C., Lawrence, B. M., Holmes, J. & Watson, M. Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecol. Lett.* **12**, 13–21 (2009).
108. Kiers, E. T. et al. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* **333**, 880–882 (2011).
The study reveals that plants and fungi form mutualistic relationships that are stable because both partners can preferentially reward each other.
109. Fellbaum, C. R. et al. Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. *Proc. Natl Acad. Sci. USA* **109**, 2666–2671 (2012).
110. Walder, F. & Van Der Heijden, M. G. A. Regulation of resource exchange in the arbuscular mycorrhizal symbiosis. *Nat. Plants* **1**, 15159 (2015).
111. Werner, G. D. A. et al. Evolution of microbial markets. *Proc. Natl Acad. Sci. USA* **111**, 1237–1244 (2014).
112. Puga, M. I. et al. Novel signals in the regulation of Pi starvation responses in plants: facts and promises. *Curr. Opin. Plant Biol.* **39**, 40–49 (2017).
113. Shi, J. et al. A phosphate starvation response-centered network regulates mycorrhizal symbiosis. *Cell* **184**, 5527–5540 (2021).
This study demonstrates a molecular basis for the self-regulation of arbuscular mycorrhizal symbiosis by phosphate starvation.

114. Das, D. et al. PHOSPHATE STARVATION RESPONSE transcription factors enable arbuscular mycorrhiza symbiosis. *Nat. Commun.* **13**, 477 (2022).
115. Wang, Z. et al. Rice SPX1 and SPX2 inhibit phosphate starvation responses through interacting with PHR2 in a phosphate-dependent manner. *Proc. Natl Acad. Sci. USA* **111**, 14953–14958 (2014).
116. Zhou, Z. et al. SPX proteins regulate Pi homeostasis and signaling in different subcellular level. *Plant Signal. Behav.* **10**, e1061163 (2015).
117. Liao, D. et al. SlSPX1-SlPHR complexes mediate the suppression of arbuscular mycorrhizal symbiosis by phosphate repletion in tomato. *Plant Cell* **34**, 4045–4065 (2022).
118. Wang, P. et al. Medicago SPX1 and SPX3 regulate phosphate homeostasis, mycorrhizal colonization, and arbuscule degradation. *Plant Cell* **33**, 3470–3486 (2021).
This work demonstrates a role for phosphate-sensing SPX proteins in plant regulation of arbuscular mycorrhizal symbiosis.
119. Kobae, Y. et al. Phosphate treatment strongly inhibits new arbuscule development but not the maintenance of arbuscule in mycorrhizal rice roots. *Plant Physiol.* **171**, 566–579 (2016).
120. Chen, A. et al. Identification of two conserved *cis*-acting elements, MYCS and P1BS, involved in the regulation of mycorrhiza-activated phosphate transporters in eudicot species. *New Phytol.* **189**, 1157–1169 (2011).
121. Lota, F. et al. The *cis*-acting CTTC–P1BS module is indicative for gene function of *LjVTI12*, a Qb-SNARE protein gene that is required for arbuscule formation in *Lotus japonicus*. *Plant J.* **74**, 280–293 (2013).
122. Jiang, Y. et al. *Medicago* AP2-domain transcription factor WRI5a is a master regulator of lipid biosynthesis and transfer during mycorrhizal symbiosis. *Mol. Plant* **11**, 1344–1359 (2018).
123. Xue, L. et al. AP2 transcription factor CBX1 with a specific function in symbiotic exchange of nutrients in mycorrhizal *Lotus japonicus*. *Proc. Natl Acad. Sci. USA* **115**, E9239–E9246 (2018).
124. Volpe, V., Dell'Aglio, E. & Bonfante, P. The *Lotus japonicus MAMI* gene links root development, arbuscular mycorrhizal symbiosis and phosphate availability. *Plant Signal. Behav.* **8**, e23414 (2013).
125. Barragán-Rosillo, A. C. et al. Genome accessibility dynamics in response to phosphate limitation is controlled by the PHR1 family of transcription factors in Arabidopsis. *Proc. Natl Acad. Sci. USA* **118**, e2107558118 (2021).
126. Rich, M. K. et al. Lipid exchanges drove the evolution of mutualism during plant terrestrialization. *Science* **372**, 864–868 (2021).
127. Whiteside, M. D. et al. Mycorrhizal fungi respond to resource inequality by moving phosphorus from rich to poor patches across networks. *Curr. Biol.* **29**, 2043–2050 (2019).
128. Van'T Padje, A., Werner, G. D. A. & Kiers, E. T. Mycorrhizal fungi control phosphorus value in trade symbiosis with host roots when exposed to abrupt 'crashes' and 'booms' of resource availability. *New Phytol.* **229**, 2933–2944 (2021).
129. Van'T Padje, A. et al. Temporal tracking of quantum-dot apatite across in vitro mycorrhizal networks shows how host demand can influence fungal nutrient transfer strategies. *ISME J.* **15**, 435–449 (2021).
130. Bhalla, K., Qu, X., Kretschmer, M. & Kronstad, J. W. The phosphate language of fungi. *Trends Microbiol.* **30**, 338–349 (2022).
131. Zhang, S. et al. A transcriptional activator from *Rhizophagus irregularis* regulates phosphate uptake and homeostasis in AM symbiosis during phosphorous starvation. *Front. Microbiol.* **13**, 1114089 (2023).
132. Zhou, X. et al. Genome-wide analysis of nutrient signaling pathways conserved in arbuscular mycorrhizal fungi. *Microorganisms* **9**, 1557 (2021).
133. Wild, R. et al. Control of eukaryotic phosphate homeostasis by inositol polyphosphate sensor domains. *Science* **352**, 986–990 (2016).
134. Jung, J.-Y., Ried, M. K., Hothorn, M. & Poirier, Y. Control of plant phosphate homeostasis by inositol pyrophosphates and the SPX domain. *Curr. Opin. Biotechnol.* **49**, 156–162 (2018).
135. Desmarini, D. et al. IP_7-SPX domain interaction controls fungal virulence by stabilizing phosphate signaling machinery. *mBio* **11**, e01920–e01920 (2020).
136. Ried, M. K. et al. Inositol pyrophosphates promote the interaction of SPX domains with the coiled-coil motif of PHR transcription factors to regulate plant phosphate homeostasis. *Nat. Commun.* **12**, 384 (2021).
137. Pipercevic, J. et al. Inositol pyrophosphates activate the vacuolar transport chaperone complex in yeast by disrupting a homotypic SPX domain interaction. *Nat. Commun.* **14**, 2645 (2023).
138. Kiers, E. T. & Van Der Heijden, M. G. A. Mutualistic stability in the arbuscular mycorrhizal symbiosis: exploring hypotheses of evolutionary cooperation. *Ecology* **87**, 1627–1636 (2006).
139. Fan, X. et al. A module centered on the transcription factor Msn2 from arbuscular mycorrhizal fungus *Rhizophagus irregularis* regulates drought stress tolerance in the host plant. *New Phytol.* **240**, 1497–1518 (2023).
140. Wang, Z., Zheng, Z., Zhu, Y., Kong, S. & Liu, D. PHOSPHATE RESPONSE 1 family members act distinctly to regulate transcriptional responses to phosphate starvation. *Plant Physiol.* **191**, 1324–1343 (2023).
141. Venice, F. et al. Symbiotic responses of *Lotus japonicus* to two isogenic lines of a mycorrhizal fungus differing in the presence/absence of an endobacterium. *Plant J.* **108**, 1547–1564 (2021).
142. Torsvik, V. & Øvreås, L. Microbial diversity and function in soil: from genes to ecosystems. *Curr. Opin. Microbiol.* **5**, 240–245 (2002).
143. Nannipieri, P. et al. Microbial diversity and soil functions. *Eur. J. Soil. Sci.* **54**, 655–670 (2003).
144. Kaiser, C. et al. Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. *New Phytol.* **205**, 1537–1551 (2015).
145. Duan, S., Declerck, S., Zhang, L. & Feng, G. Two-component system in *Rahnella aquatilis* is impacted by the hyphosphere of the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. *Environ. Microbiol. Rep.* **14**, 119–129 (2022).
146. Bender, S. F. et al. Symbiotic relationships between soil fungi and plants reduce N_2O emissions from soil. *ISME J.* **8**, 1336–1345 (2014).
147. Scheublin, T. R., Sanders, I. R., Keel, C. & van der Meer, J. R. Characterisation of microbial communities colonising the hyphal surfaces of arbuscular mycorrhizal fungi. *ISME J.* **4**, 752–763 (2010).
148. Wang, G., Jin, Z., George, T. S., Feng, G. & Zhang, L. Arbuscular mycorrhizal fungi enhance plant phosphorus uptake through stimulating hyphosphere soil microbiome functional profiles for phosphorus turnover. *New Phytol.* **238**, 2578–2593 (2023).
149. Bonfante, P. & Anca, I. A. Plants, mycorrhizal fungi, and bacteria: a network of interactions. *Annu. Rev. Microbiol.* **63**, 363–383 (2009).
This review considers plants, mycorrhizal fungi and bacteria as a whole system and illustrates the different types of interaction that occur between mycorrhizal fungi and bacteria.
150. Shi, J. et al. Dual functions of bacteria colonized on AM fungal hyphae–fixing N_2 and solubilizing phosphate. *Acta Pedol. Sin.* **58**, 1289–1298 (2021).
151. Bonfante, P. The future has roots in the past: the ideas and scientists that shaped mycorrhizal research. *New Phytol.* **220**, 982–995 (2018).
152. Bianciotto, V. et al. An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. *Appl. Environ. Microbiol.* **62**, 3005–3010 (1996).
153. Naumann, M., Schüßler, A. & Bonfante, P. The obligate endobacteria of arbuscular mycorrhizal fungi are ancient heritable components related to the *Mollicutes*. *ISME J.* **4**, 862–871 (2010).
154. Bonfante, P. & Venice, F. Mucoromycota: going to the roots of plant-interacting fungi. *Fungal Biol. Rev.* **34**, 100–113 (2020).
155. Ghignone, S. et al. The genome of the obligate endobacterium of an AM fungus reveals an interphylum network of nutritional interactions. *ISME J.* **6**, 136–145 (2012).
156. Torres-Cortés, G., Ghignone, S., Bonfante, P. & Schüßler, A. Mosaic genome of endobacteria in arbuscular mycorrhizal fungi: transkingdom gene transfer in an ancient mycoplasma–fungus association. *Proc. Natl Acad. Sci. USA* **112**, 7785–7790 (2015).
157. Sun, X. et al. Genome and evolution of the arbuscular mycorrhizal fungus *Diversispora epigaea* (formerly *Glomus versiforme*) and its bacterial endosymbionts. *New Phytol.* **221**, 1556–1573 (2019).
158. Uehling, J. K. et al. In *Evolution of Fungi and Fungal-like Organisms* (eds Pöggeler, S. & James, T.) 177–205 (Springer, 2023).
159. Lumini, E. et al. Presymbiotic growth and sporal morphology are affected in the arbuscular mycorrhizal fungus *Gigaspora margarita* cured of its endobacteria. *Cell. Microbiol.* **9**, 1716–1729 (2007).
160. Salvioli, A. et al. Symbiosis with an endobacterium increases the fitness of a mycorrhizal fungus, raising its bioenergetic potential. *ISME J.* **10**, 130–144 (2016).
161. Salvioli, A. et al. Endobacteria affect the metabolic profile of their host *Gigaspora margarita*, an arbuscular mycorrhizal fungus. *Environ. Microbiol.* **12**, 2083–2095 (2010).
162. Kuga, Y., Wu, T.-D., Sakamoto, N., Katsuyama, C. & Yurimoto, H. Allocation of carbon from an arbuscular mycorrhizal fungus, *Gigaspora margarita*, to its Gram-negative and positive endobacteria revealed by high-resolution secondary ion mass spectrometry. *Microorganisms* **9**, 2597 (2021).
163. Desirò, A. et al. *Mollicutes*-related endobacteria thrive inside liverwort-associated arbuscular mycorrhizal fungi. *Environ. Microbiol.* **15**, 822–836 (2013).
164. Brundrett, M. C. & Tedersoo, L. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytol.* **220**, 1108–1115 (2018).
165. Miyauchi, S. et al. Large-scale genome sequencing of mycorrhizal fungi provides insights into the early evolution of symbiotic traits. *Nat. Commun.* **11**, 5125 (2020).
166. Kistner, C. & Parniske, M. Evolution of signal transduction in intracellular symbiosis. *Trends Plant Sci.* **7**, 511–518 (2002).
167. Stürmer, S. L., Bever, J. D. & Morton, J. B. Biogeography of arbuscular mycorrhizal fungi (*Glomeromycota*): a phylogenetic perspective on species distribution patterns. *Mycorrhiza* **28**, 587–603 (2018).
168. Větrovský, T. et al. GlobalAMFungi: a global database of arbuscular mycorrhizal fungal occurrences from high-throughput sequencing metabarcoding studies. *New Phytol.* **240**, 2151–2163 (2023).
169. Smith, S. E. & Smith, F. A. Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia* **104**, 1–13 (2012).
170. Parihar, M. et al. The potential of arbuscular mycorrhizal fungi in C cycling: a review. *Arch. Microbiol.* **202**, 1581–1596 (2020).
171. Anthony, M. A. et al. Fungal community composition predicts forest carbon storage at a continental scale. *Nat. Commun.* **15**, 2385 (2024).
172. Hicks Pries, C. E. et al. Differences in soil organic matter between EcM- and AM-dominated forests depend on tree and fungal identity. *Ecology* **104**, e3929 (2023).
173. Weng, W. et al. Roles of *arbuscular mycorrhizal* fungi as a biocontrol agent in the control of plant diseases. *Microorganisms* **10**, 1266 (2022).
174. Babikova, Z. et al. Underground signals carried through common mycelial networks warn neighbouring plants of aphid attack. *Ecol. Lett.* **16**, 835–843 (2013).

175. Lanfranco, L. & Bonfante, P. Lessons from arbuscular mycorrhizal fungal genomes. *Curr. Opin. Microbiol.* **75**, 102357 (2023).
176. Cornell, C. et al. The arbuscular mycorrhizal fungus *Rhizophagus irregularis* harmonizes nuclear dynamics in the presence of distinct abiotic factors. *Fungal Genet. Biol.* **158**, 103639 (2022).
177. Zakem, E. J., Polz, M. F. & Follows, M. J. Redox-informed models of global biogeochemical cycles. *Nat. Commun.* **11**, 5680 (2020).
178. Rawat, P., Das, S., Shankhdhar, D. & Shankhdhar, S. C. Phosphate-solubilizing microorganisms: mechanism and their role in phosphate solubilization and uptake. *J. Soil. Sci. Plant Nutr.* **21**, 49–68 (2021).
179. Kawaka, F. Characterization of symbiotic and nitrogen fixing bacteria. *AMB Express* **12**, 99 (2022).
180. Tiedje, J. M. et al. Microbes and climate change: a research prospectus for the future. *mBio* **13**, e00800–e00822 (2022).
181. Cheng, L. et al. Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. *Science* **337**, 1084–1087 (2012).
182. Taktek, S. et al. Trapping of phosphate solubilizing bacteria on hyphae of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* DAOM 197198. *Soil Biol. Biochem.* **90**, 1–9 (2015).
183. Etesami, H., Jeong, B. R. & Glick, B. R. Contribution of arbuscular mycorrhizal fungi, phosphate-solubilizing bacteria, and silicon to P uptake by plant. *Front. Plant Sci.* **12**, 699618 (2021).
184. Qin, Y. et al. Arbuscular mycorrhizal fungus differentially regulates P mobilizing bacterial community and abundance in rhizosphere and hyphosphere. *Appl. Soil. Ecol.* **170**, 104294 (2022).
185. Kuypers, M. M. M., Marchant, H. K. & Kartal, B. The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* **16**, 263–276 (2018).
186. Hijri, M. Analysis of a large dataset of mycorrhiza inoculation field trials on potato shows highly significant increases in yield. *Mycorrhiza* **26**, 209–214 (2016).
187. Zhang, S., Lehmann, A., Zheng, W., You, Z. & Rillig, M. C. Arbuscular mycorrhizal fungi increase grain yields: a meta-analysis. *New Phytol.* **222**, 543–555 (2019).
188. Cartabia, A. et al. The arbuscular mycorrhizal fungus *Rhizophagus irregularis* MUCL 41833 modulates metabolites production of *Anchusa officinalis* L. under semi-hydroponic cultivation. *Front. Plant Sci.* **12**, 724352 (2021).
189. Zhao, Y., Cartabia, A., Lalaymia, I. & Declerck, S. Arbuscular mycorrhizal fungi and production of secondary metabolites in medicinal plants. *Mycorrhiza* **32**, 221–256 (2022).
190. Riaz, M. et al. Arbuscular mycorrhizal fungi-induced mitigation of heavy metal phytotoxicity in metal contaminated soils: a critical review. *J. Hazard. Mater.* **402**, 123919 (2021).
191. Rillig, M. C. et al. Towards an integrated mycorrhizal technology: harnessing mycorrhiza for sustainable intensification in agriculture. *Front. Plant Sci.* **7**, 1625 (2016).
192. Hontoria, C., García-González, I., Quemada, M., Roldán, A. & Alguacil, M. M. The cover crop determines the AMF community composition in soil and in roots of maize after a ten-year continuous crop rotation. *Sci. Total. Environ.* **660**, 913–922 (2019).
193. Huang, R. et al. Natural variation at *OsCERK1* regulates arbuscular mycorrhizal symbiosis in rice. *New Phytol.* **225**, 1762–1776 (2020).
This work shows that *OsCERK1* allelic variation correlates with efficiency of AMF colonization and plant growth responses to AMF, raising the question of whether counterselection for high AMF colonization occurred from the origin of plant breeding.
194. Yang, H. et al. Effects of arbuscular mycorrhizal fungi on plant growth depend on root system: a meta-analysis. *Plant Soil.* **389**, 361–374 (2015).
195. Guzman, A. et al. Crop diversity enriches arbuscular mycorrhizal fungal communities in an intensive agricultural landscape. *New Phytol.* **231**, 447–459 (2021).
196. Chu, Q. et al. Soil plant-available phosphorus levels and maize genotypes determine the phosphorus acquisition efficiency and contribution of mycorrhizal pathway. *Plant Soil* **449**, 357–371 (2020).
197. Martín-Robles, N. et al. Impacts of domestication on the arbuscular mycorrhizal symbiosis of 27 crop species. *New Phytol.* **218**, 322–334 (2018).
198. Kameoka, H. et al. Stimulation of asymbiotic sporulation in arbuscular mycorrhizal fungi by fatty acids. *Nat. Microbiol.* **4**, 1654–1660 (2019).
This breakthrough study demonstrates that fatty acids act as stimulants to induce infection-competent secondary spores in axenic culture.
199. Oddi, L. et al. Boosting species evenness, productivity and weed control in a mixed meadow by promoting arbuscular mycorrhizas. *Front. Plant Sci.* **15**, 1303750 (2024).
200. Salomon, M. J. et al. Global evaluation of commercial arbuscular mycorrhizal inoculants under greenhouse and field conditions. *Appl. Soil. Ecol.* **169**, 104225 (2022).

Acknowledgements
The authors are grateful to J. Mach for the critical reading of this manuscript, to N. Hofmann for the discussion on the 'continuum' term and to M. Novero for the assembly of Fig. 4. This work was financially supported by the National Natural Science Foundation of China (32130094, 32170116 and 42277112), Guangdong Basic and Applied Basic Research Foundation (2022A1515012013) and China Scholarship Council (202206350052).

Author contributions
S.D., E.L., P.B., X.X. and L.Z. contributed substantially to the discussion of content and wrote the manuscript. S.D., G.F. and L.Z. discussed the original logic and framework of this manuscript. S.D., P.B., X.X. and L.Z. contributed literature searching for this manuscript and reviewed and edited the manuscript before submission.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41579-024-01073-7.

Peer review information *Nature Reviews Microbiology* thanks Marcel van der Heijden, Mohamed Hijri, Pierre-Emmanuel Courty and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.