MiniReview

Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture

Jonas F. Johansson [*], Leslie R. Paul, Roger D. Finlay

Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, SE-750 07 Uppsala, Sweden

Abstract

In sustainable, low-input cropping systems the natural roles of microorganisms in maintaining soil fertility and biocontrol of plant pathogens may be more important than in conventional agriculture where their significance has been marginalised by high inputs of agrochemicals. Better understanding of the interactions between arbuscular mycorrhizal fungi and other microorganisms is necessary for the development of sustainable management of soil fertility and crop production. Many studies of the influence of mycorrhizal colonisation on associated bacterial communities have been conducted, however, the mechanisms of interaction are still poorly understood. Novel approaches including PCR-based methods, stable isotope profiling, and molecular markers have begun to shed light on the activity, identity and spatiotemporal location of bacteria in the mycorrhizosphere. This paper reviews current knowledge concerning the interactions between arbuscular mycorrhizal fungi and other microorganisms, particularly bacteria, and discusses the implications these interactions may have in sustainable agriculture.
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Keywords: Arbuscular mycorrhiza; Bacteria; Biocontrol; Mycorrhizosphere; Soil fertility; Sustainable agriculture

1. Introduction

Soil microorganisms have an important influence on soil fertility and plant health [1]. Symbiotic mycorrhizal fungi, such as arbuscular mycorrhizal (AM) fungi form a key component of the microbial populations influencing plant growth and uptake of nutrients. In addition to increasing the absorptive surface area of their host plant root systems, the hyphae of these symbiotic fungi provide an increased area for interactions with other microorganisms, and an important pathway for the translocation of energy-rich plant assimilates to the soil.

Traditionally, the influence of plant assimilates on microbial communities has been defined in relation to the rhizosphere, the narrow zone of soil surrounding living roots [2]. The rhizosphere is characterised by increased microbial activity stimulated by the leakage and exudation of organic substances from the root [3].

However, since plant roots in natural and semi-natural ecosystems are commonly mycorrhizal, the rhizosphere concept has been widened to include the fungal component of the symbiosis, resulting in the term "mycorrhizosphere" [4] (Fig. 1). The mycorrhizosphere is the zone influenced by both the root and the mycorrhizal fungus, and includes the more specific term "hyphosphere", which refers only to the zone surrounding individual fungal hyphae. Since mycorrhizas and fungal hyphae are more or less ubiquitous in natural soils, it could be argued that all soil could be included in the term "mycorrhizosphere".

The natural roles of mycorrhizosphere organisms may have been marginalised in intensive agriculture, since microbial communities in conventional farming systems have been modified due to tillage [5,6] and high inputs of inorganic fertilisers, herbicides and pesticides [1,7] (Fig. 2). Microbial diversity in these systems has been reduced [8] and the functional consequences of this loss of diversity are still uninvestigated. Increased environmental awareness has progressively led to a shift from

[*] Corresponding author. Tel.: +46-18-672720; fax: +46-18-673599.
E-mail address: jonas.johansson@mykopat.slu.se (J.F. Johansson).

Effects of mycorrhizosphere bacteria on AM fungi

10. Effects on receptivity of roots to AM/root-fungus recognition
    Meyer and Linderman 1986, Budi et al. 1999

11. Effects on fungal growth
    Ross 1980, Wilson et al. 1988, Puppi et al. 1994, Budi et al. 1999

12. Effects on germination of fungal propagules
    Mosse 1959, Daniels and Trappe 1980, Tommerup 1985, Mayo el al. 1986, Carpenter-Boggs 1995, Budi et al. 1999

13. Modification of soil chemistry

Effects of AM fungi on mycorrhizosphere bacteria

1. Supply of energy-rich C compounds via hyphae
   Andrade et al. 1997

2. pH changes

3. Competiton for nutrients
   Ravnskov et al. 1999, Wamberg et al. 2003

4. Other inhibitory (-) or stimulatory (+) compounds
   Ames et al. 1984, Secilia and Bagyaraj 1987, Puppi et al. 1994, Filion et al. 1999

5. Stimulated root growth

6. Changes in root exudation
   Ravnskov et al. 1999, Söderberg et al. 2002

7. Changes in soil structure
   Tisdal and Oades 1979, Forster and Nicolson 1981, Shreiner et al. 1997, Andrade et al. 1998

Effects of AM fungi on endophytes and soil pathogens

8. Endophytic bacteria in AM fungal structures
   Bianciotto et al. 1996, 2000

9. Effects on pathogenic fungi
   Newsham et al. 1995, Niemera et al. 1996, Citernesi et al. 1996, St-Arnaud et al. 1997, Kapoor and Mukerji 1998, Filion et al. 1999
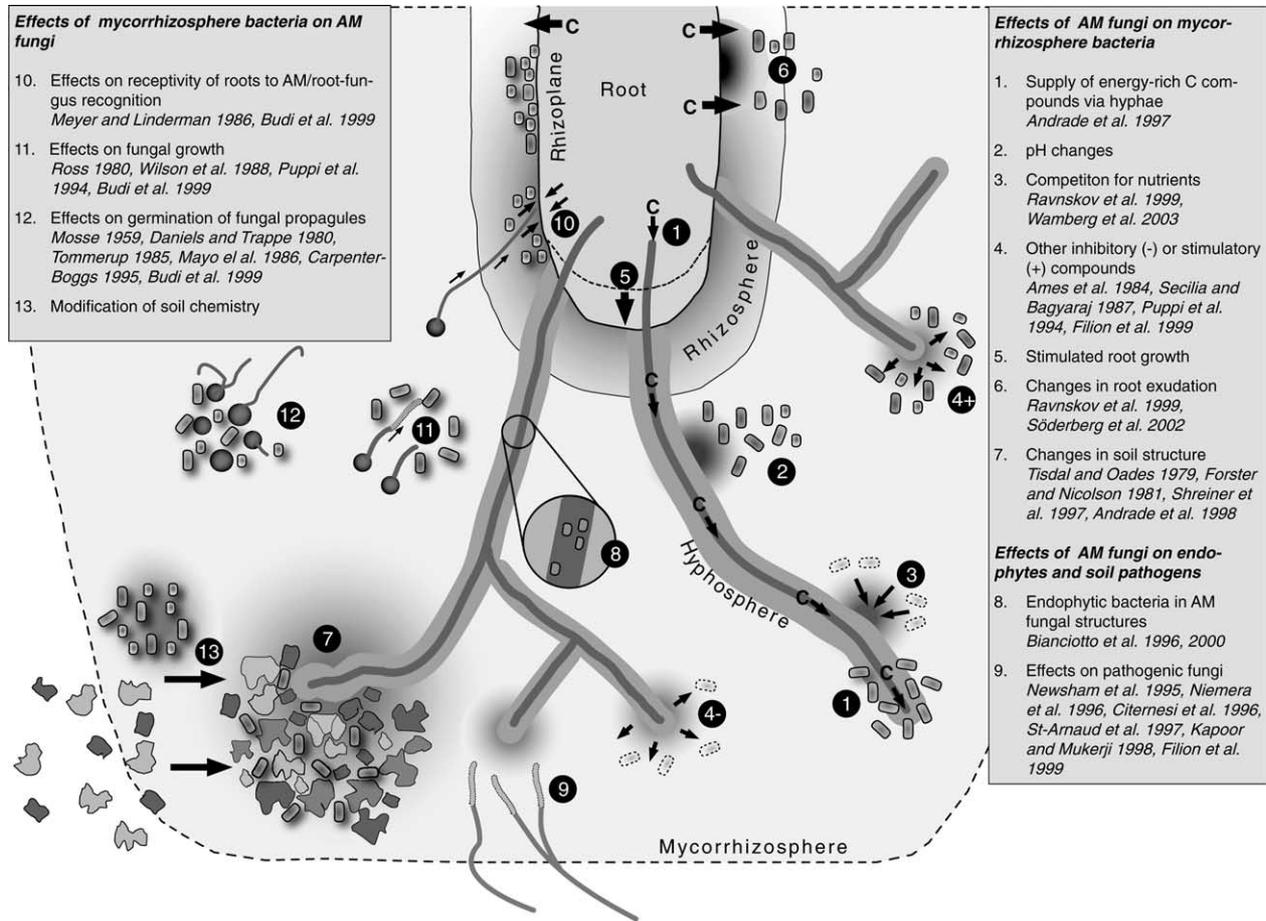
Fig. 1. Schematic view of possible interactions among different components of the mycorrhizosphere. The drawing is not to scale and underestimates the relative surface area of the external mycorrhizal mycelium.

conventional intensive management to low-input, sustainable crop production in Europe. In low-input cropping systems the natural activities of microorganisms may contribute to the biocontrol of pathogens and improved supply of nutrients, thus maintaining crop health and production. A better understanding of the interactions of soil microorganisms with each other and with plants is therefore crucial for the development of sustainable management of soil fertility and crop production.

The purpose of this review is to outline the current knowledge on microbial interactions in the mycorrhizosphere of AM plants and the potential influence of agricultural practices on the microbial communities. The review focuses on interactions between fungi and bacteria. In addition, we include a brief discussion on how this knowledge is currently used and how the understanding of microbial interactions could prove important to sustainable agriculture in the future. It does not provide a complete review of biological control research, but rather concentrates on aspects of the interactions in the mycorrhizosphere, which may have practical applications. Future perspectives of AM mycorrhizosphere research are also discussed.

2. The arbuscular mycorrhizal symbiosis

Arbuscular mycorrhizal symbiosis is the oldest (>460 million years BP) and most widespread type of mycorrhizal association. It is estimated that 250,000 species of plants worldwide, including many arable crops, are capable of forming the symbiosis [9]. The plant hosts include angiosperms, gymnosperms and pteridophytes, all having true roots [10]. Approximately 160 fungal taxa of the order Glomales (Glomeromycota) have been described on the basis of their spore morphology [11], although recent molecular analyses indicate that the actual number of AM taxa may be much higher [12,13].

During the formation of AM symbiosis the fungus penetrates the root cortical cell walls and forms haustoria-like structures (arbuscules or coils) that interface with the host cytoplasm [9]. These fungal structures (especially the highly branched arbuscules) provide an increased surface area for metabolic exchanges between the plant and the fungus. Some AM fungi also produce vesicles, which are structures, believed to function as storage organs [9]. It has been estimated that in natural ecosystems plants colonised with AM may invest 10–
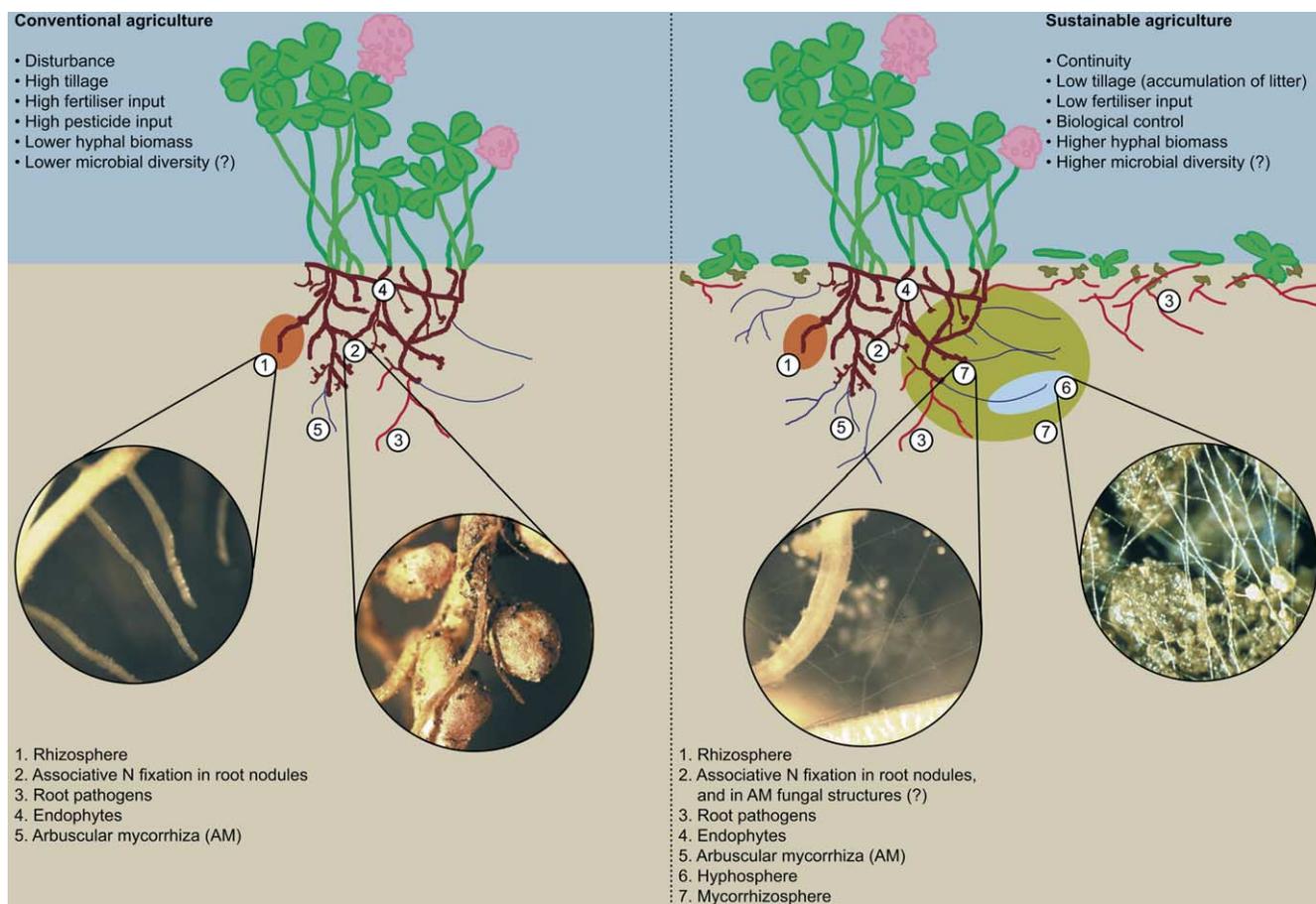
Fig. 2. Schematic view of the mycorrhizosphere concept in contrast to the rhizosphere concept: Features of conventionally managed agricultural soils versus sustainably managed agricultural soils are indicated with emphasis on mycorrhizosphere components and possible effects on them.

20% of the photosynthetically fixed carbon in their fungal partners [14]. Clearly, this represents a significant input of energy into the soil ecosystem and this carbon may be crucial to microorganisms associated with the mycorrhizosphere.

Arbuscular mycorrhizal fungi also interface directly with the soil by producing extraradical hyphae that may extend several centimetres out into the soil [15]. Extraradical hyphae can have a total surface area of several orders of magnitude greater than that of roots alone, which increases the potential for nutrient uptake, and possibly also water uptake, although results concerning the latter are still conflicting [15,16]. Hyphae of AM fungi have been shown to play an important role in soil stabilisation through formation of soil aggregates [17]. Additionally, the extraradical hyphae are generally believed to be important to the plants for acquisition of phosphorus (P) and other mineral nutrients [18]. The extraradical mycelium of AM fungi can also enhance mobilisation of organically bound nitrogen from plant litter [19].

Arbuscular mycorrhizal symbiosis can also alleviate negative effects of plant pathogens [20–23] and toxic levels of metals [24]. In addition, the extraradical hyphae may interact with other soil organisms either indirectly by changing host plant physiology, including root physiology and patterns of exudation into the mycorrhizosphere, or directly by physically and/or metabolically interacting with other organisms in the mycorrhizosphere.

3. Effects of AM fungi on mycorrhizosphere bacteria

Colonisation of plant roots by AM fungi can affect bacterial communities associated with the roots in both direct and indirect ways (Fig. 1). Direct interactions include provision of energy-rich carbon compounds derived from host assimilates, which are transported to the mycorrhizosphere via fungal hyphae, changes in pH of the mycorrhizosphere induced by the fungus, competition for nutrients, and fungal exudation of other inhibitory or stimulatory compounds. Indirect interactions can also take place in the form of mycorrhiza-mediated effects on host plant growth, root exudation, and soil structure.

Using a simple dilution plate technique, Ames et al. [25] demonstrated that a single bacterial isolate significantly increased in the mycorrhizosphere of *Glomus mosseae* inoculated plants compared with the rhizosphere of non-mycorrhizal plants, even though AM colonisation never exceeded 5.5%. Meyer and Linderman [26] also demonstrated selective differences in populations of naturally occurring taxonomic and functional groups of bacteria in the rhizosphere and the rhizoplane of mycorrhizal and non-mycorrhizal sweet corn (*Zea mays*) and subterranean clover (*Trifolium subterraneum*) plants. Secilia and Bagyaraj [27] showed that total bacterial populations in the rhizosphere of guinea grass (*Panicum maximum*) were greater in plants colonised by *Glomus fasciculatum*, *Gigaspora margarita* and *Sclerocystis dussi* than in non-mycorrhizal plants. These authors suggested that increased amino acid exudation by P-deficient mycorrhizal plants might have stimulated the growth of amino acid-requiring bacteria.

Organic compounds produced by extraradical hyphae and the hyphae themselves play a role in aggregation of soil particles [17], which could provide microsites for microbial colonisation and growth. Forster and Nicolson [28] analysed the microbial composition of such aggregates and identified a range of bacteria, actinomycetes and algae. Andrade et al. [29] used compartmented systems in which roots and hyphae were separated by fine mesh in order to investigate the qualitative and quantitative effects of AM on microbial communities in the mycorrhizosphere and the stability of soil aggregates associated with it. They found that total bacteria and P-solubilising bacteria isolated from the water-stable soil-aggregate (WSA) fraction tended to be more numerous than from the unstable fraction. Schreiner et al. [30] found increases in WSA in mycorrhizal soybean (*Glycine max*), which were dependent on the mycorrhizal fungal species involved. Differences in the bacterial communities were also found among the fungal species, suggesting that AM species may influence both WSA and bacterial composition.

Andrade et al. [31] examined bacteria associated with the mycorrhizosphere and the hyphosphere of the AM fungal species, *Glomus etunicatum*, *G. intraradices* and *G. mosseae*. The observed changes in the bacterial community in the hyphosphere were not due to the amount of AM mycelium per se, suggesting that qualitative effects (e.g., composition of exudates) of the fungal species on the hyphosphere are more important to the composition and proliferation of rhizobacteria than the quantitative development of AM mycelia in the soil.

While many studies have demonstrated qualitative and quantitative effects of AM fungi on bacterial communities, the underlying mechanisms are unclear. There are still few or no studies concerning the total amounts or spectrum of compounds released from AM mycelium. Further experiments employing spatial compartmentation of roots and mycelium are needed to distinguish between direct and indirect effects of mycorrhiza on bacterial populations. Although changed bacterial community structure has been shown in association with the mycorrhizosphere, there are no direct demonstrations of compounds produced by AM fungi, which stimulate or inhibit bacteria. There is some evidence from studies using PLFA (phospholipid fatty acid) analysis and BIOLOG™ that the effect on the bacterial populations is influenced by the mycorrhizal dependency of plants [32], however, the issue of culturability remains a general problem in studies on bacterial community structure.

4. Effects of AM fungi on fungal pathogens and N-transforming bacteria

AM fungi may also interact with other root-associated microorganisms, such as pathogenic fungi. The possible mechanisms of interaction are the same as those mentioned in the previous section. In a study by Filion et al. [33], the differential effects in vitro of a crude extract from the growth medium of the AM fungus *G. intraradices* on the sporulation of two pathogenic fungi and on the growth of two bacterial species were investigated. Conidial germination of the mycoparasitic fungus *Trichoderma harzianum* and the growth of *Pseudomonas chlororaphis* were stimulated, whereas conidial germination of the plant root pathogen *Fusarium oxysporum* was reduced, and the growth of *Clavibacter michiganensis* was unaffected. The measured effects were correlated with extract concentration and no significant influence of pH on growth or germination was detected. The authors concluded that the release of unspecified substances by the AM fungus into the growth medium was the main factor explaining the differential growth of the tested microorganisms. In other studies Citernesi et al. [34] screened bacteria isolated from 17 year old *G. mosseae* pot cultures. They found that many of the bacterial isolates within the different zones of the mycorrhizosphere were actively antagonistic against in vitro growth of the soil-borne pathogens, *Fusarium* and *Phytophthora*. Their results also suggest the possibility of integrated use of AM fungi and their associated bacteria in biological control of soil-borne pathogens.

Many authors have suggested that the ability of AM-colonised plants to better withstand an attack from root pathogens can be ascribed to an increased nutritional status in the host plant due to the presence of the AM fungus. However, there are reports which contradict this theory. In field experiments, Newsham et al. [22] transplanted *Glomus* sp.-inoculated and non-inoculated seedlings of the annual grass *Vulpia ciliata* into a natural population, and found that AM inoculation did not affect P concentrations in the plants. However, the my-

corrhiza protected the plants from the deleterious effects of *Fusarium oxysporum* infection on shoot and root growth. Apparently, the AM suppressed pathogen development in the roots. Following transplantation, comparison of root-infecting mycofloras of AM and non-AM plants revealed that AM plants had fewer naturally occurring infections of *F. oxysporum* and *Embellisia chlamydospora* [22]. It was proposed that the main benefit supplied by AM fungi to *V. ciliata* is the protection from pathogenic fungi, rather than improved P uptake. This theory was also proposed by Niemira et al. [20], who used a peat-based medium containing the AM fungus *G. intraradices* to test whether it could suppress the tuber dry rot (*Fusarium sambucinum*) in minitubers of potato (*Solanum tuberosum*). Minitubers grown in this medium had significantly less (20–90%) tuber dry rot. They were also able to demonstrate these effects in a high-input commercial greenhouse, despite the fact that AM colonisation was very low and no evidence of enhanced plant P nutrition could be found. In addition, St-Arnaud et al. [23] demonstrated that the presence of *Tagetes patula* plants colonised by the AM fungus *G. intraradices* can inhibit root pathogen development in soil and thereby reduce disease severity in co-cultured non-mycorrhizal carnation (*Dianthus caryophyllus*). In other experiments, Caron [35] observed a reduction in *Fusarium* populations in the soil surrounding mycorrhizal tomato (*Lycopersicon esculentum*) roots, and suggested that there was a potential role for AM fungi in biocontrol of soil-borne diseases.

The presence of AM fungi is known to enhance nodulation and N fixation by legumes. Mycorrhizal and nodule symbioses often act synergistically on infection rate, mineral nutrition and plant growth [36]. The increased P uptake conferred by the AM symbiosis is beneficial for the functioning of the nitrogenase enzyme of the bacterial symbiont, leading to increased N fixation and consequently promotion of root and mycorrhizal development [37]. Amora-Lazcano et al. [36] studied the response of other N-transforming microorganisms to two different *Glomus* species. The occurrence of autotrophic nitrifying bacteria in pot cultures of sweet corn colonised by the AM fungi *G. mosseae* and *G. fasciculatum* was significantly higher than in non-mycorrhizal cultures, whereas ammonifying and denitrifying bacterial populations significantly decreased in pot cultures of mycorrhizal plants. Evidently, the presence of AM fungi can modify populations of N-transforming microorganisms and these interactions may affect nutrient availability in soils [36].

Although qualitative effects of AM fungi on fungal pathogens have been repeatedly demonstrated, there is still a lack of information concerning direct effects on these pathogens. There are few or no studies on interactions of AM fungi with bacterial pathogens and, as in the case of fungal pathogens, it is often difficult to distinguish between direct effects on the pathogens and indirect effects brought about by the improved nutritional status of the mycorrhizal plants.

5. Effects of mycorrhizosphere bacteria on AM fungi

Mycorrhizosphere bacteria may affect AM fungi and their plant hosts through a variety of mechanisms (Fig. 1). Some of these have been more fully studied in ectomycorrhizal fungi [38], but possibilities include (1) effects on the receptivity of the root; (2) effects on the root-fungus recognition; (3) effects on the fungal growth; (4) modification of the chemistry of the rhizospheric soil; and (5) effects on the germination of the fungal propagules.

In an early study, Mosse [39] observed that glomalean spores germinated better on agar when bacteria were present. Later, Daniels and Trappe [40] showed that *Glomus versiforme* germinated in non-sterile soils, but not in autoclaved or irradiated soils. Germination was also enhanced following addition of kaolin or activated charcoal, indicating that the spores contained self-inhibitors, which were possibly inactivated by soil bacteria, or were immobilised by substances with a high ion exchange capacity. Similarly, Mayo et al. [41] showed that surface-sterilised spores of *G. versiforme* germinated less frequently than non-surface sterilised spores. Isolation of bacteria from these spores showed that several genera, including *Pseudomonas* and *Corynebacterium*, enhanced spore germination. Carpenter-Boggs et al. [42] tested the stimulatory effects of actinomycetes and *Streptomyces orientalis* on *Gigaspora margarita* spore germination and found that amounts of volatile compounds produced by the isolates correlated well with AM spore germination. Conversely, other studies using pasteurisation, fumigation or sterilisation of soils have demonstrated that the presence of some soil bacteria may also inhibit spore germination [43] or AM sporulation [44,45].

Nitrogen fixing bacteria clearly have the potential to influence AM fungi. The presence of genes for N fixation has been shown in endosymbiotic *Burkholderia* sp. [46], but expression of this activity at levels significantly influencing the growth of the mycorrhizal association has yet to be demonstrated. *Rhizobium* spp. may act synergistically with AM fungi on their plant hosts. Nodulation and N fixation are commonly increased in legumes following AM colonisation, probably because the mycorrhiza supplies the plant and the rhizobacteria with P, which is essential for the enzymes involved in the N fixation process. Nitrogen fixation further promotes mycorrhizal development [37]. Some mycorrhizosphere bacteria may be able to promote mycorrhizal establishment through improved spore germination [39], but so far there are no direct demonstrations of this in the

field. The colonisation enhancement may also be mutual between associated microorganisms and this has been reported following dual inoculation of *Pseudomonas* sp. and *Glomus* sp., which additionally increased the growth of the host plant in an additive manner [47].

A central problem in characterising effects of bacteria on AM fungi is the difficulty of identifying the active bacteria against a biological background of immense diversity (see Section 10). Clearly, mycorrhizosphere bacteria have a number of potential effects on AM fungi. A central remaining challenge is to determine whether effects demonstrated under laboratory conditions can be reproduced in the field.

6. Endosymbiotic bacteria of AM fungi

Many soil microorganisms are difficult to culture. Since non-culturable microorganisms may account for a large part of the soil microflora [48], the ability to identify and isolate them is important for understanding the function of these organisms in soil ecosystems. The cytoplasm of some AM fungi harbours structures called bacterium-like organisms (BLOs), however, the identification of these has been hampered since they cannot be grown on cell-free media [49]. Bianciotto et al. [50] used a combined morphological and molecular approach to demonstrate that the cytoplasm of the AM fungus *Gigaspora margarita* contains bacterial endosymbionts. Analysis of the small-subunit rRNA gene sequence of the BLOs in *G. margarita* spores led to the conclusion that these endosymbionts were closely related to the genus *Burkholderia*. In order to determine whether intracellular bacteria occur sporadically in individual AM fungal isolates or as a common feature in the family Gigasporaceae, Bianciotto et al. [51] investigated two geographically separated isolates of the species *G. margarita* and five other species in the genera *Gigaspora* and *Scutellospora*. The results showed that all investigated species except *G. rosea* contained endosymbiotic bacteria closely related to the genus *Burkholderia*. Further studies by Minerdi et al. [46] demonstrated that *Burkholderia* spp. in *G. margarita* contain genes involved in N fixation. The function and distribution of these endosymbionts remain to be explored, however, the findings suggest that these bacteria constitute a component of the fungal cytoplasm and that they must be taken into account when considering the extent of microbial diversity in ecosystems [50,51].

7. Interactions at the cellular level

Many of the mycorrhizosphere microbial interactions discussed above may involve cellular interactions that are not yet understood. Bianciotto et al. [52] demonstrated that rhizobia and pseudomonads, commonly considered to be plant growth promoting rhizobacteria (PGPR), may attach to spores and hyphae of the AM fungus *Gigaspora margarita* germinated under sterile conditions in vitro. Their results showed that the degree of attachment to fungal hyphae and inorganic surfaces was clearly different between bacterial strains. However, the bacteria that did show a higher degree of attachment did not show specificity for either fungal or inorganic surfaces. It was therefore suggested that the first stages of attachment to a surface are governed by general physiochemical parameters, such as electrostatic attraction. In contrast, cell surface structures or extracellular material of bacterial origin produced around the attached bacteria provide the mechanism for a more secure, permanent attachment to the fungal structures. This suggestion was later confirmed by Bianciotto et al. [53], who used bacterial mutants inhibited in extracellular polysaccharide production in adhesion assays in vitro to demonstrate that such mutants were strongly impaired in the attachment to mycorrhizal structures and hyphae. Using *gfp*-labelled microorganisms, Lagopodi et al. [54] observed that the biocontrol bacteria *Pseudomonas fluorescens* WCS365 and *P. chlororaphis* PCL1391 applied to tomato seedlings occupy the same sites (the groves along the junctions of the epidermal cells) on the tomato root, as does the pathogenic fungus *Fusarium oxysporum*. The biocontrol bacteria occupied these sites more rapidly than the pathogenic fungus and this could be a crucial aspect of their biocontrol ability. The use of bacterial strains with increased ability to attach to roots and hyphae, and to form protective biofilms, could have useful applications in biocontrol [53,55].

Filippi et al. [56] used transmission electron microscopy to show that bacteria were associated with the surfaces of peridium-covered spore clusters (referred to as "sporocarps"), surfaces of spores, surfaces of hyphae, and in micro-niches formed by peridial hyphae of *Glomus mosseae*. Bacterial cells were found embedded in spore walls, possibly by tunnels produced in the walls by the bacteria. The study determined that large numbers of bacteria, actinomycetes, and non-mycorrhizal fungi, some of which displayed chitinolytic abilities, occurred on the sporocarp surfaces and in the sporocarp homogenate.

In the past 10 years several attempts have been made to investigate possible physical interactions between AM fungi and bacteria in the hyphosphere [52,53,57]. Ravnskov et al. [57] studied the influence of *Glomus intraradices* on *Pseudomonas fluorescens* DF57 bacteria in hyphosphere and rhizosphere soil. They used *P. fluorescens* DF57 strain (DF57-P2) containing a chromosomal insertion of Tn5::luxAB gene in a phosphate starvation-inducible locus, in combination with a control strain (DF57-40E7) with a constitutively expressed

*lux*AB gene, to measure P starvation and metabolic activity. It was evident that the presence of AM neither induced P starvation response, nor affected the metabolic activity of the bacterium. Additionally, they found that constitutively *gfp*-labelled *P. fluorescens* did not attach to *G. intraradices* hyphae and could not use the hyphae as a carbon substrate. It was proposed that *G. intraradices* can negatively influence the growth and survival of *P. fluorescens* DF57, not only in the presence of roots, where the fungus can change the quality and quantity of root exudates, but also in the hyphosphere, where the microbes interact directly. Additional support for the antagonistic effects of *G. intraradices* on certain bacteria was reported by Wamberg et al. [58].

Intercellular interactions could be important because they allow rapid exchange of energy and nutrients between plant roots, mycorrhizal fungi and associated bacteria. The mycorrhizosphere may provide a beneficial environment for certain associated bacteria, which may in turn have positive effects on nutrient supply and control of pathogens.

8. Relevance of mycorrhizosphere interactions to sustainable agriculture

8.1. Sustainable nutrient supply

It is frequently suggested that AM may improve P nutrition, enhance N uptake, or improve disease resistance in their host plants. Other microbes, e.g., N fixing bacteria or P solubilising bacteria, may synergistically interact with AM fungi and thereby benefit plant development and growth [37]. The mycorrhizal symbiosis becomes even more important in sustainable agricultural systems where nutrient inputs are low. Under these circumstances AM mycelium (possibly in conjunction with bacteria or other fungi) could play an important role in nutrient mobilisation from crop residues. Hodge et al. [19] demonstrated that the presence of the AM symbiont *Glomus hoi* enhanced decomposition of plant litter in soil and resulted in increased N capture from the litter (^{15}N–^{13}C labelled *Lolium perenne* leaves). Hyphal growth of the fungal symbiont was also increased in the presence of the organic material. Bacteria associated with the AM can also assist in mobilising nutrients from soil. Abundant examples of this are available from bacterial-AM-legume tripartite symbiotic relationships, where diazotrophic bacteria provide fixed N not only for the plant, but also for the fungus. As previously mentioned, nodulation of legumes by N-fixing bacteria and establishment of AM often occur simultaneously and synergistically. The presence of genes for N fixation in endosymbiotic *Burkholderia* bacteria in AM hyphae has been demonstrated by Minerdi et al. [46] and suggests that there

may be a potential for improved N supply to mycorrhizal plants through fixation of atmospheric N.

Studies of tripartite symbioses are still in their infancy: more research is also needed on the possible interactions of mycorrhizal fungi with decomposition processes. As inputs of fertilisers are reduced it is increasingly necessary to exploit naturally occurring nutrient resources. These resources are distributed heterogeneously on both temporal and spatial scales, but the possible roles of mycorrhizosphere organisms in recycling these nutrients to plants are still poorly understood. There is now an increasing awareness of functional differences between different AM fungi and as our awareness of their functional capacities increases we may be able to select suitable species to maximise the ability to recycle nutrients.

8.2. Biocontrol

Microbial inoculants can be used as an alternative means for controlling pests and disease in agricultural cropping systems, permitting the reduced use of pesticides that could otherwise pose threats to human health and non-targeted organisms. The biocontrol organisms may affect AM fungi, or be affected themselves by AM fungi, in similar ways to the interactions already described above. Biocontrol agents against pathogenic fungi may in particular have negative effects on "non-target" AM fungi. The mechanisms of antagonistic interactions resulting in biocontrol may involve competition for colonisation sites or nutrients and production of fungistatic compounds. Although the literature concerning biocontrol is extensive, few studies have explicitly considered interactions involving AM fungi. Some positive effects of bacteria on AM fungal colonisation of roots may be due to antagonistic effects on competing pathogens [21], but also direct synergistic effects on mycorrhizal colonisation itself [59].

Various plant-root colonising or seed-borne *Pseudomonas* spp. have been shown to be potent microbiological control agents in plant-pathogen systems in vitro [60,61], in greenhouse [62], and in the field [62,63]. In one study, a bacterial isolate *P. chlororaphis* PCL1391 appeared to be an efficient coloniser of tomato roots and efficiently antagonised the root pathogen *Fusarium oxysporum* [64]. The bacterial strain produced a broad spectrum of antifungal substances, including phenazine-1-carboxamide (PCN), hydrogen cyanide, chitinases and proteases [64]. By knocking out the phenazine-biosynthetic operon it was shown that the mutants exhibited significantly lower biocontrol activity, indicating that this substance was an important antifungal factor for suppressing disease in tomato roots. For the same fungal and bacterial strains, tagged with green and red fluorescent protein, respectively, it was shown that the presence of the biocontrol bacteria resulted in 70–80%

reduction of the density of the hyphal network within tomato roots [65]. The effects on AM fungal hyphae were, however, not studied. In addition to producing antifungal compounds, the ability of bacteria to rapidly colonise root surfaces and thereby closely interact with pathogens may further facilitate pathogenic suppression [54].

Research on AM fungi as protective agents against pathogens has been going on for about 30 years. Many studies have been published, but the underlying mechanisms are poorly understood. Some basic mechanisms have been suggested: improvement of plant nutrition and competition for photosynthates [21], however AM-induced suppression of root pathogens and stimulation of saprotrophs and plant growth promoting microorganisms may also be important [66]. Some authors have discussed other mechanisms, which often tend to be inconsistent among studies: these include anatomical or morphological changes in the root system induced by the AM fungus; and local elicitation of plant defence mechanisms by AM fungi [67]. Due to difficulties in producing large quantities of pure culture AM inoculum, relatively few studies have explored the practical use of AM fungi as inoculants to increase plant resistance to root-rotting pathogens. Combined inoculation of AM fungi with growth stimulating bacteria may favour the inoculum production [68]. Several studies have demonstrated that some AM fungi exhibit biocontrol properties [20,22,35] against root pathogens. Whether AM fungi could be used as biocontrol agents practically, or possibly function as vectors for associated bacteria with biocontrol properties, remains to be explored.

9. Influence of agricultural management on soil microbial communities

Different management strategies introduce different types of disturbances, which may influence microbial communities in various ways. Tillage causes a physical disruption of fungal mycelia and may change physico-chemical properties of the soil. Crop rotation may cause a temporal disturbance in the presence of plant roots of a particular species. The aim of this management strategy is to disturb populations of pathogens, but it can also affect the availability of compatible host species necessary to ensure continued growth of particular mycorrhizal species and their associated mycorrhizosphere microflora. Fertilisation and application of pesticides represent chemical disturbances, and their effects depend upon application rates and involved species and soils.

Reduced tillage has been adopted more often in order to conserve soil water and reduce erosion and soil compaction caused by conventional intensive management. It is generally accepted that reducing tillage of

agricultural soils can increase early-season P uptake in crops, especially in low-input farming systems [69]. Mixing of soil may negatively affect AM colonisation of plant roots, due to disruption of the extraradical mycelium, however, non-mycorrhizal fungi may also be affected [6]. Excessive soil compaction can lead to reduced soil microbial biomass and enzyme activity with adverse implications for long-term soil health [70]. Since AM fungi may ameliorate the nutrient status of their hosts and provide protection against pathogens, it is possible that a low degree of tillage could be beneficial to AM colonisation and crop health. Conservation tillage (i.e., no tillage or less frequent tillage) tends to concentrate plant debris and consequently microbial biomass in the topsoil, and thus promotes survival of pathogens [5]. The debris itself can provide an energy source available to the pathogen prior to and during host infection. However, since disease-causing microbes constitute only a proportion of the soil microbial population, a high soil microbial activity can lead to competition effects that may ameliorate pathogen activity and survival [5].

Fontenla et al. [71] reported that AM colonisation was not inhibited when non-host plants were grown at the same time and in the same pots as host plants. However, when non-host plants were grown in the pots before the host plants, the inoculum potential and in some cases the AM colonisation of the host plants were decreased. The authors concluded that non-host species may produce substances that inhibit the establishment of AM fungi in host roots. These compounds appear to affect the AM fungi before they become established in the root and are apparently non-systemic in nature. Another possibility, however, is that the presence of the non-host plants may have modified the bacterial community in the pots, which in turn adversely influenced the growth and colonisation of the AM fungus.

Using soils from conventional, integrated and organically managed farms, Knudsen et al. [72] investigated soil suppressiveness against the root pathogen *Fusarium culmorum*. They found that soils from integrated and organically managed systems harboured higher microbial biomass, including the pathogen population, and concluded that specific organic amendments, such as mulching with straw and the practice of using legumes as a break-crop in cereal cultivation, may contribute to a build-up of soil-borne pathogens.

Only a few studies have investigated the long-term effects of management history on microbial communities. Mäder et al. [73] compared AM colonisation in plots with 7-year identical crop rotation and tillage schemes that differed only in the amount and type of applied fertiliser. Root length colonised by AM fungi was 30–60% higher in plants grown in soils from low-input farming systems than those grown in conventionally fertilised soils. Additionally, soil biological

activity and microbial biomass were higher in low-input soils than in conventionally managed soils. Soil aggregate stability was 10–60% higher in the low-input plots and was positively correlated with microbial biomass [8]. Thus, the nutrient input regime alone could influence structure and activity of microbial communities.

In a Swedish study, the variation in AM colonisation rates of three plant species found in semi-natural grasslands with different soil management histories was compared [74]. AM colonisation was significantly higher in semi-natural pastures with a long continuous management regime, compared to sites with a short or interrupted management regime (new cultural pastures). Furthermore, there was a significant positive correlation between plant species diversity and colonisation by mycorrhiza, possibly due to the fact that a long continuous management is associated with an increasing likelihood of successful dispersal of both plant and fungal species.

There are also strong indications that AM fungi have the potential to determine plant community structure [75] and vice versa [76]. AM fungi may affect the nature of weed communities in agro-ecosystems in a variety of ways, including changing the relative abundance of mycotrophic and non-mycotrophic weed species. There is thus a potential role for AM fungi in weed management [77].

In conclusion, conservation tillage or low levels of soil disturbance promote AM fungal development. Under these conditions plant pathogen inoculum may build up, but the improved AM fungal development may in turn control the development of pathogens and weeds. In sustainable agricultural systems the resident soil microflora becomes ever more important for ecosystem processes such as nutrient cycling and pest control. The influence of management practices on soil microbial functional groups is an important issue that needs further investigation if the full potential of these organisms is to be appreciated.

10. Outlook

Since the concepts of ''rhizosphere'' and ''mycorrhizosphere'' were coined it has been recognised that microbial populations may vary in different fractions of soil and in the various zones of the rhizosphere and the mycorrhizosphere. Many earlier studies relied on dilution plate counts to enumerate and describe microbial populations. Such methods, however, only detect culturable organisms. A large proportion of mycorrhizosphere bacteria remains unculturable, and it is therefore difficult to assess the microbial diversity in the mycorrhizosphere and the relative contribution of unculturable microorganisms to the interactions in the mycorrhizosphere. AM fungi themselves cannot be grown in pure culture, but root organ cultures [78] are routinely used to culture AM fungi in vitro and can be used for investigating the interactions of AM fungi with their biotic and abiotic environment [32,33].

The analysis of rRNA genes has lately become an important tool for studying the diversity of soil bacterial [79,80] and mycorrhizal [13] communities in different ecosystems. The use of fatty acid patterns of phospholipids and lipopolysaccharides (reviewed by Zelles [81]) and the utility of ergosterols as bioindicators of fungi in soil [82] have also been useful tools in the characterisation of microbial communities. Genetic markers, e.g., *gfp* or genes coding for various forms of luciferase [83,84], or viability stains have enabled direct counts of microorganisms using luminometry, flow cytometry or microscopy [52,85].

Recently, Borneman [86] described a method to identify active, but non-culturable cells in environmental samples based on their ability to incorporate the thymidine nucleotide analogue bromodeoxyuridine (BrdU) during DNA synthesis. After incubation with BrdU, the total DNA was extracted and the DNA containing BrdU was isolated by immunocapture, revealing notable differences in the bacterial communities among the different P supplementation treatments and total DNA banding patterns.

An increasing number of studies make use of PCR-based methods like denaturing gradient gel electrophoresis (DGGE) [79] or terminal-restriction fragment length polymorphism (T-RFLP) [80] for characterising complex soil bacterial communities. The advantage of DGGE is that it recognises very small differences in the nucleotide sequence, allowing a description of the community structure expressed as band patterns on a gel. This method also allows extraction and sequencing of the individual bands in the gel gradient. The advantage of T-RFLP is that it reveals the community structure without requiring culture or cloning. In common with DGGE, it gives a description of the species composition and an estimate of the relative abundance of taxa in the sample, based on the abundance of different restriction fragments detected by laser-induced fluorescence on an automated gene sequencer.

Whilst the above methods enable the in situ study of microbial communities with improved resolution, additional information is still often required about the functional capacities of identified taxa. Stable isotope profiling (SIP) now provides a promising method for describing the fraction of the community that is functionally active in metabolising a particular substrate containing one or several stable isotopes [87].

There is an inherent difficulty in determining whether mycorrhizosphere bacteria are specifically associated with roots or mycorrhizal fungi, or they simply form opportunistic associations with a range of other organisms. Fluorescent antibodies allow specific detection of

cells when used in conjunction with flow cytometry or fluorescence microscopy [88]. However, these methods are often destructive since samples need to be fixed, and therefore continuous in situ monitoring of cells cannot be performed. New molecular techniques such as tagging of microbes with marker genes in combination with flow cytometry or microscopy have enabled non-destructive, direct visual study of microorganisms in situ [57,83]. It has been recently shown that a *gfp*-tagged *Bacillus cereus* isolate from fallow field soil, incorporating BrdU, appears to attach to AM hyphal fragments [89]. This work raises the interesting question of the extent to which different bacteria obtain their carbon saprotrophically from dead hyphae or in association with intact mycorrhizal hyphal systems supplying assimilates derived from their plant hosts. Visualisation of microbial cells using fluorescent viability stains and confocal microscopy [50,85], visualisation of fungal structures in naturally autofluorescent AM fungal species [90], as well as scanning electron microscopy [91] can be used for studying spatial interactions between bacteria and fungi. However, additional methodologies are necessary to elucidate physiological interactions.

Although the composition of microbial communities in the various parts of the mycorrhizosphere in different ecosystems has been described and discussed in many papers, the underlying mechanisms behind the interactions are still poorly known. To a great extent, this has been due to difficulties in culturing and monitoring AM fungi and associated microorganisms. In particular, the spatial complexity and possible temporal variation in the mycorrhizosphere represent formidable obstacles. However, methodological advances have greatly improved the monitoring of the fate and behaviour of microorganisms in both natural and artificial systems. An intriguing field of research that needs to be further explored is the role of cell-to-cell interactions of microorganisms. The ability of organisms to attach to and form biofilms on hyphae, roots and other surfaces could contribute to their successful application in biocontrol. Fungal hyphae may play important roles for the distribution of natural bacterial populations and could therefore act as vectors for bacteria in microbial inocula. Studying the spatiotemporal stability of such bacterial-fungal associations would provide more information on this potential and improve our understanding of microbial interactions, which may be important for the development of sustainable management of soil fertility and crop production.

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