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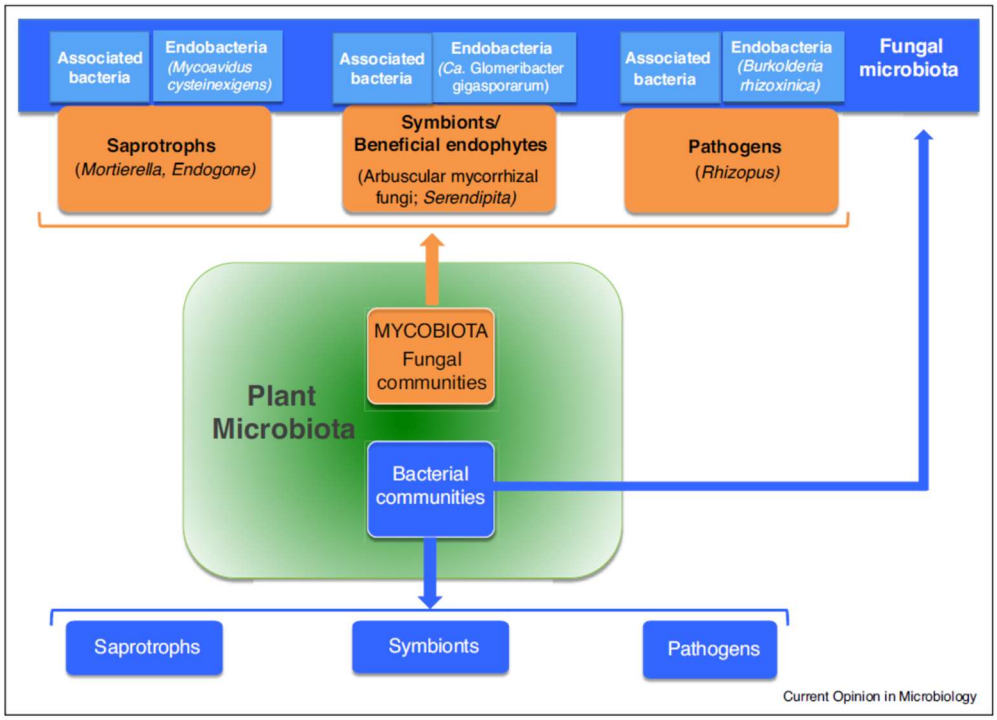
Eukaryotes host numerous intracellular and associated microbes in their microbiota. Fungi, the so-called Mycobiota, are important members of both human and plant microbiota. Moreover, members of the plant mycobiota host their own microbiota on their surfaces and inside their hyphae. The microbiota of the mycobiota includes mycorrhizal helper bacteria (for mycorrhizal fungi) and fungal endobacteria, which are critical for the fungal host and, as such, likely affect the plant. This review discusses the contribution that these oftenoverlooked members make to the composition and performance of the plant microbiota.

Introduction

The Fungal kingdom encompasses a plethora of eukaryotic species that proliferate in diverse environments; fungi also have important roles as components of the microbiota, where they act as symbionts, endophytes, parasites, or saprotrophs. Characterizing the microbiota of diverse species across kingdoms has revealed an unexpected double nature of the fungi in the microbiome: they colonize higher eukaryotes from humans to plants [1,2]. In the mean time, as with all other eukaryotes, fungi host their own microbiota, consisting of microbial communities that adhere to the hyphal surface, develop among the pseudotissues produced by hyphal aggregation, or colonize the fungal cytoplasm. In this review, we illustrate the double role played by fungi in the microbiota (Figure 1).



The flow chart offers a map to the Mycobiota as a component of the more complex Plant Microbiota. The fungal communities consist of saprotrophic, symbiotic and pathogenic fungi. On their own, these fungi may associate with soil bacteria or host endobacteria, which represent the more intimate form of interaction. These bacterial communities in their whole add a further level of complexity to the microbial component of the Plant Microbiota.

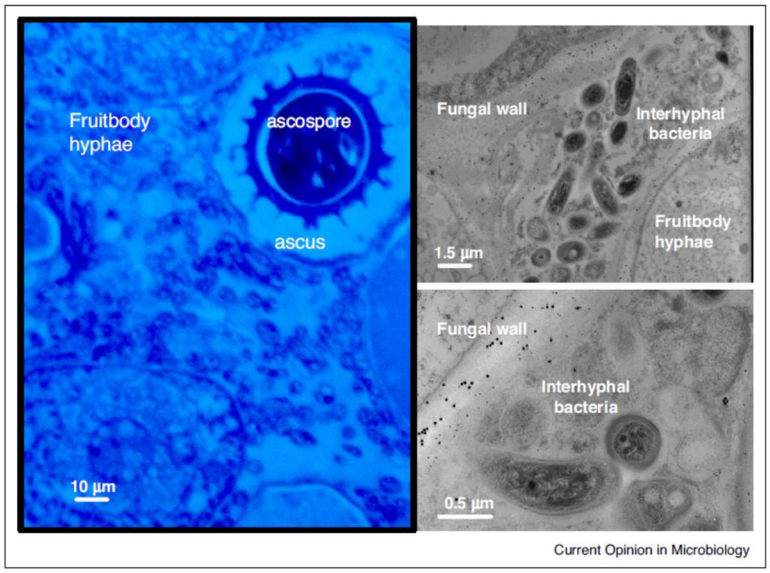
Mycobiota: fungi of the plant microbiota

The knowledge that fungi live strictly associated with plants in diverse niches, particularly in the rhizosphere, dates back more than 100 years [3]. However, only a minor part of the mycobiome is cultivable, in line with what is known about fungal diversity, where only a small portion of the estimated 3.8 million species [4] are in collections (http://www.wfcc.info/ccinfo/home/). Most of our knowledge of plant-associated fungi therefore comes from molecular analysis, where the internal transcribed spacer (ITS) of the nuclear rRNA operon is used as the official taxonomic barcode for fungi [5], providing species-level taxonomic delineation for most groups. Emerging 'omics' techniques, as well as the concept of the microbiota as an additional plant genome (alongside the nuclear and organellar genomes), offer new views of fungal diversity. The numbers of operational taxonomic units (OTUs) units increase with sequencing and sampling depth [6], suggesting that many members of the mycobiota remain to be discovered. Fungi proliferate in different environments (soil, air, water) and with different nutritional strategies (saprotrophic, biotrophic, parasitic), but the highest numbers of fungi are found to be plantassociated and in the soil. These findings suggested a remarkable fungal/plant species ratio of 17/1. Moreover, these approaches revealed a variety of fungal communities associated with myriad plants in diverse environments [7]. Pioneering reports on the plant microbiota focused on identifying *Arabidopsis thaliana* bacterial assemblages [8], but recent papers consider eukaryotic and prokaryotic components of the microbiota. For example, Bergelson *et al.* [9] grew a worldwide panel of *A. thaliana* accessions and found that fungi influence root microbiota structure. Ascomycota and Basidiomycota are more common in leaves than in roots, whereas Mortierellomycota are moderately enriched in the root microbiota. Irrespective of their qualitative differences, the leaf and root microbiotas had similar fungal richness. Moving from identity to functions, Almario *et al.* [10] identified 15 fungal taxa consistently present in the root of *Arabis alpina*, including a Helotiales taxon that colonizes the root endosphere and transfers phosphate to the plant. Similar functions have been described for the endophyte *Colletotrichum tofieldiae* [11] and an endophytic strain of *Fusarium solani* was found to protect against root and foliar pathogens [12]. Emerging work is therefore discovering novel, beneficial members of the mycobiota in addition to long-standing studies on mycorrhizal fungi. Living in association with plants and exploring the soil with their network of extraradical hyphae make mycorrhizal fungi a perfect example of the plant microbiota. Their diversity in the most different environments has been deeply investigated [13]. Notwithstanding some pitfalls and potential biases when applied to fungal communities [7], high-throughput sequencing has shown that fungi are unexpectedly diverse, important members of the plant microbiota. The challenge for the future will be to unravel the complex interactions among fungi and neighboring bacteria, and their effects on host physiology and metabolism [14].

From nutrient transfer to truffles: fungal-associated bacterial communities

Mycobiota-associated bacteria have diverse effects on their interacting fungi and plants, from nutrient transfer to production of aromatic metabolites. Mycorrhiza helper bacteria (MHB) were the first to be acknowledged for their positive effects; identified by Garbaye [15], they interact with ecto- and arbuscular mycorrhizal fungi (AMF), and belong to very diverse taxa including Proteobacteria such as *Pseudomonas* and Oxalobacteracea, Actinomycetes such as *Streptomyces*, and Firmicutes such as *Bacillus* [16,17]. MHB may enhance mycorrhizal functions, provide nutrients to the fungus and plant, and promote defenses. For example, the fructose exuded by the AMF *Rhizophagus irregularis* stimulates phosphatase expression and secretion in the MHB *Rahnella aquatilis*, thus promoting the mineralization of organic phosphorus (i.e. phytate) into inorganic phos- phorus [18]. Even if established in fully artificial conditions, this system reveals an interesting cooperation between AMF

and bacteria. High-throughput sequencing gave a wider description of MHB communities: Iffis *et al.* [19] identified the domi-nant AMF-associated bacterial OTUs in the roots of plants growing in hydrocarbon-polluted soils. Vik *et al.* [20] explored the bacterial community composition of the ectomycorrhizal roots of *Bistorta vivipara* and concluded that Actinobacteria were significantly more abundant in ectomycorrhizas than in soil. A detailed profile of bacterial communities associated with *Pinus sylvestris* roots colonized by different fungi demonstrated that each ectomycorrhizal root harboured distinct bacterial communities [21]. Other root-associated fungi have their own microbiota. Glancing at a truffle under an electron microscope in the pre-microbiota era was a shocking experience that revealed diverse bacteria proliferating around the aggre- gating hyphae forming the fruiting body (Figure 2).



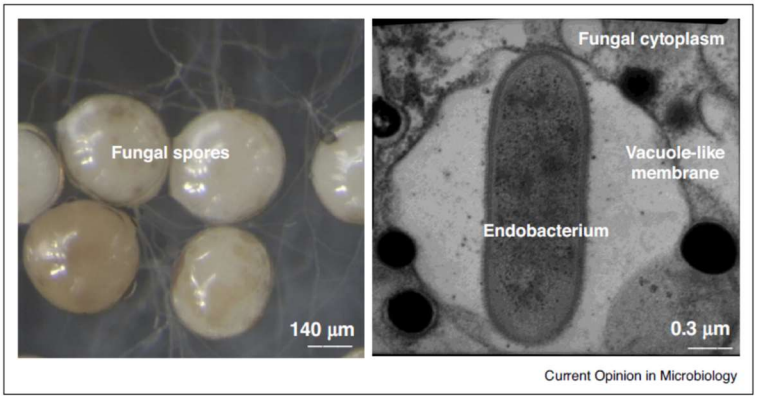
An example of fungal-associated bacteria: on the left, a section from a fruitbody of a truffle, an ectomycorrhizal fungus, is seen under light microscope. The fruitbody consists of aggregating sterile hyphae which surround ascospore-containing asci. When seen at transmission electron microscope (on the right), the inter-hyphal space appears to be filled up by bacterial colonies, very diverse for shape and size. At higher magnification, the bacterial ultrastructure becomes apparent. The fungal wall is labeled by wheat germ agglutinin linked to colloidal gold particles, which detect the chitinous component of the truffle hyphal wall. Pictures by courtesy of Raffaella Balestrini.

The existence of this complex system of bacteria and fungi opened the question of whether we are smelling and tasting bacteria or the precious truffles. Indeed, the *Tuber borchii* bacterial communities are dominated by a-proteo- bacteria and b-proteobacteria [22], which produce sul-phur-containing volatiles such as thiophene derivatives, characteristic of the captivating aroma of truffles. Many other bacterial communities associate with Basidiomycota fruiting bodies, from *Chantarellus* to *Tricholoma* [23,24]; soil is a major source of associated taxa, but the fungal host has a strong effect. Bacteria inhabiting fungal fruiting bodies may be selected based on their metabolic func-tions and habitat requirements [23]; for example, growth-promoting bacteria such as *Dietzia*, *Ewingella*, *Pseudomonas*, *Paenibacillus*, and *Rodococcus* could be positively selected for their beneficial effects on fungal growth. Bacterial–fungal interactions (BFIs) occur in many niches and affect biogeochemical cycles, plant and animal health, as well as drug, food, and toxin production (reviewed in Deveau *et al.* [25]). In the fungal microbiota, ‘fungiphiles’ explore soil niches using saprotrophic fungi as substrates [26] while others use mycorrhizal fungi as a highway to reach plant organs [27]. The phyllosphere microbial community is also strongly influenced by the interaction between microorganisms: particular taxa, including fungi, act as ‘hub microbes’ due to their rele-vance in

shaping the plant microbiota [28]. In another special environment, cheese rinds, *Serratia* isolates disperse on fungal networks by swimming in the liquid layers formed on *Mucor* hyphae [29]. Indeed, by mecha-nisms including flagella-mediated motility, fungal-associated bacterial dispersal can shift the cheese rind microbiota composition by promoting the growth of motile over non-motile community members.

Life on the inside: endobacteria of fungi

In addition to their surface bacteria, numerous fungi have cytoplasmic endobacteria. They represent the most extreme and specialized type of BFI since, in many cases, these bacteria have lost their capacity to live indepen-dently, have experienced a strong genome reduction, and exploit the fungal cytoplasm as a niche to complete their life cycles [25,30 _,31,32]. Bacteria with this intracellular habit are transmitted by diverse strategies: *Listeria* patho-gens in human cells and Phytoplasma in plant cells often colonize their host cells by horizontal transmission [33,34]. By contrast, the beneficial bacteria that live in insect tissues and complement the host diet with essential nutrients [35] are often transmitted vertically and have been maintained by co-evolution events. Fungi offer a wide range of examples of endobacteria. Endobacteria have been detected in Ascomycota and Basidiomycota [25], even if their presence seems to be transient, and often only supported by detection of their 16S ribosomal DNA. For these reasons, and because they are cultivable, they are described as facultative endobac- teria. A good example is given by the *Rhizobium radiobacter* strain F4 detected inside *Serendipita indica*, but able to induce plant growth like the conventional plant growth-promoting rhizobacteria [36]. In contrast to the Ascomycota and Basidiomycota, the basal group of Mucoromycota [37] contains endobacteria that have been consistently detected and are vertically transmitted. The endobacteria include rod-shaped Beta- proteobacteria (*Burkholderia*-related endobacteria, BRE) and coccoid-shaped Mollicutes (Mycoplasma-related endobacteria, MRE). These microbes have been detected in *Gigaspora* (Figure 3), *Diversispora*, *Rhizophagus*, *Geosiphon pyriforme* (Glomeromycotina), *Rhizopus*, *Endogone* (Mucoromycotina), and *Mortierella* (Mortierellomycotina).



An example of fungal endobacteria: on the left, a group of *Gigaspora margarita* spores + during the germination process, as seen under the steromicroscope ×100. On the right, the endobacterium *Candidatus* Glomeribacter gigasporarum is detected inside a fungal-like vacuole.

Some BRE and MRE genomes have been sequenced (Table 1) [38–44] revealing common features: reduced genomes (BRE: 3.7–1.8 Mbp; MRE: 0.6–1.3 Mbp), loss of biosynthetic capabilities related to primary metabolism, and specialization in fungal metabolite uptake.

Table 1

List of fungal endobacteria, whose genomes have been so far sequenced, together with their fungal host. When compared to the cultivable *Rhizobium (Agrobacterium) radiobacter*, hosted by a Basidiomycete, the genomes of endobacteria hosted by Mucoromycota are reduced, as well as their free-living capacities

Endobacterium	Bacterial taxonomy	Endobacterium genome size (Mb)	Cultivability	Fungal host	Fungal nutritional strategy	Fungal taxonomy	Ref.
Rhizobium (*Agrobacterium*) *radiobacter*	Gram - *Rhizobiaceae*	5.6	Cultivable	*Piriformospora indica*	Endophyte	Basidiomycota	[36]
Burkholderia rhizoxinica	Gram - *Burkholderiaceae*	3.75	Limited	*Rhizopus microsporus*	Opportunistic plant/human pathogen	Mucoromycotina	[44]
Mycoavidus cysteinexigens B1-EB	Gram - *Burkholderiaceae*	2.79	Limited	*Mortierella elongata*	Saprotroph	Mortierellomycotina	[50]
M. cysteinexigens AG77	Gram - *Burkholderiaceae*	2.64	Limited	*Mortierella elongata*	Saprotroph	Mortierellomycotina	[39]
Candidatus Glomeribacter gigasporarum	Gram - *Burkholderiaceae*	1.8–2.00	Uncultivable	*Gigaspora margarita BEG 34*	Obligate symbiont (AMF)	Glomeromycotina	[42]
Mycoplasma-related endobacteria (MRE)	*Mycoplasmataceae*	2.9 (3 phylotypes)	Uncultivable	*Endogone sp.* FLAS F-59071	Saprotroph/symbiont	Mucoromycotina	[55]
Mycoplasma-related endobacteria (MRE)	*Mycoplasmataceae*	1.8 (2 phylotypes)	Uncultivable	*Jimgerdemannia lactiflua*	Saprotroph/symbiont	Mucoromycotina	[55]
Mycoplasma-related endobacteria (MRE)	*Mycoplasmataceae*	0.9	Uncultivable	*Jimgerdemannia flammicorona* AD002	Saprotroph/symbiont	Mucoromycotina	[55]
Candidatus Moeniiplasma glomeromycotorum (MRE)	*Mycoplasmataceae*	0.7–1.3	Uncultivable	*Claroideoglomus etunicatum*	Obligate symbiont (AMF)	Glomeromycotina	[40]
Candidatus Moeniiplasma glomeromycotorum (MRE)	*Mycoplasmataceae*	0.7–1.3	Uncultivable	*Racocetra verrucosa*	Obligate symbiont (AMF)	Glomeromycotina	[40]
Candidatus Moeniiplasma glomeromycotorum (MRE)	*Mycoplasmataceae*	0.7–1.3	Uncultivable	*Rhizophagus clarus*	Obligate symbiont (AMF)	Glomeromycotina	[40]
Mycoplasma-related endobacteria (*Dh* MRE)	*Mycoplasmataceae*	0.71	Uncultivable	*Dentisculata heterogama* FL65	Obligate symbiont (AMF)	Glomeromycotina	[41]
Mycoplasma-related endobacteria (*De* MREI-1)	*Mycoplasmataceae*	0.63	Uncultivable	*Diversispora epigea*	Obligate symbiont (AMF)	Glomeromycotina	[38]
Mycoplasma-related endobacteria (*De* MRE-2)	*Mycoplasmataceae*	0.61	Uncultivable	*Diversispora epigea*	Obligate symbiont (AMF)	Glomeromycotina	[38]

Burkholderia rhizoxinica, the BRE of *Rhizopus microsporus*, uses host-derived lipids for energy, but *Mycoavidus cysteinexigens* and *Candidatus* Glomeribacter Gigasporarum (*Ca*Gg), the BRE of *Mortierella elongata* and *Gigaspora margarita*, respectively, import fungal amino acids and use fungal organic acids for energy. Most BRE have retained secondary metabolite gene clusters and secretion systems. *B. rhizoxinica*, which has limited free-living capacities, relies on a Type II secretion system (TIISS) to re-invade fungal hyphae and diffuse horizontally [45]; other endobacteria that are thought to be exclusively vertically transmitted retain TIISS and TIIISS for unknown functions [39,42]. All known BREs retained several toxin/antitoxin operons in their genomes, but this has not been documented in MREs [46]. At least for *Ca*Gg, these gene clusters are finely regulated across the life cycle of *G. margarita*: the bacterium overexpresses toxin in the spores and expresses more of the antitoxin during AMF symbiosis. Therefore, endobacteria likely adapted to survive inside their hosts by modulating potentially dangerous activities. Lastly, and remarkably, despite being strongly reduced, MRE genomes contain a number of horizontally transferred genes of fungal origin. The impact of the MRE on the fungal host remains mostly undiscovered, but recent findings indicate that MRE may have adopted a non-lethal parasitic lifestyle in Mortierellomycotina [47]. By contrast, BRE and Mucor-omycota fungi have been more deeply characterized [39,48–50]. *B. rhizoxinica* supports the pathogenic ability of *R. microsporus* by synthesizing a powerful toxin, rhizoxin, which affects rice health [51]. *M. cysteinexigens* has been detected in many *Mortierella* isolates; surprisingly it decreases the growth of its host under laboratory condi- tions, probably due to lipid depletion. However, it may increase fungal competitiveness through secondary metabolite biosynthesis, including a toxin predicted to have insecticidal activity [52]. Some *Mortierella* species grow on insect exoskeletons; they may have been the ancestral hosts of MRE currently hosted by Mucoromy- cota [47]. These observations shed light on the origin of this association and on the potential contribution of *M. cysteinexigens* to its host's ecological success, challenging the hypothesis of an exclusively parasitic interaction. *Ca*Gg, closely related to *M. cysteinexigens*, lives inside the AMF *G. margarita*, where it positively influences pre-symbiotic growth and increases lipid storage [53,54]. Omics and biochemical analyses revealed that *Ca*Gg leads to higher ATP production and more efficient responses to oxidative stress [49,55,56]. Evidence emerging from these studies suggests that the fungal counterpart can survive without its endobacterium, and not all individuals from the same

species harbor endobacteria. The same is not true for the endobacteria, which likely have strategies for maintenance inside the host population, avoiding trans- mission bottlenecks and genetic drift [32]. In *B. rhizoxinica* these strategies include horizontal transmission and dispersal through manipulation of host sexuality. BRE and MRE have been useful for exploring the origin of Mucoromycota–endobacteria interactions [38,43,47]. On the basis of examination of endobacteria diversifica- tion, which seems to be encompassed by the diversity of their Mucoromycota hosts, Bonfante and Desiro` [30], suggested an invasion event that predates the diversifica- tion of Mucoromycota (550–700 MYA). The striking dis-tribution of endobacteria in Mucoromycota may be due to their aseptate mycelium, which could favour diffusion and transmission [30]. Moreover, the Mucoromycota genomes possess up to 3% 6-methyladenine (6 mA) [57], a DNA modification that regulates gene expression and is rather common in bacteria. The shared use of 6 mA may have allowed the endobacteria to manipulate the Mucoromycota genome [32]. Endobacteria are emerging as more widespread than expected in fungal isolates. To better decipher their role, the next step will be to set up metagenomics protocols that detect their presence in natural environments, as suggested by the pioneering results of Bodenhausen *et al.* and Lastovetsky *et al.* [58,59] Indeed, these new results suggest that fungi may act as vectors of bacteria that increase plant microbiota complexity, while their endobacteria operate as multipliers of fungal genetic variabil-ity, providing diversity for natural selection.

Mycobiota in the future: discoveries and agricultural applications

Mycobiota and fungal-associated bacterial communities may have applications in agriculture as part of the micro-bial revolution, the widespread use of beneficial microbes to improve crop yields and prevent diseases. Fungi and their associated bacteria may be used to produce inocula to coat seeds [60], but the development of synthetic microbial consortia represents a new target for high qual-ity crops [61]. Many AMF have already been used in in field experi-ments, leading to remarkable successes [62]. Like some soil bacteria [63], AMF with their endobacteria may represent a source of novel natural products, such as antibiotics and other pharmaceutical compounds. How-ever, new approaches like microfluidics [64] have to be developed to set up the experimental conditions where fungi and bacteria interact leading to the synthesis of these potential new molecules. As is done for the human microbiota [65], it will be crucial to use multiple culture conditions for the plant micro-biota, and to push a rebirth of classical microbiological techniques to grow currently uncultivable microbes. Like opening Russian nesting dolls, exploring the complex interactions of plants, their mycobiota, and the microbiota of the mycobiota is vastly expanding our understanding of what it means to be a holobiont.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

1. Nash AK, Auchtung TA, Wong MC, Smith DP, Gesell JR, Ross MC, Stewart CJ, Metcalf GA, Muzny DM, Gibbs RA *et al.*: The gut mycobiome of the human microbiome project healthy cohort. Microbiome 2017, 5:153.

*2 Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez LP, Tringe SG: Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. New Phytol 2016, 209:798-811.
*The research was one of the first to include fungal communities as component of Agave microbiota.
3. Bonfante P: The future has roots in the past: the ideas and scientists that shaped mycorrhizal research. New Phytol 2018, 220:982-995.

4. Hawksworth DL, Lu¨ cking R: Fungal diversity revisited: 2.2 to 3.8 million species. Microbiol Spectr 2017, 5.

5. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Consortium FB: Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. PNAS 2012, 109:6241-6246.

*6. Peay KG, Kennedy PG, Talbot JM: Dimensions of biodiversity in the earth mycobiome. Nat Rev Microbiol 2016, 14:434-447.
*An excellent and very informative review to get a good general knowledge of fungal diversity on the basis of molecular tools.

7. Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L: Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev Microbiol 2019, 17:95-109.

8. Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E *et al.*: Revealing structure and assembly cues forArabidopsis root-inhabiting bacterial microbiota. Nature 2012, 488:91-95.

9. Bergelson J, Mittelstrass J, Horton MW: Characterizing both bacteria and fungi improves understanding of the Arabidopsis root microbiome. Sci Rep 2019, 9:24.

*10. Almario J, Jeena G, Wunder J, Langen G, Zuccaro A, Coupland G, Bucher M: Root-associated fungal microbiota of nonmycorrhizal Arabis alpina and its contribution to plant phosphorus nutrition. Proc Natl Acad Sci U S A 2017, 114:E9403- E9412.

*11 Hiruma K, Gerlach N, Sacrista´ n S, Nakano RT, Hacquard S, Kracher B, Neumann U, Ramı´rez D, Bucher M, O'Connell RJ *et al.*: Root endophyte Colletotrichum tofieldiae confers plant fitness benefits that are phosphate status dependent. Cell 2016, 165:464-474.
*These two excellent experimental papers demonstrate how plant micro-biota studies lead to the detection of beneficial endopytes which may improve plant mineral nutrion in not AM plants.

12. Skiada V, Faccio A, Kavroulakis N, Genre A, Bonfante P, Papadopoulou KK: Colonization of legumes by an endophytic Fusarium solani strain FsK reveals common features to symbionts or pathogens. Fungal Genet Biol 2019, 127:60-74.

13. Brundrett MC, Tedersoo L: Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol 2018, 220:1108-1115.

14. Chialva M, di Fossalunga AS, Daghino S, Ghignone S, Bagnaresi P, Chiapello M, Novero M, Spadaro D, Perotto S, Bonfante P: Native soils with their microbiotas elicit a state of alert in tomato plants. New Phytol 2018, 220:1296-1308.

*15. Garbaye J: Tansley review no. 76 helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol 1994, 128:197-210.
*The review opened a new productive research field showing how fungal-associated bacteria may enhance mycorrhizal traits.

16. Scheublin TR, Sanders IR, Keel C, van der Meer JR: Characterisation of microbial communities colonising the hyphal surfaces of arbuscular mycorrhizal fungi. ISME J 2010, 4:752-763.

17. Turrini A, Avio L, Giovannetti M, Agnolucci M: Functional complementarity of arbuscular mycorrhizal fungi and associated microbiota: the challenge of translational research. Front Plant Sci 2018, 9:1407.

18. 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 2018, 12:2339-2351.

19. Iffis B, St-Arnaud M, Hijri M: petroleum contamination and plant identity influence soil and root microbial communities while AMF spores retrieved from the same plants possess markedly different communities. Front Plant Sci 2017, 8:1381.

20. Vik U, Logares R, Blaalid R, Halvorsen R, Carlsen T, Bakke I, Kolstø A-B, Økstad OA, Kauserud H: Different bacterial communities in ectomycorrhizae and surrounding soil. Sci Rep 2013, 3:3471.

21. Marupakula S, Mahmood S, Jernberg J, Nallanchakravarthula S, Fahad ZA, Finlay RD: Bacterial microbiomes of individual ectomycorrhizal Pinus sylvestris roots are shaped by soil horizon and differentially sensitive to nitrogen addition. Environ Microbiol 2017, 19:4736-4753.

22. Splivallo R, Deveau A, Valdez N, Kirchhoff N, Frey-Klett P, Karlovsky P: Bacteria associated with truffle-fruiting bodies contribute to truffle aroma. Environ Microbiol 2015, 17: 2647-2660.

23. Pent M, Hiltunen M, Poldmaa K, Furneaux B, Hildebrand F, Johannesson H, Ryberg M, Bahram M: Host genetic variation strongly influences the microbiome structure and function in fungal fruiting-bodies. Environ Microbiol 2018, 20:1641-1650.

24. Oh S-Y, Lim YW: Root-associated bacteria influencing mycelial growth of Tricholoma matsutake (pine mushroom). J Microbiol 2018, 56:399-407.

25. Deveau A, Bonito G, Uehling J, Paoletti M, Becker M, Bindschedler S, Hacquard S, Herve´ V, Labbe´ J, Lastovetsky OA *et al.*: Bacterial-fungal interactions: ecology, mechanisms and challenges. FEMS Microbiol Rev 2018, 42:335-352.

26. Warmink JA, Nazir R, van Elsas JD: Universal and species-specific bacterial "fungiphiles" in the mycospheres of different basidiomycetous fungi. Environ Microbiol 2009, 11:300-312.
27. Bianciotto V, Andreotti S, Balestrini R, Bonfante P, Perotto S: Mucoid mutants of the biocontrol strain Pseudomonas fluorescens CHA0 show increased ability in biofilm formation on mycorrhizal and nonmycorrhizal carrot roots. Mol Plant Microbe Interact 2001, 14:255-260.

28. Agler MT, Ruhe J, Kroll S, Morhenn C, Kim S-T, Weigel D, Kemen EM: Microbial hub taxa link host and abiotic factors to plant microbiome variation. PLoS Biol 2016, 14:e1002352.

29. Zhang Y, Kastman EK, Guasto JS, Wolfe BE: Fungal networks shape dynamics of bacterial dispersal and community assembly in cheese rind microbiomes. Nat Commun 2018, 9:336.

*30. Bonfante P, Desiro` A: Who lives in a fungus? The diversity, origins and functions of fungal endobacteria living in Mucoromycota. ISME J 2017, 11:1727-1735.
*The review presents some hypothesis on the origin of fungal-endobac-terial symbioses when Mucoromycota are considered.

31. Olsson S, Bonfante P, Pawlowska TE: Ecology and evolution of fungal-bacterial interactions. In The Fungal Community: Its Organization and Role in the Ecosystem, edn 4. Edited by Dighton J, White JF.CRC Press; 2017.

32. Pawlowska TE, Gaspar ML, Lastovetsky OA, Mondo SJ, Real- Ramirez I, Shakya E, Bonfante P: Biology of fungi and their bacterial endosymbionts. Ann Rev Phytopathol 2018, 56: 289-309.

33. Zhang T, Abel S, Abel Zur Wiesch P, Sasabe J, Davis BM, Higgins DE, Waldor MK: Deciphering the landscape of host barriers to Listeria monocytogenes infection. Proc Natl Acad Sci U S A 2017, 114:6334-6339.

34. Tomkins M, Kliot A, Mare´ e AF, Hogenhout SA: A multi-layered mechanistic modelling approach to understand how effector genes extend beyond phytoplasma to modulate plant hosts, insect vectors and the environment. Curr Opin Plant Biol 2018, 44:39-48.

35. Moran NA, McLaughlin HJ, Sorek R: The dynamics and time scale of ongoing genomic erosion in symbiotic bacteria. Science 2009, 323:379-382.

36. Guo H, Glaeser SP, Alabid I, Imani J, Haghighi H, Ka¨ mpfer P, Kogel K-H: The abundance of endofungal bacterium Rhizobium radiobacter (syn. Agrobacterium tumefaciens) increases in its fungal host Piriformospora indica during the tripartite sebacinalean symbiosis with higher plants. Front Microbiol 2017, 8:629.

37. Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML, Bonito G, Corradi N, Grigoriev I, Gryganskyi A *et al.*: A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia 2016, 108:1028-1046.

38. Sun X, Chen W, Ivanov S, MacLean AM, Wight H, Ramaraj T, Mudge J, Harrison MJ, Fei Z: Genome and evolution of the arbuscular mycorrhizal fungus Diversispora epigaea (formerly Glomus versiforme) and its bacterial endosymbionts. New Phytol 2019, 221:1556-1573.

39. Uehling J, Gryganskyi A, Hameed K, Tschaplinski T, Misztal PK, Wu S, Desiro` A, Vande Pol N, Du Z, Zienkiewicz A *et al.*: Comparative genomics of Mortierella elongata and its bacterial endosymbiont Mycoavidus cysteinexigens. Environ Microbiol 2017, 19:2964-2983.

40. Naito M, Morton JB, Pawlowska TE: Minimal genomes of mycoplasma-related endobacteria are plastic and contain host-derived genes for sustained life within Glomeromycota. Proc Natl Acad Sci U S A 2015, 112:7791-7796.
41. Torres-Corte´ s G, Ghignone S, Bonfante P, Schu¨ ßler A: Mosaic genome of endobacteria in arbuscular mycorrhizal fungi: transkingdom gene transfer in an ancient mycoplasma-fungus association. PNAS 2015, 112:7785-7790.

42. Ghignone S, Salvioli A, Anca I, Lumini E, Ortu G, Petiti L, Cruveiller S, Bianciotto V, Piffanelli P, Lanfranco L *et al.*: The genome of the obligate endobacterium of an AM fungus reveals an interphylum network of nutritional interactions. ISME J 2012, 6:136-145.

43. Chang Y, Desiro` A, Na H, Sandor L, Lipzen A, Clum A, Barry K, Grigoriev IV, Martin FM, Stajich JE *et al.*: Phylogenomics of endogonaceae and evolution of mycorrhizas within Mucoromycota. New Phytol 2019, 222:511-525.

44. Lackner G, Moebius N, Partida-Martinez L, Hertweck C: Complete genome sequence of Burkholderia rhizoxinica, an Endosymbiont of Rhizopus microsporus. J Bacteriol 2011, 193:783-784.

*45. Moebius N, U¨zu¨m Z, Dijksterhuis J, Lackner G, Hertweck C: Active invasion of bacteria into living fungal cells. eLife 2014, 3: e03007.
*One of the few experimental researches performed on fungal endobac- teria: it elegantly shows how B. rhizoxinica enters fungal cells.

46. Salvioli di Fossalunga A, Lipuma J, Venice F, Dupont L, Bonfante P: The endobacterium of an arbuscular mycorrhizal fungus modulates the expression of its toxin-antitoxin systems during the life cycle of its host. ISME J 2017, 11:2394-2398.

*47. Desiro` A, Hao Z, Liber JA, Benucci GMN, Lowry D, Roberson R, Bonito G: Mycoplasma-related endobacteria within Mortierellomycotina fungi: diversity, distribution and functional insights into their lifestyle. ISME J 2018, 12:1743- 1757.
*An interesting contribution revealing how Mortirella maintained in cultures may host MREs and how they can be cured by applying antibiotics.

48. Li Z, Yao Q, Dearth SP, Entler MR, Castro Gonzalez HF, Uehling JK, Vilgalys RJ, Hurst GB, Campagna SR, Labbe´ JL *et al.*: Integrated proteomics and metabolomics suggests symbiotic metabolism and multimodal regulation in a fungal- endobacterial system. Environ Microbiol 2017, 19:1041-1053.

49. Salvioli A, Ghignone S, Novero M, Navazio L, Venice F, Bagnaresi P, Bonfante P: Symbiosis with an endobacterium increases the fitness of a mycorrhizal fungus, raising its bioenergetic potential. ISME J 2016, 10:130-144.

50. Partida-Martinez LP, Groth I, Schmitt I, Richter W, Roth M, Hertweck C: Burkholderia rhizoxinica sp. nov. and Burkholderia endofungorum sp. nov., bacterial endosymbionts of the plant-pathogenic fungus Rhizopus microsporus. Int J Syst Evol Microbiol 2007, 57:2583-2590.

51. Scherlach K, Busch B, Lackner G, Paszkowski U, Hertweck C: Symbiotic cooperation in the biosynthesis of a phytotoxin. Angew Chem Int Ed Engl 2012, 51:9615-9618.

52. Sharmin D, Guo Y, Nishizawa T, Ohshima S, Sato Y, Takashima Y, Narisawa K, Ohta H: Comparative genomic insights into endofungal lifestyles of two bacterial endosymbionts, Mycoavidus cysteinexigens and Burkholderia rhizoxinica. Microbes Environ 2018, 33:66-76.

53. Lumini E, Bianciotto V, Jargeat P, Novero M, Salvioli A, Faccio A, Be´ card G, Bonfante P: Presymbiotic growth and sporal morphology are affected in the arbuscular mycorrhizal fungus Gigaspora margarita cured of its endobacteria. Cell Microbiol 2007, 9:1716-1729.

54. Salvioli A, Chiapello M, Fontaine J, Hadj-Sahraoui AL, Grandmougin-Ferjani A, Lanfranco L, Bonfante P: Endobacteria affect the metabolic profile of their host Gigaspora margarita, an arbuscular mycorrhizal fungus. Environ Microbiol 2010, 12:2083-2095.

55. Dearth SP, Castro HF, Venice F, Tague ED, Novero M, Bonfante P, Campagna SR: Metabolome changes are induced in the arbuscular mycorrhizal fungus Gigaspora margarita by germination and by its bacterial endosymbiont. Mycorrhiza 2018, 28:421-433.

56. Venice F, de Pinto MC, Novero M, Ghignone S, Salvioli A, Bonfante P: Gigaspora margarita with and without its endobacterium shows adaptive responses to oxidative stress. Mycorrhiza 2017, 27:747-759.

57. Mondo SJ, Dannebaum RO, Kuo RC, Louie KB, Bewick AJ, LaButti K, Haridas S, Kuo A, Salamov A, Ahrendt SR *et al.*: Widespread adenine N6-methylation of active genes in fungi. Nat Genet 2017, 49:964-968.

58. Bodenhausen N, Somerville V, Desiro` A, Walser J-C, Borghi L, Heijden MGA, van der, Schlaeppi K: Petunia- and Arabidopsis- specific root microbiota responses to phosphate supplementation. Phytobiome J 2018, 3:112-124 http://dx.doi. org/10.1101/400119.

59. Lastovetsky OA, Ezekiel A, Mondo SJ, Toomer KH, Zhang A, Johnson LM, Pawlowska TE: Distribution and population structure of endobacteria in arbuscular mycorrhizal fungi at North Atlantic dunes. ISME J 2018, 12:3001-3013.

60. Lugtenberg B (Ed): Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture. Springer International Publishing; 2015.

61. Kong Z, Hart M, Liu H: Paving the way from the lab to the field: using synthetic microbial consortia to produce high-quality crops. Front Plant Sci 2018, 9.

62. Rodriguez A, Sanders IR: The role of community and population ecology in applying mycorrhizal fungi for improved food security. ISME J 2015, 9:1053-1061.

63. Crits-Christoph A, Diamond S, Butterfield CN, Thomas BC, Banfield JF: Novel soil bacteria possess diverse genes for secondary metabolite biosynthesis. Nature 2018, 558:440-444.

64. Millet LJ, Aufrecht J, Labbe´ J, Uehling J, Vilgalys R, Estes ML, Miquel Guennoc C, Deveau A, Olsson S, Bonito G *et al.*: Increasing access to microfluidics for studying fungi and other branched biological structures. Fungal Biol Biotechnol 2019, 6:1.

65. Lagier J-C, Dubourg G, Million M, Cadoret F, Bilen M, Fenollar F, Levasseur A, Rolain J-M, Fournier P-E, Raoult D: Culturing the human microbiota and culturomics. Nat Rev Microbiol 2018, 16:540-550 http://dx.doi.org/10.1038/s41579-018-0041-0.