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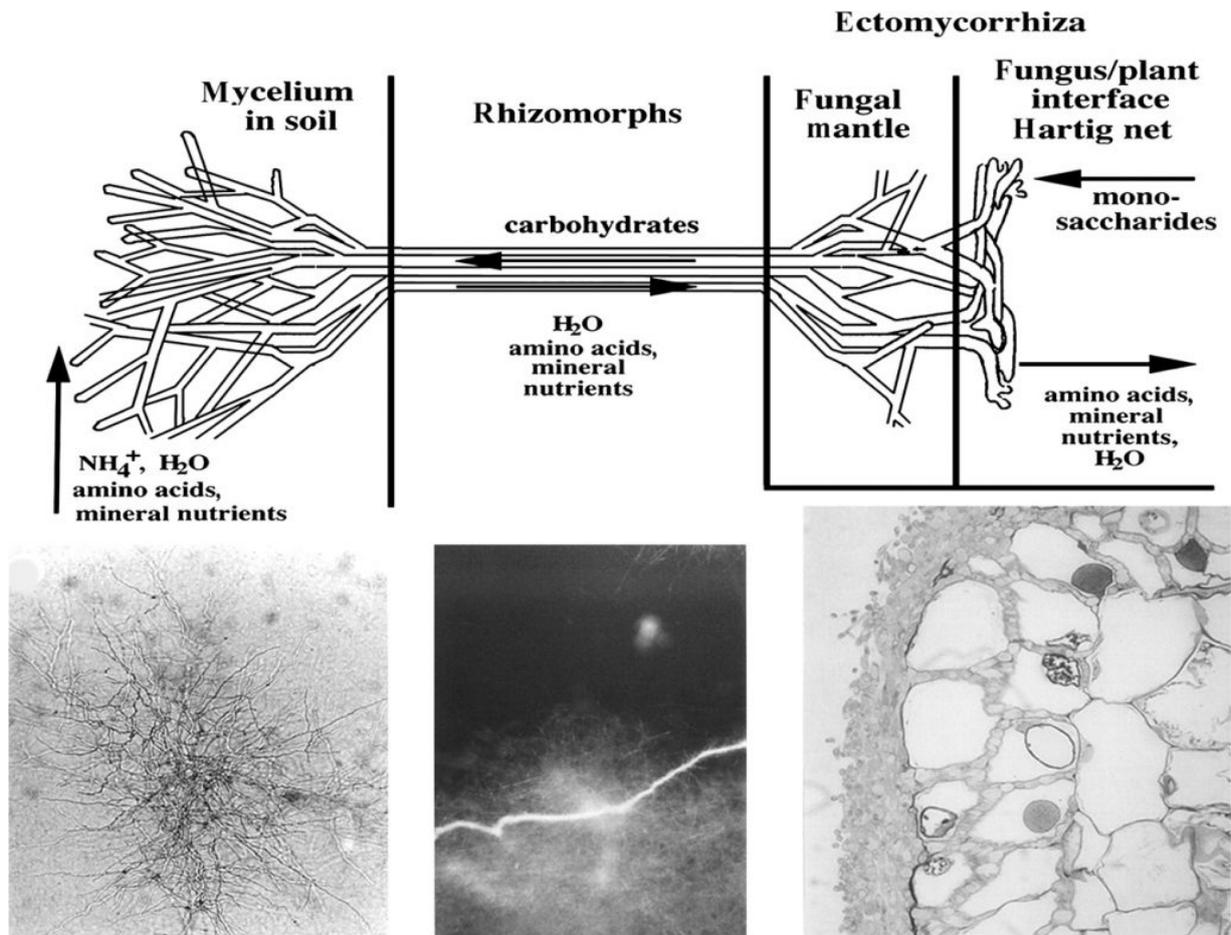
Mycorrhizal fungi: biodiversity and use in agriculture

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The mycorrhizal fungi relevant for agriculture include (a) a group of ectomycorrhizal (EM) symbionts of trees and shrubs (e.g. the “truffles”, Ascomycota belonging to *Tuber spp.*, and the edible Basidiomycota such as *Boletus spp.*) forming a mantle around the plant root apex and (b) a group of obligate endosymbionts (phylum Glomeromycota) of plant roots, called AMF or arbuscular mycorrhizal fungi, forming mutualistic symbioses with about 80% of land plant species, including many agricultural crops. There are other groups of endomycorrhizas with more limited range of plant symbionts, i.e. Ericales and Orchidaceae. Mycorrhizal fungi are considered natural bio-fertilizers, providing the plant with nutrients (e.g. assimilable phosphorus, sulphates, ammonium), water, and protection against pathogens, in exchange for photosynthetic plant products, e.g. organic carbon, such as glucides.



Scheme of an ectomycorrhizal fungal colony (without fruit bodies). Shown is a scheme of an ectomycorrhizal fungal colony (upper part) and photographs of the respective fungal structures (lower part, from left to right: soil-growing hyphae, rhizomorph, ectomycorrhiza).

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The mechanism of mutual benefit is relatively simple: the fungal mycelium that emerges from the root canopy acquires water and nutrients from larger soil volumes that are inaccessible to roots, i.e. beyond the root depletion zone. The fungal hyphae, which colonize the plant root cortex (AMF) or the external cortex or epidermal cells (EM) on one side and elongate into the bulk soil on the other side, are much thinner than the plant roots, hence able to penetrate smaller pores and explore more soil volumes. Furthermore, AM fungi can also have a direct effect on the ecosystem: they contribute to reducing emissions of N_2O , to improving plant tolerance to drought and salinity, to ameliorating the soil structure and

aggregation, and to driving the structure of plant communities and productivity (Berruti et al. 2016).



Cultivation of truffles

Photo:static.wixstatic.com/media/04884c1b7251837cf9798c0de6b26a59.wix_mp_1024

There are 6.000 species of ectomycorrhizal fungi. However, only a relatively restricted number has been subjected to extensive study for their exploitation in agriculture. Among the latter group, the species of the genus *Tuber*, namely *T. aestivum* Vitt., *T. borchii* Vitt., *T. brumale* Vitt., *T. dryophilum* Tul., *T. maculatum* Vitt., *T. macrosporum* Vitt., *T. magnatum* Pico, and *T. melanosporum* Vitt. The interest in truffles is driven by their high market price market, their unique aroma, and the limited “cultivable” areas. The most significant steps of our knowledge in the last three four decades include (i) development of artificially inoculated plants (with “crude inoculum” or pure *Tuber spp.* cultures) in the nursery, ready for transplant to the open field, (ii) development of DNA probes for unambiguous identification of truffle species (*Tuber borchii* Vitt., *T. brumale* Vitt., *T. dryophilum* Tul., *T. magnatum* Pico, *T. maculatum* Vitt., *T. melanosporum* Vitt., *T. puberulum* Berk e Br., *T. indicum* Cooke e Mass., and *T. indicum var. himalayensis*), (iii) application of proteomics for identification of local races, (iv) elucidation of the trophic and pedoclimatic factors affecting the

physiology and growth of sporocarps (i.e. the edible part of the truffles), (v) the improvement of technology for storage of mature sporocarps over time; patents have been filed covering the production of natural truffle aroma by pure cultures of ruffle associated bacteria, (vi) experimental evidence that bacteria are constantly present in the sporocarps at a density of 10^5 - 10^8 cells /g d.w.

Currently the recognized roles of the associated bacteria include their involvement in spore germination and hyphal differentiation leading to sporocarp formation, protection of the truffle against pathogens, stimulation of mycorrhization, hyphal growth and mycelial net formation, and truffle aroma formation in *T. aestivum*, *T. borchii*, *T. magnatum*, and *T. melanosporum* (only bacteria can synthesize volatile compounds containing sulphur, e.g. thiophene; Vahdatzadeh et al. 2015).



Tuber borchii Vitt., in soil. These truffle spores not only give your tree the capacity to host a highly prized fungi; the treatment will also improve your trees health by improving its capacity to fight infections and unlock otherwise unavailable nutrients from the soil.

Photo:

www.totallytruffles.co.uk/store/p19/Tuscany_truffle_%28Tuber_borchii%29_spores_in_soil_%28100g%29.html

Since the first truffle plantations were established in Italy and France in the 80's, many field studies have been carried out to improve their productivity and sustainability. It is now widely accepted that the success of a truffle plantations is related to the mycorrhizal status of the host trees over the years, from inoculated seedlings to truffle-producing trees, and that the microbiome of a truffle orchard plays an essential role for the sporocarp production, growth and yield per plant.

-The arbuscular mycorrhizal fungi exclusively colonize the plant root cortex and form highly branched structures inside the cells, i.e. the arbuscules, which are considered the functional site of nutrient exchange (Balestrini et al., 2015). The specificity of this mutualistic relationship is not fully elucidated, although studies of meta-analysis of data clearly show that the use of AMF inoculation in the field leads to benefits for yield and quality of the crops produced. As an example, Pellegrino et al. (2015) have shown that field AMF inoculation of wheat increases: aboveground biomass, grain yield, harvest index, aboveground biomass, P concentration and content, straw P content, aboveground biomass N concentration and content, grain N content and grain Zn concentration. Similar experimental evidence is being obtained for legumes, other cereals, coffee, and potatoes. Unfortunately, since AM fungi are obligate symbionts, they cannot be cultivated so far in pure culture. Therefore the production of inoculants represents a quite challenging agro-industrial process, particularly in the quality-control phase. In addition, it is clear from the most recent findings that inoculants based on microbiomes, instead of solely AM fungi, are better performing

as bio-fertilizers (Nutti and Giovannetti 2015). Therefore the choice of the appropriate inoculant can be one of the factors which ultimately affect the success of the inoculation, after a careful selection of the favorable plant/niche/fungus/microbiome combinations.



Tuber melanosporum, Vitt.,
spores in compost

Photo:

http://www.totallytruffles.co.uk/store/p26/Black_truffle_%28Tuber_melanosporum%29_spores_in_compost_%28100g%29.html

Despite its enormous potential, the application of AMF in agriculture has not been fully adopted by farmers so far. Berruti et al. (2016) underline that “since indigenous AMF have been demonstrated to be equally or even better performing than commercial or culture collection isolates, farmers are encouraged to autonomously produce their AMF inocula, starting from native soils. This makes the bio-fertilization technology more likely to be affordable for farmers, including those in developing countries who need their cropping system to be as highly sustainable as possible”.