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Review article

Agricultural practices to improve nitrogen use efficiency through the use of arbuscular mycorrhizae: basic and agronomic aspects

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Highlights

- Nitrogen cycling in agroecosystems is heavily dependent upon arbuscular mycorrhizal fungi present in the soil notably when they are in undisturbed
- It is necessary to investigate which agricultural practices could be favorable to maximize the benefits of AMF in order to improve crop nitrogen use efficiency.
- We describe, how improving colonization by AMF through the reduction of soil perturbation could be helpful for increasing crop nitrogen use efficiency.

ABSTRACT

Nitrogen cycling in agroecosystems is heavily dependent upon arbuscular mycorrhizal fungi (AMF) present in the soil microbiome. These fungi develop obligate symbioses with various host plant species, thus increasing their ability to acquire nutrients. However, AMF are particularly sensitive to physical, chemical and biological disturbances caused by human actions that limit their establishment. For a more sustainable agriculture, it will be necessary to further investigate which agricultural practices could be favorable to maximize the benefits of AMF to improve crop nitrogen use efficiency (NUE), thus reducing nitrogen (N) fertilizer usage. Direct seeding, mulch-based cropping systems prevent soil mycelium disruption and increase AMF propagule abundance. Such cropping systems lead to more efficient root colonization by AMF and thus a better establishment of the plant/fungal symbiosis. In addition, the use of continuous cover cropping systems can also enhance the formation of more efficient interconnected hyphal networks between mycorrhizae colonized plants. Taking into account both fundamental and agronomic aspects of mineral nutrition by plant/AMF

symbioses, we have critically described, how improving fungal colonization through the reduction of soil perturbation and maintenance of an ecological balance could be helpful for increasing crop NUE.

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4. Improving plant N uptake and utilization with arbuscular mycorrhizal fungi

1. The contribution of mycorrhizal fungi to plant nitrogen nutrition

Mineral fertilizers such as nitrogen (N) are presently the main source of nutrients applied to soils, even if the contribution of animal manure remains important in areas where there are livestock nearby. Following the Green Revolution in the 1960s, N fertilizers synthesized by the Haber–Bosch process have been used extensively to increase crop yield, allowing the production of food for nearly half of the world population [1].

Despite an almost ten-fold increase in the application of mineral N fertilizers, the overall increase in yield has been less than 3-fold [2]. This indicates that N use efficiency (NUE), defined as the yield obtained per unit of available N supplied by the soil and by added N fertilizer, has declined considerably over the last 50 years. NUE is composed of the uptake efficiency, that is the ability of plants to take up N from the soil and the utilisation efficiency, that is the ability of plants to use N to produce biomass, grain in particular. It has therefore become crucial that NUE should be improved worldwide, for both environmental and economic benefits [3]. One of the main reasons for the decline in NUE, has been that most modern crops were bred in the presence of non-limiting mineral fertilization conditions, in particular N. Thus, the opportunities to select productive genotypes under low levels of

mineral or organic fertilization conditions have been missed. This includes the ability of genotypes to develop non-symbiotic N₂-fixing and symbiotic AMF (arbuscular mycorrhizal fungal) associations [4]. It will be necessary to enhance NUE in countries which do not have the benefit of the intensive use of N fertilizers, by the selection of productive genotypes that can grow under low N conditions, notably in tropical regions. It has also suggested that in additions to breeding strategies for improving crop NUE, changes in government policy, including a decrease in N fertilizer usage, are needed to reduce the effect of N inputs on human health, climate and ecosystems [3].

More than 50% of the N applied to the soil is not used by the crop plant and may be taken up by the soil microbiome [5]. In the intensive agricultural production systems that are currently used worldwide, this uptake is likely to shift N accumulation towards the bacterial biomass. N is also lost by volatilization [6] or by leaching [7]. In some agroecosystems N volatilization can be as high as 50% [8]. The loss of N, mainly in the form of nitrate (NO₃⁻), [9]), which can run off into the surface water or flows into the groundwater can have a detrimental impact on living organisms notably animals and humans [10]. In addition, NO₃⁻ can have a major effect on the environment by causing eutrophication of freshwater and marine ecosystems [11] and through the emission of N gases such as N₂O in heavily N fertilized agricultural soils, which has a potent greenhouse effect [9, 12].

It will be necessary to increase agricultural production by 1.7-fold in 2050, if we are to feed the growing world population (FAO; <http://www.fao.org/nr/nr-home/en/>). The detrimental impact of the overuse of N fertilizers on the environment can be minimized if it is accompanied by sustainable agricultural practices, such as fertilizer use rationalization [13]. In addition, the use of crop rotation, the establishment of ground cover and the burial of crop residues will be of value, preferably with plants that fix dinitrogen (N₂) fixing and utilize AMF symbiotic associations [13]. Paradoxically, N₂ fixation, which is mainly carried out by

the symbiotic association between seed and forage producing legumes and Rhizobiaceae [14], is one of the most important sources of reduced N in agricultural systems [15]. This is also why intensive research is currently being conducted to expand symbiotic N₂ fixation to a greater diversity of crop plants [16]. An increasing number of studies have shown that conservation tillage using no-till and permanent plant cropping systems also significantly enhances the potential of plant colonization by AMF in comparison with conventional tillage [17]. The occurrence of even more efficient tripartite symbioses between legumes, Rhizobiaceae and AMF has also been suggested [18]. Thus, either the use of legumes as cover crops and the development of no-till farming or both, could also be attractive to increase NUE through the beneficial action of AMF on N uptake efficiency, with regards to both soil N availability and N transfer to the host plant. The development of such sustainable agricultural practices implies that more studies are needed for a better understanding of the biological mechanisms involved in the establishment and functioning of more efficient symbioses with Rhizobiaceae and AMF as they share a common initial signaling pathway when they develop a symbiotic association with a plant [19]. The knowledge gained from such studies could be used to develop future breeding and farming strategies to select and grow highly productive crops using less synthetic N fertilizer [20]. However, it will be necessary to take into account that legumes require more phosphate (P) fertilizer than cereals [21], and many are more demanding of water [22], notably in areas where water scarcity is increasing. Therefore, plant breeders and agronomists will need to determine whether increasing N₂ fixation is economically justified as, in contrast to atmospheric and synthetic N, P is not inexhaustible.

2. The complexity of nutrient uptake in a plant-fungal symbiosis

Many excellent reviews have described extensively our current knowledge of the role of mycorrhizal fungi in the uptake of nutrients by plants, notably N [23] and P [24]. In these

reviews it has been emphasized that AMF play an essential role in the nutrient uptake of the majority of land plants, including many important crop species. The contribution of the AMF symbiosis to P nutrition has focused on the interplay between direct P uptake *via* the roots and uptake *via* the AMF pathway.

Whether AMF contribute similarly to the N nutrition of the host plant is discussed, taking into account that AMF can actively transfer N to their host [23], even through the contribution of AMF to plant P uptake is usually much larger than the contribution to plant N uptake [24]. Moreover, the greatest AMF benefits are realized under low P and high N fertilization, substantiating the greater role of AMF in P uptake than in N uptake [25]. Therefore, the relative availability of P and N is an important driver of AMF structure and function and of the impact of AMF on host plants, whether they are present as a single species or as a community of species in various ecosystems [26].

Therefore, we will briefly cover the assimilation of mineral nutrients in mycorrhizal symbiotic associations by focusing this section on N transport and only summarizing current knowledge and future perspectives. Roots colonized by AMF have two uptake pathways for nutrients. One involves the plant, whilst the other involves the fungal symbiotic partner. The plant root takes up minerals *via* high or low affinity transport systems, allowing the uptake of nutrients present in low concentrations, or those with a low mobility such as P [27]. The AMF possess only high affinity transport systems towards P located in the extraradical mycelia for nutrient uptake, or in the hyphae colonizing the root cortex, for their translocation. The mechanisms involved in the control of P transport by the plant and the fungal uptake systems are well characterized [24, 27]. In contrast, it remains to be demonstrated if, in a similar manner to P uptake, the plant NO_3^- or ammonium (NH_4^+) transport systems are down regulated when the plant is colonized by AMF [23]. Whether the fungi with their own NO_3^- or NH_4^+ transporters are able to provide enough N for optimal plant growth and development, also remains to be

determined, knowing that inorganic N taken up by the fungi can be incorporated into amino acids that are further transferred to the plant [28]. Fungi are also able to obtain substantial amounts of N from decomposing organic material that can enhance their fitness to grow in such an environment (see section 3.1 for details). Moreover, the large biomass and high N demand of AM fungi means that they represent a global N pool equivalent in magnitude to fine roots and play a substantial role in the N cycle [29].

For example, several amino acids such as asparagine and arginine are taken up in greater amounts from soil organic matter and transferred to the plant when *Sorghum bicolor* is colonized by AMF, suggesting the occurrence of multiple transporters in the fungus [30]. Therefore, more research will be required to characterize these putative amino acid transporters and to determine to what extent they contribute to NUE improvement in the symbiotic association. Establishing whether there is competition for N between the plant and the AMF and identifying the underlying regulatory control mechanisms seems to be a key issue for optimizing the efficiency of the symbiotic association, notably when there is low N availability in the soil [31]. Individual or combined “omics”-based techniques [32] will provide means to decipher some of the regulatory control mechanisms. However, the interpretation and integration of the generated transcriptomic, proteomic and metabolomic data under agronomic conditions will be difficult, due to the occurrence of complex mycelial networks in a single species, that are even more complex in mixtures of several species. The traits of both the roots and mycorrhizal fungi need to be jointly considered for studying regulation and optimizing nutrient foraging by the symbiotic association [33]. Initially the physiological and molecular mechanisms involved in inorganic or organic N uptake by the fungi and their transfer to the plant, should be investigated using a single plant species including N₂ fixing legumes and a single fungal partner. This research should be carried out before glasshouse or field studies using natural soils, when interaction with the microbiome

and the neighboring plants are much more complex. Such investigations could be first conducted using microcosms [34] and well-controlled gnotobiotic systems similar to those recently developed for non-symbiotic N₂-fixing associations [35], (Figure 1). These two experimental systems could then be adapted to study the interaction between a mixture of several plant species and multiple fungal partners both at the molecular and physiological levels, thus gradually increasing the complexity of the plant/AMF symbiotic association. Further on, the qualitative and quantitative impact of N nutrition and the nature of the soil used to study the plant/AMF interactions will need to be assessed in order to determine if the knowledge gained from these experimental systems can be transferred to the field under agronomic conditions.

3. The roles of arbuscular mycorrhizal fungi in agroecosystems

The association between AMF and host plants is an ancient symbiosis that arose on the earth more than 400 million years ago [36]. Such associations are based upon a succession of biological events that can lead to a number of beneficial effects in both natural and agricultural ecosystems [37]. Mycorrhizal fungi can be involved in seedling growth [38], litter decomposition [39], and soil aggregation [40]. In this section, we will discuss the three main requirements necessary for the successful establishment of an AMF symbiosis in agricultural soils: 1) density of propagules within the rooting zone; 2) chemical communication between plants and AMF hyphae; and 3) diversity of soil AMF communities.

Over the last few decades, agricultural practices have been monitored for their negative impacts on ecosystem services. These ecosystem services include soil nutrient supply, water retention and water filtration [41]. The diversity of AMF in agricultural systems is much lower than in natural systems, especially in those conventionally managed [42]. However, the shift in the composition of the AMF observed in crops when the soil is less disturbed is not

necessarily beneficial for crop productivity, notably when the fallow forest soils of tropical countries are replaced by conventional cropping systems [43]. Therefore, we will critically describe which of the three key biological requirements listed above need to be considered as essential in terms of agricultural management for optimal and sustainable crop productivity.

3.1 The importance of propagule density and mycelial networks

The establishment of a mycorrhizal symbiosis is undoubtedly associated with the density of propagules in the soil. Propagules are the sources of AMF inoculants such as spores, colonized root fragments and hyphae [44]. Propagule density is defined as the number of viable AMF propagules in a given volume of soil that can potentially colonize the roots of the host plants. The presence of infective hyphae and their ability to colonize roots in order to form specialized intraradical structures for nutrient exchange (arbuscules) and storage (vesicles) is important (Fig. 2). The AMF extraradical networks originating from individual plant root systems are highly interconnected, due to the occurrence of numerous anastomoses within the networks themselves [45]. Anastomoses between contiguous mycorrhizal networks can occur in the vicinity of the root systems of different plant species [46]. In addition, one or several mycorrhizal fungal species can colonize different plant species, forming connections within a common fungal network. This network originates from hyphae colonizing neighboring roots and from hyphal fusions between previously independent mycelia [47]. One of the direct consequences of the establishment of such interconnected networks, is the possible transfer of nutrients such as N from one plant to another, even those plants belong to different taxa [37, 47]. The possibility that within plant populations, AMF networks confer an advantage to individuals that are slightly larger than their neighbors also needs to be taken into account. However, in addition to the effects of differences in plant size, inter-specific plant competition is more difficult to predict, as different combinations of species-specific

traits may either amplify or decrease nutrient competition in a mycorrhizal network. Therefore, future research needs to be developed to investigate the roles of these networks in the inter-plant transfer of plant signals and allelochemicals [48].

Considerable evidence suggests that AMF can also affect the nature of weed communities in agroecosystems, by changing the relative abundance of mycotrophic host weed species and the non-hosts weed species [49]. Through a variety of mechanisms, weed/AMF interactions may reduce the crop yield losses caused by weeds and increase the positive effects of weeds on soil quality and beneficial organisms. Therefore, AMF could provide a new means of ecologically-based weed management [49], by taking into account the compatibility between the crop, the weed and the fungi.

Among agricultural practices, it has been observed that the detrimental effects of fungicides on AMF development largely depend on the mode of action of the active molecule(s) present in the fungicide and the specificity of the AMF-fungicide interaction. The results obtained indicate that the application of an AMF inoculant should not be close to a systemic seed-applied fungicide and placing the AMF inoculant in a separate furrow is generally recommended [50].

There is evidence that tillage with high fertilization inputs and frequent fallow periods disturbs the development of AMF, leading to a decrease in the abundance of viable AMF propagules, including spores and infective hyphae [51]. In particular, tillage strongly decreases the density of AMF propagules and thus reduces host colonization [52]. Top soil is the major storage pool of AMF propagules [53]. It is thus not surprising that a no-till system that does not involve the physical disturbance of top soil, stimulates an increase in propagule density leading to better colonization, as compared to plowing and disc harrow cultivation. In contrast, under no-till conditions due to the lack of soil structure disruption, soil penetration resistance (SPR), a mechanical property that may be used as an indicator of soil compaction,

can remain significantly higher. Such penetration resistance depends both on the type of soil and on the timing of the no-till cultivation system. If the SPR is not too high, it is not necessarily detrimental for root colonization by the AMF propagules present under no-till conditions, in comparison to those conventionally cultivated [17]. However, when there is a critical increase of soil compaction in soils managed under no-till conditions, it could limit root growth and development and thus reduce yield [54]. Whether soil aeration provided by tillage practices could be beneficial for increasing the abundance of AMF propagules is another interesting question. However, it has been reported that well-aerated non-tilled soils are more resistant to erosion forces and can have better aeration and water infiltration due to the presence of heterogeneous patches of C that enhance microbial diversity [55]. Such findings indicate that compared to tillage, no-till is an agricultural practice that is able to increase the number of viable propagules. Therefore, developing alternative agricultural practices such as reduced tillage could decrease the deleterious effects of conventional agriculture both on the development and growth of AMF propagules and on the colonization of the host by the fungi. In line with this proposal, it was suggested that the hyphal network may be important, particularly when spore abundance is low, as the density of fungal propagules remained the same under low and high inputs of irrigation or fertilizers [56].

The finding that reduced soil physical disturbance favors AMF colonization, explains why following two years of experimentation there was a significant increase in the AMF colonization rate from stem elongation to flowering of the wheat and triticale host plants. Such an increase in AMF colonization again confirms that the previously produced extraradical mycelium acted as a source of inoculum [57]. The maintenance of a potential AMF inoculum over winter, when both live root density and biomass are maintained in cover crop mixtures, represents a key element for increasing the colonization process in the subsequent cultivated crop. Consequently, in the following spring, there was an increase in

the spore density and AMF colonization rate of sunflower and maize [58]. In addition, it was observed that no-till increased the external mycorrhizal mycelium length in comparison to conventional tillage in a maize-wheat rotation [59]. It is thus advisable to improve AMF life cycles throughout crop rotation by direct seeding of mulch-based cropping systems, as mechanical soil disturbance is avoided and the plant covering period is maximized (see Fig. 3 for technical examples of management in the field). The lack of mechanical disturbance under no-till conditions has sometimes been associated with changes in weed communities, inducing higher weed pressure that has required an increased use of herbicides (see [60] for review).

As described in section 2, the extraradical hyphae of AMF are able to take up and assimilate inorganic N [61], originating from either N fertilizer or released as the result of decomposition of patches of organic matter. The inorganic N sources NH_4^+ and in particular NO_3^- in the soil, are relatively highly mobile, as compared to P. Thus it was believed that the extraradical mycelia network was unlikely to significantly increase access to mineral N resources in the soil [23]. However, ^{15}N -labelling studies have shown that the high affinity NH_4^+ uptake system of the AMF *Rhizophagus irregularis* has a five times higher affinity for the cation than plants, thus allowing the AMF to take up NH_4^+ from the soil, even under conditions of low N supply [62]. Despite the frequent assumptions that due to their obligate biotrophic life cycle, AMF are unable to exploit soil organic N sources, some AMF are able to acquire up to 30% of the organic N present in organic soil patches [63]. Combined ^{15}N -and ^{13}C -labeling studies, in which the litter comprised plants previously treated with $^{13}\text{CO}_2$ and $^{15}\text{NO}_3^-$, revealed that when the AMF has access to a patch containing organic matter, it was enriched with the ^{15}N -tracer, but not with ^{13}C . Such a finding confirms that AMF do not have saprotrophic capabilities. However, they are able to acquire a substantial amount of N originating from the decomposition of organic matter, which unlike P that is readily available in a patch of organic matter [29], must be first mineralized before it is transferred to the fungi [29]. Even if AMF

do not contribute directly to organic decomposition, they have an indirect role in the decomposition process through modification of the microbial community of decomposers in the litter by supplying C originating from the neighboring plants [64]. Using ^{15}N -labelled organic residues, it was shown that AMF-induced decomposition processes accelerated N mineralization from organic matter [65]. Thus, the greater root colonization of wheat by AMF, leading in some cases to an increase in N uptake [17], could be explained by the fact that the soils under no-till conditions are characterized by higher contents of organic C and N in the upper soil layer [66].

The possible nutrient exchange from one plant to another by means of hyphal networks is of importance in intercropping systems, since it has been suggested that these networks are able to transfer the N acquired by symbiotic N_2 -fixing cover crop plants to neighboring plants that do not develop N_2 -fixing symbiotic associations [37]. Labeling the legume cover crop with $^{15}\text{N}_2$ under glasshouse or field conditions, should demonstrate that the fixed ^{15}N is transferred to the neighboring non-legume plants, by measuring the enrichment in the ^{15}N -tracer both in their roots and shoots.

Therefore, both the choice of species in cover crops mixtures, as well as their sowing density represents key technical issues. These greatly impact on the turnover of propagules, highlighting the importance to a farmer in deciding to what extent AMF can contribute to the productivity of the main crop. For instance, Brassicaceae such as mustard are often used as cover crops to protect soils during the winter and prevent NO_3^- leaching in European croplands. However, the release of isothiocyanates by mustard residues can suppress the ability of native AMF inocula to colonize the following crops [67]. Other cover crops such as radish (another Brassicaceae) which also has a high ability to capture NO_3^- , do not have this detrimental effect on AMF colonization, indicating that the choice of the cover crop is important [68]. Only a few field studies utilizing maize and wheat have been used to evaluate

the impact of contrasting management practices on the density of AMF propagules [56, 57]. These studies confirmed that the extraradical mycelia previously produced acted as a source of inoculum. In addition, they showed that colonization responded more strongly to tillage practices than to the combination of the maize or wheat crop with the preceding crop namely *Medicago sativa*, in particular in low fertility soils. To optimize AMF colonization under no-till conditions, it will be necessary to conduct more large-scale field experiments in different locations using several combinations of crops, cover crops and inoculants both under low and high N input. Such field experiments will provide additional and reliable information on the type of cover crop to be used and its management in a wide range of environmental conditions.

3.2 Interactions between arbuscular mycorrhizal fungi and plant roots

It is well established that plants modify the composition of the soil in the vicinity of their roots, through active efflux or passive diffusion of C compounds from root cells, a process called rhizodeposition [69]. Such a process leads to a proliferation of microorganisms within the roots, but also at their surface and adjacent to them [69]. Given that 5 to 50 % of the total photosynthetically fixed C is transported by exudates in the vicinity of the roots [70], it represents a significant loss for the plant, with a concomitant loss of yield. Rhizodeposits play important roles both in plant defense mechanisms and as attraction systems for microorganisms, as they are able to trigger negative interactions with parasitic plants, pathogens and invertebrate herbivores and positive interactions with beneficial bacteria and AMF [71]. Plants actively interact with soil microbial communities by releasing signaling molecules that control potential colonization by the surrounding microorganisms [70]. Consequently, the microbial community living within the rhizosphere is strongly responsive to the root exudates [72]. The root exudates released by host plants enhance AMF spore

germination [73], as well as hyphal growth and branching [74]. In contrast, the root exudates that are produced by incompatible host plants have no effect, or even inhibit hyphal growth [75]. The putative attractive effect of root exudates on AMF hyphae is important, as it allows the rapid colonization of a potential host plant, either from a germinating spore or from a previously colonized root [76].

Strigolactones and their derivative metabolites have been found to be released by the roots in the rhizosphere and are thought to be involved both in the attraction of AMF [77] and in the stimulation of hyphal branching [78]. The potential use of strigolactones as indirect biofertilizers that can stimulate the colonization of roots by AMF and thus the delivery of nutrients to plants has been recently reviewed [79]. The growth and branching of AMF hyphae depend on the composition of the root exudate, which also depends on root architecture [80], soil structure [81] and the plant species, including their physiological status [82]. Mycorrhizae are also strongly influenced both by the nutritional status of the host plant and by soil nutrient availability [83]. Under controlled low N and P nutrition, both the production and the relative proportions of strigolactones present in root exudates can be modified [84]. The quantitative extraction of strigolactones from root exudates collected from the soil in a field experiment is technically much more difficult. However, it will be interesting to study the impact of strigolactones on AMF colonization, by using for example mutants deficient in strigolactone synthesis or those that can be induced to produce greater amounts of the hormone [85].

Agricultural practices, together with the type of soil and the nature of the crop species control the composition and thus the biological activity of the rhizosphere microbiota, including AMF [86]. For instance, it has been shown that the beneficial effect of AMF on cereal and potato nutrition is markedly reduced when high rates of N, P and K fertilizer are applied, or when green manure is incorporated directly, without previous composting [87]. In contrast, low P

and N fertilization are favorable for the establishment of an AMF symbiosis. Moreover, a low-input cropping system in which legume species are integrated in the crop rotation without any mineral N supply or with half the recommended rate, boost the contribution of AMF to both soil quality and plant productivity [17,87]. How such reduced mineral fertilizer availability, notably N, stimulates AMF biological activities is not known. Paradoxically, soils containing a high organic N content promoted both root colonization by AMF and N transfer to *Plantago lanceolata* plants by means of the mycelial network [63]. Therefore, the type of N fertilization is an important factor that controls both AMF colonization and biological activity in terms of N uptake efficiency [34]. Using the controlled experimental systems described in Figure 1 [34, 35], it will be interesting, to study the physiological and regulatory mechanisms that control N uptake by the fungi and its transfer to the host plant when the mineral N is low, or when there are high concentrations of amino acids or carbohydrates present in the growth medium.

Tillage is another important parameter that influences the interactions between the host plant and the soil microbiota, notably when there is a tripartite symbiosis between legumes, Rhizobiaceae and AMF [18]. A number of nodulated N₂-fixing legumes such as *Medicago sativa* or *Phaseolus vulgaris* when cultivated in undisturbed soil, were colonized faster by AMF compared to those grown in plowed soil. In such tripartite symbioses, AMF is the most demanding symbiotic partner in terms of C supplied by the host plant. Therefore, the N necessary for building up the photosynthetic machinery provided by the symbiotic Rhizobiaceae, must be efficiently assimilated by the host plant. The C supplied by the *Phaseolus vulgaris* plant allows a rapid growth of the AMF that in return efficiently supplies P to the nodulated legume [88]. Therefore, to increase legume N utilization and thus plant productivity, cropping systems that limit the physical disturbance of the soil and that stimulate both AMF colonization and N uptake, must be encouraged (Fig.2). In addition, exploiting the

genetic diversity of a given legume species will be a way to find the most efficient tripartite symbiotic partners for both N and P acquisition.

3.3 Agronomic importance of the diversity of arbuscular mycorrhizal fungi

The view of a one fungus–one plant mycorrhizal association does not hold ecologically, since most plant roots are colonized by multiple fungi that are most of the time not host-specific, as they can simultaneously colonize different plants forming a mycorrhizal network [37]. Selection of the appropriate AMF species and of their complementary effects contributed to the improvement of biomass production of *Trifolium pratense* and *Lolium multiflorum* by up to 85% in comparison to the inoculation with a single fungal species [89]. These results also indicate that below-ground diversity may act as an insurance for maintaining plant productivity under differing environmental conditions [89]. For example, it has been shown that the two fungi *Rhizophagus intraradices* and *Claroideoglossum claroideum* can simultaneously colonize *Medicago truncatula* and *Allium porrum* plants thus increasing plant productivity more than if only one fungus was present [90]. In addition, the separate foraging properties of the different fungal species reduce the competition for nutrients between individual host plants, which further enhances the complementary effect of AM fungal communities [91]. Therefore, inoculating plants with several AMF species will be well worth testing either under controlled conditions (see section 2 and Fig.1) or in the field using different tillage systems under low and high N fertilizer inputs, which can range from no additional mineral N fertilization to the optimal recommended rate for a given crop, which is on average around 180kgN ha⁻¹.

However, agricultural soils are generally dominated by the presence of only a few AMF taxa belonging to the order *Glomerales* [92]. The intensive farming systems currently used worldwide induce over several years a specific [93] and persistent selection pressure [94]

on the structure of the AMF community which can lead to a competitive dominance by only a few species. Such AMF species can be transferred from plant to plant plants through fragmented hyphal networks and colonized roots pieces, thus indicating that intensive tillage can favor such species [95]. Therefore, more work is required to evaluate the impact of different tillage regimes on the diversity of AMF communities. It is possible that tillage by modifyng the distribution, of the fungal communities in the soil, favors the trophic interactions between different species. Whether soil organic matter content under different tillage regimes has an impact on both AMF colonization and on the composition of AMF communities also remains to be determined, although it has been shown that organic C content does not markedly modify the AMF spore populations [92].

Using cover crops containing a mixture of different plant species can supply a broad spectrum of potential hosts that are able to enhance AMF diversity in soils and to favor their complementary effects. The simultaneous use of no-till and cover crops increased the AMF spore density and species richness both in the upper and lower clay soil layers and 33 AMF species were found under this management practice [96]. Therefore, improving our knowledge of the complex interactions occurring within the AMF community with respect to the recognition and the colonization of a single host plant is essential. This should also be carried out with a population of host plants, in order to implement efficient agricultural management strategies to stabilize or increase plant productivity using a minimum of inputs ranging from no additional fertilization, 50% and up to 75% of the recommended rate. This can be achieved by estimating the functional diversity of the AMF species that are found in agricultural soils with different management practices and by measuring their impact on plant productivity [97].

4. Improving plant N uptake and utilization with arbuscular mycorrhizal fungi

The ability of AMF to increase nutrient uptake through the hyphal network offers an interesting strategy to limit the rather inefficient use of applied N by crops. AMF could play a useful role in the recycling of soil N by varying the availability of soil mineral N, through changes in the composition of the rhizosphere microbial community by modifying the development of denitrifying, nitrifying and diazotrophic symbiotic or free-living bacteria. This could be due to the changes in resources and abiotic conditions as well as the generation of allelopathic interactions mediated by the AMF [98]. However, the potential effects of AMF on the neighboring bacterial community is a very complex process and more research is required to evaluate the importance of these effects on soil N recycling and if they have a significant impact on plant N nutrition. Modeling studies [99] could be a way to gain a system-level understanding of the role of AMF and nutrient availability on bacterial diversity in soil ecosystems.

In the last two decades, numerous investigations describing the impact of AMF on grasses have been carried out. However, in many cases it has proved difficult to obtain a general consensus concerning the mode of N management of the symbiotic association. This is probably because very different experimental systems and symbiotic associations were used, encompassing a wide variety of different plant and fungal species and even plant genera originating from different habitats [100]. Studies need to be first conducted in an appropriate microcosm and well-controlled experimental systems involving one fungal species and one plant partner, such as those presented in section 2 (Fig. 1), [34, 35] using increasing N concentrations in order to understand the mechanisms of C/N exchange in AMF [100]. Using such a kind of experimental systems, it will be possible to assess the benefit of a given plant/fungal symbiotic association and then to select and study the best ones by means of holistic, physiological and systems biology approaches including modeling studies [99]. Changes in plant gene expression, enzyme activities and physiological traits following AMF

colonization could then be studied under various N nutritional conditions as already performed under drought stress conditions [101]. Such investigations could be extended to large-scale biology studies similar to those recently used to link plant physiology to yield in maize [102].

In some recent investigations performed under semi-controlled conditions in a mineral N-rich soil and when atmospheric CO₂ was increased twice, a positive impact on wheat N uptake, N accumulation and NUE has been reported in the presence of AMF [103]. Such important findings highlight the role that AMF could play for enhancing NO₃⁻ uptake in response to the current CO₂ increase in the atmosphere [104].

Residual, organic material present in the soil was also able to increase hyphal growth, independently of the host plant, which probably explains why in the absence of tillage and of N fertilization, AMF development was improved [17] as well as fungal spore density [105]. Moreover, N uptake and aboveground biomass production by the plants did not vary significantly between tilled plots fertilized with mineral N at the recommended rate and no-till plots that had not been fertilized with mineral N (Figure 4). No-till farming is thus a sustainable agricultural system that can allow, by promoting organic nutrient utilization, gradual reduction in N fertilizer use. by promoting AMF growth, whilst at the same time increasing NUE [17, 106]. There is therefore a strong potential for developing the use of micorrhiza in sustainable agriculture and a need to further develop appropriate technologies adapted to conservation agriculture for many crops and in various environments and temperate and tropical climatic conditions [107]. However, to optimize the use of these technologies, it will be necessary to evaluate the optimal rate of N fertilizers inputs reduction depending on the cultivated crop, the previous crop rotation and determine if the presence of cover crops improves crop NUE and yield.

The beneficial effect of AMF inoculation using several fungal species on N uptake was recently highlighted in a meta-analysis of more than 300 field experiments. The data showed that field inoculation could be profitable for large-scale agronomic applications [108], whilst in another recent study it was shown that the genetic and functional diversity of AMF must be taken into account [109]. High, medium and low performance fungal isolates were distinguished in terms of biomass production and both N and P nutrition, thus indicating that the adaptability of the fungus to the host plant plays an important part in the symbiotic performance of both partners. In a similar way to P transport [110], it will be important to establish whether plant genetic variability and AMF genotype interactions are important determinants for the mineral and organic N uptake systems both on the fungi and host plants. These will be key issues for both understanding the genetic basis of NUE under no-till conditions and when N fertilization is lowered. Such studies can be developed using a quantitative genetic approach similar to that conducted to identify chromosomal regions and genes involved in the control of NUE in maize [111].

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Captions

Fig. 1. Two possible controlled systems to study interactions between plants and AMF. (A) Microcosm in which an individual plant is colonized by an AMF within one compartment of the microcosm, and a ^{15}N -labelled organic matter patch is placed into an adjoining patch compartment (shown in blue). Half of the microcosm units contain a $0.45\ \mu\text{M}$ mesh (vertical gray dotted line) to prevent the roots crossing from the plant compartment to the patch compartment. Adapted from [34]. (B) Gnotobiotic system allowing the inoculation of plants such as maize with AMF under sterile conditions. The glass bottle can be closed with a rubber stopper allowing injection of gases or of ^{15}N -labelled or unlabelled compounds to the growth medium as well as measuring N_2 fixation using the acetylene reduction assay. The growing substrate is composed of clay beads allowing an easy recovery of the roots and of the fungal hyphae. Adapted from [35].

Fig. 2. Common bean roots (*Phaseolus vulgaris* L.) colonized by arbuscular mycorrhizal fungi. (A) Hyphae colonizing the roots (black arrow). (B) Vesicles for nutrient storage (white arrow). (C) Arbuscules for nutrient exchange (black arrow). (D) Non-colonized roots. Roots were stained by trypan blue. The scale bar is the same in panel A, B, C and D.

Fig. 3. Conceptual representation of relationships between agricultural practices and AMF in soils, leading to either efficient or inefficient plant/fungal symbioses. Agricultural practices are displayed on the figure center with A: Low N and P fertilization, B: Conventional high N and P fertilization, C: No-till system, D: Conventional moldboard plowing, E: Winter cover cropping system including leguminous species in plant mixtures and F: Winter bare fallow. The beneficial (green left side) or deleterious (red right side) effects of the above listed practices on the establishment of AMF in soils are schematically summarized in 1:

Interconnected mycorrhizal plants (main crops and cover crops) in hyphae networks, 2: AMF propagule density and diversity, 3: Hyphae branching and growth, 4: Root colonization by AMF leading to symbiosis. The green left panels show that the AMF-related traits 1, 2, 3 and 4 are all higher under low N and low P fertilization inputs under no-till conditions in the presence of cover crops.

Fig. 4. Effect of tillage (till) and nitrogen (N) fertilization on the percentage of wheat root length colonized by arbuscular mycorrhizal fungi (AMF) and on plant N uptake. A field experiment performed over a 5-year period demonstrated that both the absence of tillage and of nitrogen (N) fertilization improved AMF colonization of wheat roots (bars). This was due to the presence of organic material in the soil efficiently that was efficiently used by the AMF as under no-till conditions. N uptake is represented in dotted lines (---■---). Aboveground biomass production was 7.13 ± 0.65 in No-Till-N, 6.07 ± 0.30 in No-till+N, 4.88 ± 0.27 in Till-N and 7.02 ± 0.43 in Till+N. The +N plots were fertilized with 160 kg N ha^{-1} , whereas the -N plots were not fertilized. Mineral N already present in the soil before sowing was 17 kg N ha^{-1} . N uptake and aboveground biomass production did not vary significantly between N-fertilized and N-unfertilized plots. In contrast, both N uptake and above ground biomass were 30% lower when mineral N fertilizers were not added during conventional tillage due to the reduction in AMF colonization. Values are the mean of four replicated plots for each of the four treatments (see 17 and 105 for experimental details).





