



Deciphering Nitrogen Fixing Role Of Various Fungal Species – A Study

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Article History	Abstract
Received: 30/09/2023 Revised: 15/10/2023 Accepted: 30/10/2023	<p>Nitrogen-fixing fungi play a key role in the ecosystem by helping to provide plants with the nitrogen they require to survive, improving soil quality, and reducing the need for synthetic fertilizers. They have the potential to be used as biofertilizers. Fungi like <i>Pleurotus</i> sp. are reportedly capable of fixing nitrogen (N₂). It would seem that only a very small number of free-living fungal species have the ability to fix nitrogen. Some examples of nitrogen-fixing fungi include <i>Azotobacter</i>, <i>Clostridium</i>, and <i>Frankia</i>. Arbuscular mycorrhizal fungi (AMF) and Lichens are symbiotic nitrogen fixing fungi. Among the higher plants mycorrhizal infections of the roots are exceedingly common, endotrophic mycorrhiza obtain their energy supply from the exudate of the host plant, and indirectly from sunlight. It would seem very probable that if they possess the ability to fix nitrogen, the quantity fixed may be of considerable economic importance. However, it may be concluded that the efficiency of nitrogen fixation greatly depends on phosphorus availability. Since the vesicular arbuscular endophytes are efficient in phosphorus uptake, the vesicular arbuscular-mycorrhizal fungi may play an important role for associative-symbiotic nitrogen fixation, especially in phosphorus deficient soils. The review article mainly focuses on the ability of various fungal species to fix nitrogen and the probable mechanism of the phenomenon.</p> <p>Keywords: <i>fungal strains, nitrogen fixation, enzymes, symbiotic, soil microbe, mechanism</i></p>
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INTRODUCTION:

The soil microbes plays a key role in determining the productivity and structure of plant communities. The productivity in plant is generally higher in communities with Arbuscular Mycorrhizal Fungi than in communities without Arbuscular Mycorrhizal Fungi. Other advantages of Arbuscular Mycorrhizal Fungi, such as pathogen resistance, may also contribute to this boost in productivity apart from increased phosphorus uptake. The species *Piptadeniagonoacantha* found in southern and southeastern Brazil, is economically and

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socially useful because it is used in the energy, cellulose, and paper sectors, among other fields. This species is also used considerably in degraded site restoration projects because it can biologically fix nitrogen. (Júnior, 2016). Plant mutualisms provide essential services and resources, from pollination to nutrient supply, drought resistance, and pathogen protection. One important group of mutualists that associate with plants is nitrogen-fixing bacteria. Finally, as many plants that associate with nitrogen fixing bacteria also associate with mycorrhizal fungi, it is possible that filters from these two symbionts act non-additively in limiting plant establishment on islands. Synergistic limitation, where plant species that associate with both types of symbionts are less likely to colonize islands than expected from the two filters individually, may occur given that synergistic effects on plant growth has been found for co-inoculation with mycorrhizal fungi and N-fixing bacteria (Delavaux, 2022). However, meta-analyses suggest that synergistic impacts on plant growth may be limited to perennial plants, which may reduce the likelihood of finding synergistic limitations on island colonization. To date, the possibility of a plant island colonization filter strengthened by multiple mutualisms has not been tested. In particular, recent work suggests that biogeographical patterns of plants are consistent with the dispersal limitation of mycorrhizal fungi, particularly arbuscular mycorrhizal (AM) fungi, filtering out Arbuscular Mycorrhizal plants species on islands (Delavaux, 2022). Among the true fungi, the genus *Aspergillus* and, to a lesser extent, *Penicillium* have received the greatest attention. Certain fungi capable of generating mycorrhiza on the roots of higher plants have also received considerable interest. (ALLISON, 1934). Arbuscular mycorrhizal fungi (AMF), which belong to the phylum *Glomeromycota*, have been shown to provide several benefits to their host plants, including increased nutrient uptake, increased plant tolerance, and stimulation of carbon (C) and mineral nutrient cycles (Zhou, 2023).

MATERIALS AND METHODS:

The right information for this review article was found by searching in PubMed, PubMed Central, Google Scholar, ResearchGate, and Google for published research works on Nitrogen fixing role of various fungal species. These research works took the form of original studies and review articles from all over the world. Only data that have been published were considered. Information acquired from reputable sources of publications on the subject is one of the inclusion criteria. Other languages were not included in the study.

DISCUSSION

Nitrogen Uptake By Arbuscular Mycorrhizal Fungi:

Arbuscular Mycorrhizal plants roots obtain nutrients in two ways: directly through the root epidermis and root hairs, or through symbiotic interfaces provided by arbuscules or hyphal/ arbuscule coils generated inside the root cortical cells. It has been proposed that mycorrhizal symbiosis is responsible for nitrogen uptake by plants regardless of the host type with which they are linked. (Javaid, 2010). Nitrogen uptake by Arbuscular Mycorrhizal fungus can be classified into two types:

- (1) organic N uptake and
- (2) inorganic N uptake.

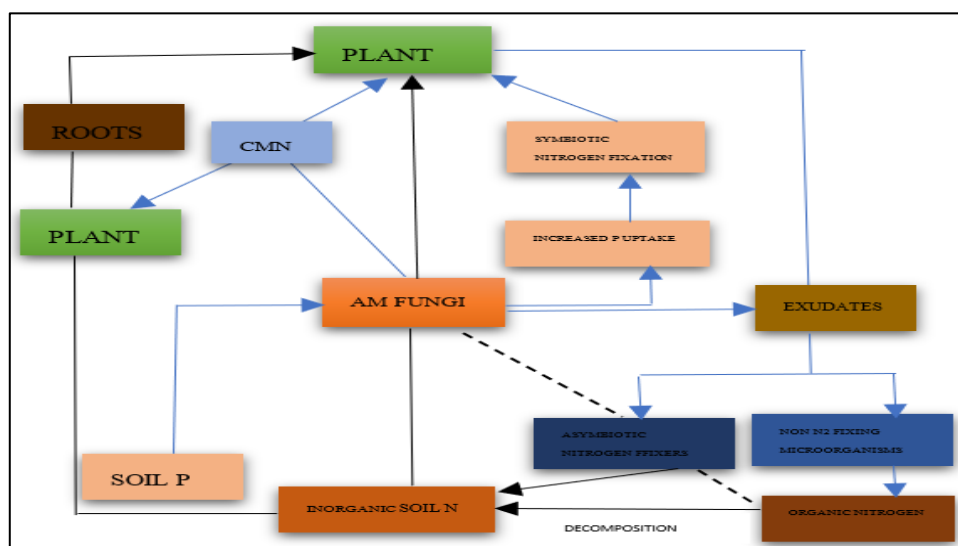


Fig 1: Role of arbuscular mycorrhizal (AM) fungi in direct and indirect nitrogen (N) nutrition of plants

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- Direct uptake and transfer of soil Nitrogen by plant roots or the extraradical hyphae of Arbuscular Mycorrhizal fungi
- Increased availability of inorganic nitrogen in the soil due to the enhanced decomposition of organic nitrogen and/or dinitrogen (N₂) fixation by symbiotic microorganisms
- Improved symbiotic nitrogen fixation in leguminous and actinorhizal plants through improved plant phosphorus (P) nutrition.
- Interplant transfer of nitrogen between plants by common mycelial network (CMN).
- As the direct decomposition of organic matter by Arbuscular Mycorrhizal fungi is not well resolved it is shown by broken line arrow.

Mechanism:

Emergence of AM fungus mycelia from the root system enables acquisition of minerals and nutrients from the soil which are rather unreachable to roots (Smith et al. 2004; Berruti et al. 2016). However, it is the extra-radical hyphae of AM fungi that regulates the uptake of nutrients specifically N and P to the plants (George et al. 1995). The hyphae are very thin as compared to the roots of plants and thus they easily penetrate into minute pores (Allen 2011). The exchange of minerals, nutrients, and carbohydrates within the roots occurs at the interface of host plant and the fungal hyphae (Berruti et al. 2016).

The colonization of mycorrhizal fungi in turn modifies the characteristics of plants which play a role in nutrient uptake and thus, it impacts the accumulation of nutrients by the plants associated with mycorrhiza. The modifications include enhanced nutrition of phosphorus to mycorrhizal plants or might also be Phosphorus independent. However, the most common impact post colonization of mycorrhiza is modification of the ratio of root and shoot (George 1994a,1994b) and the specific length of roots (George et al. 1995).

Colonization of the root cortex by the AM fungal hyphae and formation of highly branched edifices within the cells, i.e., arbuscules, which are deemed to be the operative site of exchange of nutrients (Balestrini 2015; Berruti et al. 2016). The colonization of mycorrhiza enhanced the percentage of amino acids and organic acids within the roots and the shoots of *Phleum pratense* (Clapperton and Reid 1992) while few amino acid concentrations were increased in *Plantago lanceolata* (Gange and West 1994; George et al. 1995). Emergence of AM fungus mycelia from the root system enables acquisition of minerals and nutrients from the soil which are rather unreachable to roots (Smith et al. 2004; Berruti et al. 2016). However, it is the extra-radical hyphae of AM fungi that regulates the uptake of nutrients specifically N and P to the plants (George et al. 1995). The hyphae are very thin as compared to the roots of plants and thus they easily penetrate into minute pores (Allen 2011). The exchange of minerals, nutrients, and carbohydrates within the roots occurs at the interface of host plant and the fungal hyphae (Berruti et al. 2016).

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The emergence of Arbuscular Mycorrhizal fungal mycelia from the root system allows for the uptake of minerals and nutrients from the soil that are unreachable to roots. However, the uptake of nutrients, particularly Nitrogen and Phosphorus, by the plants is regulated by the extra-radical hyphae of Arbuscular Mycorrhizal fungus (Vats, 2021). Because hyphae are much thinner than plant roots, they can easily enter minute pores. The interface between the host plant and the fungal hyphae is the region where minerals, nutrients, and carbohydrates are exchanged within the roots. The colonization of mycorrhizal fungi, in turn, affects the properties of plants that play a role in nutrient intake, influencing the nutrient accumulation by plants connected with mycorrhiza. The modification includes improved phosphorus nutrition for mycorrhizal plants or could even be phosphorus independent. The most prevalent change following mycorrhizal colonization, are the changes in the ratio of roots to shoots and the specific length of roots. The colonization of the root cortex by the Arbuscular Mycorrhizal fungal hyphae and the production of highly branched edifices within the cells, i.e., arbuscules, are thought to be the operative site of nutrient exchange. Mycorrhizal colonization enhanced the percentage of amino acids and organic acids in *Phleum pratense* roots

and shoots, while only a few amino acid concentrations were increased in *Plantago lanceolata*. Therefore, it was believed that the colonization of mycorrhiza had no direct impact on N metabolism. demonstrated that more than 50% of the plant's nitrogen requirements were met by mycorrhiza's interaction with the root system of the plant. Arbuscular fungus can use nitrogen that is released from both organic and inorganic sources. During mineralization, the AM fungus enters plant tissues through vascular bundles and use nitrogen released by soil microbes (Vats, 2021).

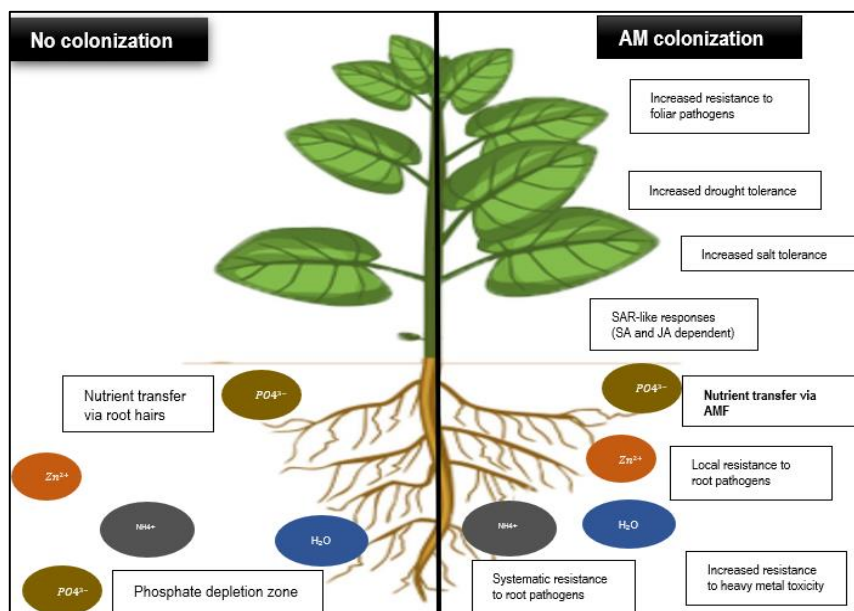


Fig 2: Positive Effects Of Arbuscular Mycorrhizal (AM) Colonization

Arbuscular mycorrhizal fungi (AMF) have a hyphal network that extends beyond the depletion zone, allowing them to access a larger region of soil for phosphate uptake. A zone of mycorrhizal phosphate depletion will form surrounding Arbuscular Mycorrhizal hyphae. Nitrogen (ammonium) and zinc are two more nutrients that have been shown to improve assimilation in Arbuscular Mycorrhizal roots. Tolerance to numerous abiotic and biotic stresses can be gained by colonization through induction systemic acquired resistance.

Fungi As Symbiont:

Fungi can denitrify in both symbiotic and nonsymbiotic ways. Mycorrhizae are the structures formed by fungi and plant roots association, that improves the plants ability to absorb nutrients gradually from the soil. Arbuscular Mycorrhizal Fungi has a limited ability to acquire organic nitrogen but can boost plant nitrogen uptake. However, the nitrogen that mycorrhizal plants obtain from organic matter is doubled by the synergistic interaction between Arbuscular Mycorrhizal Fungi, *Rhizophagus irregularis*, and soil microbial communities. When compared to non-mycorrhizal plants grown in the absence of soil microbial communities, the increase in nitrogen uptake is ten times greater (Singh, 2021). Arbuscular Mycorrhizal Fungi linked with plants is destroyed by intense agriculture practices. The symbiotic relationship between Arbuscular Mycorrhizal Fungi and plants has potential for commercial use as a biofertilizer in place of chemical fertilizers. These biofertilizers improve the nitrogen and organic content of soil and plant nutrition absorption (total N, P, and K). An animal-fungus interaction has showed Nitrogen fixation activities in recent study. Nitrogen fixation in the ant-microbe symbiosis of the leaf cutter ant is an example of a completely novel nitrogen source in neotropical ecosystems (Pinto-Tomas et al. 2009). Another example of nitrogen fixation is the symbiotic relationship between termites and fungus, in which termites meet their nitrogen needs by utilizing the atmospheric Nitrogen fixing capacity of diazotrophic bacteria. This is expected to eliminate the need for bacterial symbionts to fix Nitrogen. As a result, fungi play a distinct and diversified role in the nitrogen cycle.

Enhanced Phosphorus Nutrition:

Plants have evolved intricate methods to promote Phosphorus uptake in soil to overcome unfavourable situations by creating symbiotic partnerships with Arbuscular Mycorrhizal fungi. It is now well documented that mycorrhizal colonization can improve plant root Phosphorus absorption. Increasing the amount of

fertilizer available Phosphorus frequently increases nitrogen-fixation, resulting many to believe that the role of Arbuscular Mycorrhizal fungi in nitrogen-fixation enhancement is to improve host Phosphorus nutrition . (Javaid, 2010) .The Phosphorus content of nodules of mycorrhizal plants is often higher than that of non-mycorrhizal plants, which may explain why mycorrhizal legume plants have higher nitrogen-fixing activity. Additionally, different species of Arbuscular Mycorrhizal fungi have distinct effects on the growth and nitrogen-fixation responses of legumes.

Significance Of Fungus In Nitrogen Fixation:

Fungi not only have a beneficial symbiotic connection with plants, creating mycorrhiza with the roots, but also play a part in the nitrogen cycle through denitrification and nitrogen fixation. The nitrogen dioxide reductase gene (nosZ) is found in denitrifying soil bacteria and transforms N₂O to N₂. Despite the fact that fungi lack this gene, they can execute denitrification and co-denitrification in a variety of soils by utilising the nitric oxide reductase cytochrome p450nor and Nirk genes. As a result, fungi produce N₂O as an end product of denitrification rather than Nitrogen as bacteria do. Thus, denitrification and co-denitrification, dominated by soil fungi, result in the release of organic and inorganic nitrogen to the atmosphere in the form of nitrous oxide and nitrogen gas, which maintain the nitrogen cycle (Singh, 2021). Therefore, it can be inferred that many of the fungal species play a vital role in the release of biological nitrogen in the forms of Nitrogen and Nitrous oxide gases into the atmosphere, in addition to the synergistic relationship of mycorrhiza which gives phosphorus to the soil microbiome. Fungi serve as a nitrogen cycle regulating unit.

MICROORGANISM	ROLE IN NITROGEN CYCLE	RELATIONSHIP
<i>Fusarium oxysporum</i>	Denitrification	Commensals/symbiotic/pathogenic
<i>Fusarium solani</i>	Co-denitrification	Non-symbiotic/pathogenic
<i>Bipolaris sorokiniana</i>	Denitrification and co-denitrification	Non-symbiotic/pathogenic
<i>Cylindrocarpontokinense</i>	Denitrification	

Table 1:- List of fungi and their role in Nitrogen cycle

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CONCLUSION

This study expands our understanding of Arbuscular Mycorrhizal Fungi community and their relationships with diazotrophs and their potential impacts on biological nitrogen fixation. *P. gonoacantha* is extremely reliant on Arbuscular Mycorrhizal Fungi, particularly for nodulation. Arbuscular mycorrhizae have the potential to significantly boost legume crop growth, yield, nodulation, and nitrogen fixation. The efficacy of a composite inoculation of symbiotic rhizobia or asymbiotic free-living nitrogen fixers and Arbuscular Mycorrhizal fungi largely dependent on the compatibility of the interacting partners. Furthermore, Arbuscular Mycorrhizal fungi can improve the nitrogen fixing effectiveness of free-living diazotrophs in the rhizosphere of legumes. It is also found that the Arbuscular mycorrhizal fungi community can influence the nitrogen fixation process in mangrove ecosystems by controlling microbial extracellular enzyme activity and altering the microenvironment of the mangrove rhizosphere. Furthermore, Arbuscular Mycorrhizal fungi and diazotrophs produced mostly positive co-occurrences, which may increase nitrogen fixation efficiency in

mangrove habitats. The nitrogen uptake structures of fungi have huge affinity for nitrogen uptake through the soil but the regulation of the expression of nitrogen transporters of Arbuscular Mycorrhizal fungi is still unspecified. As a result, further more field research are needed to determine their significance for plant growth and maximize the benefits of such natural affordable resources.

Conflict of Interest: There is no conflict of interest declared by the authors.

Author Contributions: Acquisition and interpretation of data is done by Uzma Juned. Conception, design and revising of the article are done by Dr. Pritha Pal.

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