

Distinctive Arbuscular Mycorrhizal Fungi Communities in the Rhizosphere of Selected Tropical Trees Grown in an Arboretum

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Abstract

This study was conducted at the Forestry Arboretum of the University of Port Harcourt Teaching and Research Farm, to assess Arbuscular Mycorrhizal Fungi community characteristics in some selected tropical trees. Soil and root samples were collected from the Rhizosphere of these trees at and transferred to the laboratory for extraction and identification and percentage colonization of AMF. Results showed that *Nauclea diderrichii* had the highest AMF population (345.0g/dwt) followed by *Anona muricata* (310.0g/dwt) while *Irvingia gabonensis* had the lowest (165.8g/dwt) AMF population. *Gmelina arborea* was observed to have the highest percentage AMF colonization of 36.17% while *Irvingia gabonensis* had the lowest percentage AMF colonization of 10.76%. A total of six AMF genera were identified from the rhizosphere of the selected trees namely; *Acaulospora*, *Rhizophogus*, (*Glomus*), *Scutelospora*, *Gigaspora*, *Entrophospora* and *Funnelformis*, of which *Rhizophagus* was the highest occurring under all the trees with values ranging from 82.8 – 160.6g/dwt followed by *Acaulospora* (50.6 – 165.2g/dwt). The least occurring AMF genera was *Entrophospora* (0 – 1.6g/dwt). The results indicated that AMF can establish effective symbiotic relationship with these tree species and this symbiotic interaction is beneficial to the development of the trees.

Keywords: Arboretum, Arbuscular Mycorrhizal Fungi, Tropical Trees, Rhizosphere

INTRODUCTION

A flourishing soil is home to a wide population of microorganisms, such as fungi, bacteria, and archaea (Chen *et al.*, 2018). These microorganisms play a major role in decomposition processes, nutrient cycling, and plant growth promotion (Harrier and Watson, 2016). Additionally, soil microbes such as Arbuscular Mycorrhizal Fungi (AMF) are in charge of the biological fertility of the soil (Chen *et al.*, 2018) and play a significant role in determining soil performance by breaking down plant residues and materials to raise the soil's organic matter content and enhance soil quality (Rillig *et al.* 2019).

The majority of AMF species are members of the Glomeromycotina subphylum of the Mucoromycota phyla, which is made up of numerous orders and 25 taxa, including the Glomerales, Archaeosporales, Paraglomerales, and Diversisporales (Spatafora *et al.*, 2016). The unique symbiotic association that fungi have with higher plants is known as mycorrhiza, and according to fossil evidence, arbuscular mycorrhizal associations first appeared between 400 and 450 million years ago (Barwant *et al.*, 2025).

Arbuscular Mycorrhizal Fungi are soil-borne fungi that can considerably improve plant nutrient uptake and resilience to numerous abiotic stress conditions (Sun *et al.*, 2018), and as obligatory biotrophs, they complete their life cycle by consuming lipids and photosynthetic products from trees (Jiang *et al.*, 2017). In the

rhizosphere, AMF produce spores and hyphae, and when AMF and plant roots build a hyphal network, it greatly increases the roots' access to a vast soil surface area, which improves plant growth (Bowles *et al.*, 2016). Also, in order to improve stress tolerance, AMF can control the bacterial and fungal makeup of the rhizosphere and cultivate specific advantageous microorganisms (Zhu *et al.*, 2016), and by colonizing roots and forming a bond with the substrate, AMF inhibits root infections, increases food availability, and produces plant growth hormones (Barwant *et al.*, 2025).

The most complex microhabitat is unquestionably the rhizosphere, which is made up of soil, plant roots, and a diverse range of bacteria, fungi, eukaryotes, and archaea (Hakim *et al.*, 2021). It is commonly recognized that tree species and the rhizosphere have an impact on the biomass, activity, and communities of live microorganisms associated with trees (Lladó *et al.*, 2018; Bardgett and Caruso, 2020). Trees affect the characteristics of soil through a number of mechanisms, including root penetration, the addition of organic matter through root litter, and the exudation of ions and organic compounds, (De Gannes *et al.*, 2016). Trees can also alter the soil microbial composition and/or community organization by modifying the litter chemistry and site microclimate (Bardgett and Caruso, 2020). Additionally, they can impact the surrounding soil by secreting or releasing a variety of substances known as rhizodeposits, which are primarily made up of organic

acids, carbohydrates, secondary metabolites, and amino acids (Bardgett and Caruso, 2020).

Furthermore, trees have a substantial impact on the population, composition, and structure of soil organisms because of their capacity to enhance biotic and abiotic stress resistance, encourage plant growth, remediate contaminated soils, recycle nutrients, regulate soil fertility and weather, mineralize rock, and carry out other functions that lessen the need for fertilizers and pesticides in agriculture (Hakim *et al.*, 2021). Tree diversity and abundance also serve as ecological factors to assess soil health and productivity (Bardgett and Caruso, 2020).

Several studies have generally indicated that Arbuscular Mycorrhizal Fungi can improve soil quality and enhance plant growth and productivity (Chen *et al.* 2018; Rillig *et al.* 2019). However, knowledge of the diversity of AMF associated with tropical trees species in planted forest is scanty and there have been relatively few studies on AMF colonization in tree species especially in the Arboretum in Port Harcourt. Hence, this research assessed AMF community characteristics and association with selected tropical trees grown in an arboretum.

MATERIALS AND METHODS

Study Area

The study area was Port Harcourt metropolis, which is situated in the humid rainforest region of southern Nigeria, and lies between latitude 4.5°N and longitude 7.0°E on an elevation of 18m above sea level. This research was conducted at the Faculty of Agriculture, Teaching and Research Farm at the Forestry Arboretum unit. The Arboretum was started in the year 2011, it covers a total area of 15,996.90m with several trees stands of various species identified by a name tag (Eludoyin *et al.*, 2015).

Selection of Sample Trees

Five tropical tree species [Teak (*Tectona grandis*), Soursop (*Anona muricata*), White Teak (*Gmelina arborea*), African Peach (*Nauclea diderrichii*) and Bush Mango (*Irvingia gabonensis*)] were selected for this study.

Soil and Root Sampling

Soil and root Sampling was conducted in May 2023 and consisted of collecting soils and root samples from the Rhizosphere of each tree species. Soil and root samples were collected from a depth of 0 to 30cm, using a hand trowel and a spade. A total of 25 samples were collected, 5 samples from the Rhizosphere of each of the 5 tree species. Samples were collected and placed into properly labeled polythene bags and transferred to the laboratory for analysis.

Extraction of AMF Spores

The AM fungal spores were separated from the soil by wet sieving and decanting technique. 50g of rhizospheric soil sample was mixed in 200ml of distilled water in a large beaker. After 1hr, the contents of the beaker were decanted through sieves which were arranged in a descending order from 200µm to 25µm size. The process was repeated thrice, until the upper layer of the soil suspension was transparent. The retained material on the sieve was decanted into a beaker with a stream of water and estimation of spores was done.

Identification of Mycorrhiza Fungi

Identification of AMF spore was performed by morphological observation of color, shape, size, hyphal attachment, spore ornamentation and spore reaction towards Meizer's solution (Schubler and Walker, 2010). Spore enumeration was conducted under a stereo microscope and spore identification was conducted under a microscope with 200x magnification.

AMF Root Colonization

For the analysis of mycorrhizal colonization in the plants, the root samples were washed free of soil and cut into 1cm long bits, cleared in 2.5% KOH at 90°C for 20-30 minutes, rinsed in water, acidified with 5N HCL and stained in lactophenol containing 0.05% trypan blue. 50 segments approximately stained root samples were mounted on slides and examined for AM colonization under a compound microscope at 10 x 10 Magnification, and percent root colonization was calculated (Dhar and Mridha, 2012). Percent root colonization was determined using the following formula:

$$\% \text{Root colonization} = (\text{No of positive segments} \div \text{No of segments observed}) \times 100.$$

Statistical Analysis

Data collected from the various parameters were subjected to analysis of variance (ANOVA) at $P \leq 0.005$, and Mean difference was separated using least significant difference at 5% using Gen-stat package.

RESULTS

AMF Population/Spore Numbers

Results on the total Arbuscular Mycorrhizal Fungi population and spore numbers in Rhizosphere soils of the selected tree species are presented on Figure 1. AMF population ranged from 165.8-345.0g/dwt under the trees selected. Highest spore number (345g/dwt) was recorded in the rhizosphere of *Nauclea diderrichii*, while the lowest spore number was recorded in the rhizosphere of *Irvingia gabonensis*. AMF spore numbers were significantly different ($P \leq 0.05$) under the selected tree species (Figure 1) and followed the order; *Nauclea diderrichii* > *Anona muricata* >

Tectona grandis > *Gmelina arborea* >
Irvingia gabonensis, respectively.

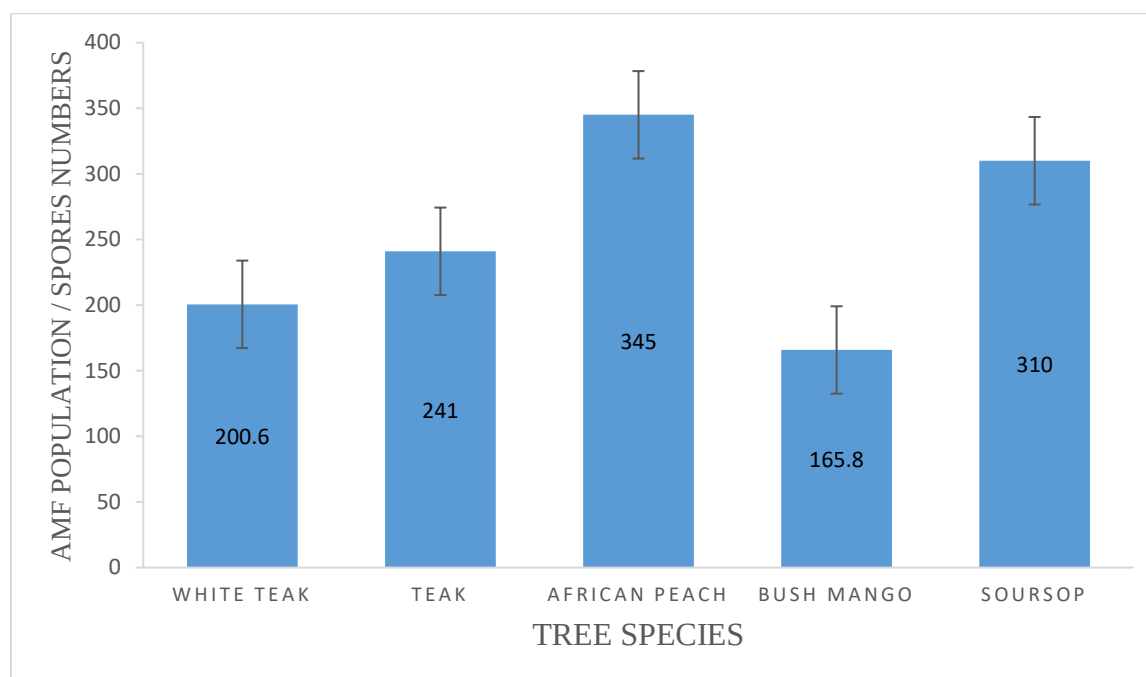


Figure 1: AMF Total Spore Numbers (100g/dwt) Under the Selected Species.

AMF Diversity

Results on the individual mycorrhizal fungi species identified from the rhizosphere soils of the selected trees species is presented on Table 1. A total of six Arbuscular Mycorrhizal Fungi morphological types were identified belonging to six different genera, these include *Acaulospora*, *Rhizophogus*, *Scutelospora*, *Gigaspora*, *Entrophospora* and *Funnelformis* (Table 1). *Rhizophogus* was observed to have the highest population of AMF spores under all trees species ranging from 111.8-

160.6g/dwt, except under *Anona muricata*, where *Acaulospora* was 165.2 spores/100g of soil. *Entrophospora* was almost absent under all trees species except under *Gmelina arborea* where 1.6 spores/100g of soil was recorded (Table 1). The Mycorrhiza genera for spore abundance spores/100g of soil followed the order; *Rhizophogus* > *Acaulospora* > *Funnelformis* > *Scutelospora* > *Gigaspora* > *Entrophorspora*.

Table 1: Individual AMF Species Population and Diversity under the various Tree species

Tree Species	<i>Acaulospora</i>	<i>Rhizophagus</i>	<i>Scutelospora</i>	<i>Gigaspora</i>	<i>Entrophospora</i>	<i>Funnelformis</i>	Total Spore 100g/dwt
White Teak (<i>Gmelina arborea</i>)	51.4c	115.4b	0.0	0.4b	1.6a	33.8b	200.6b
Teak (<i>Tectonagran dis</i>)	58.2c	132.0b	7.4b	2.2b	0.0	41.2a	241.0b
African Peach (<i>Naucleadide rrichii</i>)	85.4b	160.6a	26.6a	22.6a	0.0	49.8a	345.0a
Bush Mango (<i>Irvingiagabo nensis</i>)	50.6c	82.8c	0.6b	0.8b	0.0	31.0b	165.8c
Soupsop (<i>Anonamuric ata</i>)	165.2a	111.8b	2.4b	1.2b	0.0	29.4b	310.0a

Means with the same letters were not significantly different at $p \leq 0.05$

AMF Colonization Status

Results on the Total root count, Total root colonized and Percentage Arbuscular Mycorrhizal Fungi colonization are presented on Table 2 and figure 2. The roots of the five trees selected were all infected by mycorrhizal fungi hyphae and arbuscules during the study, total number of roots ranged from 105.4 – 108.0, while total root infected ranged from 11.4- 38.0 under the various trees respectively (Table 2). Percentage AMF colonization

ranged from 10.76 – 36.17, with the highest colonization observed in *Gmelina arborea* while *Irvingia gabonensis* recorded the lowest % AMF colonization (figure 2). The % AMF colonization under the various trees followed the order; *Gmelina arborea* > *Anona muricata* > *Tectona grandis* > *Irvingia gabonensis* > *Irvingia gabonensis*.

Table 2: AMF Colonization Status under Various Trees Species

Tree Species	Total root count	Total root colonized
White Teak (<i>Gmelina arborea</i>)	107.2a	38.0a
Teak (<i>Tectona grandis</i>)	108.0a	29.4b
African Peach (<i>Nauclea diderrichii</i>)	107.2a	25.8b
Bush Mango (<i>Irvingia gabonensis</i>)	105.4a	11.4c
Sour sop (<i>Anona muricata</i>)	107.0a	29.0b

Means with the same letters were not significantly different at $p \leq 0.05$

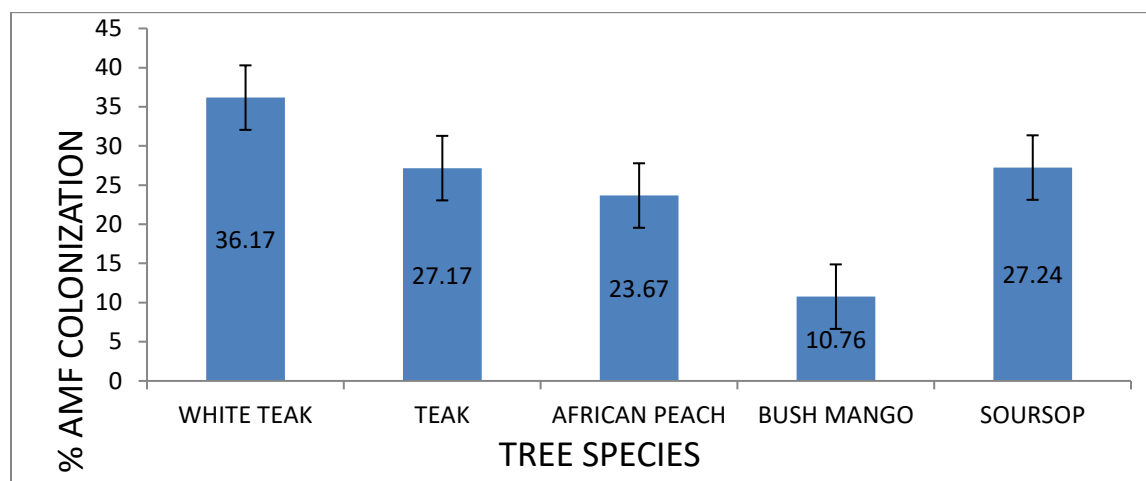


Figure 2: Percentage AMF Colonization of the Various Trees

DISCUSSION

Soil microbial populations support the productivity of forested soils by encouraging the exchange of chemicals between forest flora and soil microorganisms (Gardner *et al.*, 2023). Conversely, plants engage in a range of interactions with organisms that live in the soil that span the whole ecological spectrum (competitive, neutral, commensal, and mutualistic) (Jacoby *et al.*, 2017), hence, the relationships between plants and soil microorganisms reflect the effects of plant variety on ecosystem function (Gardner *et al.*, 2023).

In this study, it was observed that *Nauclea diderrichii* had the highest Arbuscular Mycorrhizal Fungi population followed by *Anona muricata*, and AMF spore numbers were recorded in the following order under the various trees; *Nauclea diderrichii* > *Anona muricata* > *Tectona grandis* > *Gmelina arborea* > *Irvingia gabonensis* (Figure 1). The relatively large canopy and broad leaves of *Nauclea diderrichii* and *Anona muricata* may account for the high AMF association with

them, since large canopy and broad leaves promotes a shaded atmosphere which according to Rodriguez-Echeverria *et al.* (2017) encourages AMF association. Some tree species' capacity to sustain large AMF populations may be due to their root exudates, which promote mycorrhizal spore germination (De Gannes *et al.*, 2016). Also, compared to trees with border leaves and a greater leaf area, *Irvingia gabonensis* recorded a low population of AMF unlike *Nauclea diderrichii* which recorded the highest (Figure 1). As opined by Rodriguez-Echeverria *et al.* (2017), the low association of AMF with *Irvingia gabonensis* might be due to the tree's firm roots and small leaves which exposes the soil surrounding the rhizosphere to sunlight, resulting in drier soils.

The diversity of AMF species within a soil ecosystem is essential for the functional resilience of plant-microbe interactions because diverse AMF communities can offer a wider range of benefits to the host plant (Chen *et al.* 2018; Rillig *et al.* 2019). Additionally, environmental physiological factors may have

an impact on the variety of AMF within tree communities' roots (Hakim *et al.*, 2021). In our study, a total number of 6 genera of Arbuscular Mycorrhizal Fungi were identified; *Acaulospora spp*, *Rhizosphaera spp* (*Glomus*), *Scutelospora spp*, *Gigaspora spp*, *Entrophospora spp* and *Funneliformis spp*, and under all the trees, *Rhizoglyphus* (*Glomus*) was the most occurring (Table 1). Arguably, the high occurrence of *Rhizoglyphus* (*Glomus*) could be due to their smaller spore size, which makes it easier for them to produce more spores in a short amount of time. This study's fungal community structure is similar to that of the non-native evergreen conifer that Gao *et al.* (2021) reported in their study. Additionally, our argument that tropical trees have some influence on the structure of fungal communities is supported by the differences in fungal diversity under the trees under study, and the position of Sasse *et al.* (2018) and Dukunde *et al.* (2019), that tree species significantly affect the beta-diversity of the fungal community structure, and their impact on fungal diversity is higher in plantations than in abiotic settings. Also, Gao *et al.* (2021) suggested that there was a strong correlation between fungal communities and tree species, which supports our position.

Furthermore, our study showed that the five tree species examined were colonized by Arbuscular Mycorrhizal Fungi. However, the AMF colonization status varied significantly depending on the planted tree species (Table 2). Also, it was observed that *Gmelina arborea* had the highest percentage of AMF colonization (Table 2) and this might be because this tree had higher nutrient intake, particularly for the less mobile elements; better tolerance to biotic and abiotic stress; and

increased N-nutrition because of the synergistic interactions among AMF (Hakim *et al.*, 2021). Also, the percentage of AMF colonization of the various trees was observed in the following order: *Gmelina arborea* > *Anona muricata* > *Tectona grandis* > *Nauclea diderrichii* > *irvingia gabonensis* (Figure 2), and the highest percentage colonization in *Gmelina arborea* may be attributed to its rooting morphology; *Gmelina arborea* grows very deep, roots well in drained base rich soil and have a greater dependence on mycorrhiza association. The variance in percentage colonization among the tree species in our study is supported by the results of Lladó *et al.* (2018) and Bardgett and Caruso (2020), who argued that the biomass, activity, and communities of living microorganisms associated with trees differ depending on the species and rhizosphere, support. Additionally, Bardgett and Caruso (2020) proposed that trees can alter the community organization and/or soil microbial makeup.

CONCLUSION

In the studied Arboretum, the roots of the selected trees were colonized by Arbuscular Mycorrhizal Fungi also, in the rhizosphere soils of the examined tree species, abundant spore numbers were observed. *Acaulospora spp* and *Rhizoglyphus spp* were the highest AMF genera identified. AMF had the highest spore number in *Nauclea diderrichi* and highest percentage colonization in *Tectona grandis* while *Nauclea diderrichi* had the highest population of weed cover. AMF fungi play an essential role in plant and tree growth, they also improve soil quality and texture through better plant and tree rooting capacity. Therefore, AMF are vital endosymbionts playing effective roles in tree productivity, diversity, and regeneration of tree communities and the overall functioning of the ecosystem. The biological and functional

diversity of AMF is vitally important to forest ecosystems and can be decisive for tree community structures and productivity.

REFERENCES

- Bardgett, R. D. and Caruso, T. (2020). Soil Microbial Community Responses to Climate Extremes: Resistance, Resilience and Transitions to Alternative States. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 375(1794), 20190112
- Barwant, M., Singh, B., Sharma, S., Gore, N. & Mohammad, A. (2025). Role of Mycorrhizal Association in Leguminous Plant Growth, In P. Mathur, A. Gupta (eds.), *Recent Trends and Applications of Leguminous Microgreens as Functional Foods*, (pp.277-295).
- Bowles, T.M., Barrios - Masias, F.H., Carlisle, E.A., Cavagnaro, T.R. and Jackson, L.E. (2016). Effects of Arbuscular Mycorrhizal on Tomato Yield, Nutrient Uptake, Water Relations, and Soil Carbon Dynamics under Deficit Irrigation in Field Conditions. *Sci. Total Environ.* 566, 1223-1234.
- Chen, Q.L., An, X.L., Li, H., Su, J.Q. and Zhu, Y.G. (2018). Long-term organic farming promotes differential resistance and resilience of soil microbial community under herbicide exposure. *Environmental Pollution*, 241, 387-396.
- de Andrade, L.A., Santos, C.H.B., Frezarin, E.T., Sales, L.R. & Rigobelo, E.C. (2023). Plant growth-promoting rhizobacteria for sustainable agricultural production. *Microorganisms*, 11, 1088.
- De Gannes, V., Bekele Dipchansingh D., *et al.*, (2016). Microbial Community Structure and Function of Soil Following Ecosystem Conversion from Native Forests to Teak Plantation Forests. *Front Microbiol.* 7:1976.
- Dhur, P.P. and Mridha, M.A.U. (2012). Arbuscular mycorrhizal associations in different forest tree species of Hazarikhil forest of Chittagong, Bangladesh. *J. For. Res.* 23,115-122.
- Dong, H., Huang, L., Zhao, L., Zeng, Q., Liu, X., Sheng, Y., Shi, L., Wu, G., Jiang, H. and Li, F. A. (2022). Critical review of mineral-microbe interaction and co-evolution: Mechanisms and applications. *Natl. Sci. Rev.*, 9, nwac128.
- Dukunde, A., Schneider, D., Schmidt, M., Veldkamp, E. and Daniel, R. (2019). Tree Species Shape Soil Bacterial Community Structure and Function in Temperate Deciduous Forests. *Front. Microbiol.*, 10, e17000
- Eludoyin, O., Oladele, A. and Iyanda, O. (2015). Mapping and Assessment of Ethno Medicinal Trees in Built Up Areas - University of Port Harcourt, Nigeria. *South-east European forestry*, 6.15-10.
- Gao, Y., Wang, X., Mao, Z., Yang, L., Jiang, Z., Chen, X. and Aubrey, D. P. (2021). Changes in Soil Microbial Community Structure Following Different Tree Species Functional Traits Afforestation. *Forests*, 12 (8), 1018. <https://doi.org/10.3390/f12081018>
- Gardner, T. G., Frene, J. P., Lawson, S. S., Lue Sue, N. D., Handy, J. & Crawford, R. H. (2023). The Impact of Tree Species on Microbial Community Structure and Soil Function on Forest Plantations in

- the Central Hardwoods Region (CHR). *Forests*, 14 (5), 859. <https://doi.org/10.3390/f14050859>
- Hakim, S., Naqqash, T., Nawaz, M. S., Laraib, I., Siddique, M. J., Zia, R., Mirza, M. S. and Imran, A. (2021). Rhizosphere engineering with Plant Growth Promoting Microorganisms for Agriculture and Ecological Sustainability. *Front. Sustain. Food Syst.* 5:617157.
- Harrier, L. A. and Watson, C. A. (2016). The role of arbuscular mycorrhizal fungi in sustainable crop production. *Agricultural Research*, 6 (2), 101-109.
- Jacoby, R., Peukert, M., Succurro, A., Koprivova, A. & Kopriva, S. (2017). The role of soil microorganisms in plant mineral nutrition—Current knowledge and future directions. *Front. Plant Sci.*, 8, 1617.
- Jiang, Y.N., Wang, W.X., Xie, Q.J., Liu, N., Liu, L.X. and Wang, D.P. (2017). Plants Transfer Lipids to Sustain Colonization by Mutualistic Mycorrhizal and Parasitic Fungi. *Science* 356, 1172–1175.
- Lladó, S., López-Mondéjar, R. and Baldrian, P. (2018). Drivers of Microbial Community Structure in Forest Soils. *Applied Microbiology and Biotechnology*, 102 (10), 4331-4338.
- Rillig, M. C., Aguilar-Trigueros, C. A., Camenzind, T., Cavagnaro, T. R., Degrune, F., Hohmann, P. and Yang, G. (2019). Why farmers should manage the arbuscular mycorrhizal symbiosis. *The New Phytologist*, 222 (3), 1171-1175.
- Rodriguez-Echeverria, S., Teixeira, H., Correia, M., Timoteo, S., Heleno, R., Opik, M. and Moora, M. (2017). Arbuscular mycorrhizal fungi communities from tropical Africa reveal strong ecological structure. *New Phytologist*, 213: 380–390 DOI: 10.1111/nph.14122
- Sasse, J., Martinoia, E. & Northen, T. (2018). Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends Plant Sci.*, 23, 25–41.
- Schuler, A. and Walker, C. (2010). The Glomeromycota: A Species List with New Families and New Generation. Edinburgh and Kew: The Royal Botanic Garden Kew, Munich and Oregon State University.
- Spatafora, J.W., Chang, Y., Benny, G.L., Lazarus, K., Smith, M.E., Berbee, M.L., Bonito, G., Corradi, N., Grigoriev, I. & Gryganskyi, A. (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia*, 108, 1028–1046.
- Sun, Z., Song, J., Xin, X., Xie, X. and Zhao, B. (2018). Arbuscular Mycorrhizal Fungal Proteins 14-3-3- are Involved in Arbuscule Formation and Responses to Abiotic Stresses during AM Symbiosis. *Front. Microbiol.* 5, 9–19.
- Zhu, C., Ling, N., Guo, J., Wang, M., Guo, S. and Stien, Q. (2016). Impact of Fertilization Regimes on AMF Community Composition were Correlated with Organic Matter Composition in Maize Rhizosphere Soil. *Frontiers in Microbiology*. 7, PP; 18-40.