

## Arbuscular Mycorrhizal Fungi Communities And Root Colonization Of Crops In The Rhizosphere Of A Continuous And Crop Rotation Cropping System In Derived Savannah, Nigeria

Adeyemi NO<sup>1\*</sup>, Atayese MO<sup>1</sup>, Olubode AA<sup>2</sup>

<sup>1</sup>Department of Plant Physiology and Crop Production, Federal University of Agriculture, Abeokuta, P.M.B.2240, Alabata, Ogun State, Nigeria;

<sup>2</sup>Department of Soil Science and Land Management, Federal University of Agriculture, Abeokuta, P.M.B.2240, Alabata, Ogun State, Nigeria.

\*Author for Correspondence: [adeyemisworld@gmail.com](mailto:adeyemisworld@gmail.com)

### ABSTRACT

The evaluation of the effects of cropping practices on arbuscular mycorrhizal fungi (AMF) in soil is of great importance in sustainable agriculture. This study investigated the spore density, root colonization and composition of native AMF in soils under two different cropping systems (continuous cropping and crop rotation) in a derived Savannah, Nigeria. Rhizosphere soils and root samples were collected from five fields to assess AMF spores density and root colonization. The spore density and root colonization was highest in field plot under crop rotation practice (273.3 spores per 50 g dry soil and 82.7 respectively). Maize and soybean cultivation significantly produced higher spore density and root colonization compared to sesame and sunflower. AMF spores of *Glomus*, *Gigaspora*, *Acaulospora*, and *Scutellospora* species were identified based on morphological characteristics. *Glomus* specie was the most dominant genus in soils with highest relative abundance of 68.7% followed by *Acaulospora* (19.8%) and *Scutellospora* (13.5%), with lowest relative abundance of AM spores observed for *Gigaspora* (5.6%) and unknown genera (2.5%). The results contribute to a better understanding of AMF composition as influenced by the cropping practices and host plants, and could be valuable in regulating the AMF community structure, and providing a primary basis for sustainable crop production.

**Keywords:** Arbuscular mycorrhizal fungi, community composition, crop rotation, root colonization, spore density

### INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are important obligate symbionts, which form association with over 90% terrestrial plants (Smith and Read, 2008; Brundrett, 2002). The roots of cultivated plants are generally colonized by AMF and when establishing symbiosis, the proliferation of mycelial hyphae into the soil increases the prospecting volume with respect to plant essential plant nutrients such as nitrogen and phosphorus (Hill et al. 2010; Smith and Read, 2008; Wagg et al. 2011). AMF also promotes drought resistance and water uptake (Auge et al. 2001) and protect plants against soil pathogens (Sikes et al. 2009). AMF gains a carbon and the host plant gains several benefits from infection including enhanced uptake, improved water relations (Smith and Read, 2008). It has been reported that the soil mycelium of AMF was coated with a mucilaginous substance that helps in soil

particles aggregation (Mosse, 1986). AMF play a central role in many ecological processes, influencing soil fertility, decomposition, cycling of minerals and organic matter, as well as plant health and nutrition (Finlay, 2008).

The quantification of the spores has been very useful for evaluating the level and diversity. It is important as AMF play important roles in agro-ecosystem productivity and sustainability. Variation in the population of these fungi and their symbiosis with plant roots is related to several factors including agricultural practices and host plant. A number of factors including agricultural practices have been reported to influence AMF in rhizosphere of crops (Lin et al. 2012; Oehl et al. 2010). Several agricultural practices such as land-use type, crop rotation, tillage and fertilization have been reported to have significant effect on soil properties and microbial diversity including AMF (Alguacil et al. 2008; Xiang et al. 2016; Tsiafouli et al. 2015). The microbes present in

the soils have been shown to be influenced by these practices, thereby affecting different soil ecosystem processes and possibilities to develop sustainable agriculture to meet growing global demand for food (Bender et al. 2016; van der Heijden et al., 2008). Increasing the diversity of AMF communities in agroeco systems has been suggested to have the ability to boost crop growth, nutrient uptake, contribute to P nutrition and can maximize the benefits from AMF (van der Heijden et al. 1998; Verbruggen et al. 2013). In the context of sustainable agriculture, farmers are constrained by the global economic crisis to try to reduce the input of organic fertilizers using AMF inocula (Berruti et al. 2016).

It has also been reported that continuous cropping resulted in rapid sporulation of detrimental AMF species, which leads to a decline in beneficial AMF species and decreases crop performance over time (Johnson et al. 1991). Intensive agricultural practices on soil such as continuous cropping can affect AMF community composition (Schnoor et al. 2011). However, there are conflicting results on how AMF communities respond to such practices (Jansa et al. 2014). The distribution of AMF in different ecological regions as influenced by different agricultural practices and host plants have been investigated by several researchers. However, there is limited knowledge about AMF composition and root activities in derived Savannah of Nigeria vegetation. The understanding on how different agricultural practices commonly carried out in this agro-ecology affect AMF community composition and activities is essential for their management.

In this study, the effect of different agricultural practices was examined on the community composition, spore density and root colonization of AMF in soils of a derived Savannah of southwest Nigeria. The finding of the present study being one of the few studies to assess AMF communities in this agro-ecology, will contribute to a better understanding of the native AMF communities associated with maize, soybean, sesame and sunflower under two different agricultural practices (crop rotation and continuous cropping).

## MATERIALS AND METHODS

### Study Setting

Field plots located at the Teaching and Research Farms, Federal University of Agriculture Abeokuta, Southwest Nigeria (Latitude 7° 15'N, Longitude 3° 28'E, altitude of 75 m a.s.l.) were studied during the fifth year of establishment (2012) in late cropping season (August – December 2012). The field plots were five (P1 – P5), replicated thrice with an area of 8 m × 5 m (40 m<sup>2</sup>) each. Information about the study plots are presented in Table 1.

Plots	Cropping systems	Crops
Plot 1 (P1)	Continuous cropping	Maize
Plot 2 (P2)	Continuous cropping	Soybean
Plot 3 (P3)	Continuous cropping	Sesame
Plot 4 (P4)	Continuous cropping	Sunflower
Plot 5 (P5)	Crop rotation	Maize- soybean- sesame- sunflower

Crops were grown under two cropping system practices (continuous cropping and crop rotation). The effect of the cropping practices was investigated in the five plots, P1 – P5, while host crop effects (maize, soybean, sesame and sunflower) was studied in P1 – P4.

### Roots and soil sampling

The roots of the crops and rhizosphere soil samples were collected at monthly interval between August and December 2012. Soil samples were collected at six points randomly on each plot at a depth of 10 cm to 20 cm, bulked together to form a composite sample. Root samples were collected from five plants per plot. The collected samples of soils and roots were kept at 4°C until microscopic analysis in laboratory.

### AMF spore extraction and morphological identification

AMF spores were extracted from 50 g of soil using the modified wet sieving method of

Giovannetti and Mosse, (1980). A sample of air-dried field soil was mixed with distilled water. The resulting mixture was passed through 250, 150 and 40  $\mu\text{m}$  sieves. Soil materials retained by the 250, 150 and 32  $\mu\text{m}$  sieves were washed into centrifuge tubes using a small stream of distilled water. Tubes were centrifuged at 4000 rpm for 2 minutes. The supernatants were decanted and subjected to sucrose centrifugation (70% (w/v)) gradient and centrifuged at 4000 rpm for 2 minutes. The supernatant was passed through the 40  $\mu\text{m}$  sieve, washed with distilled water and transferred to new Petri dishes. Spores, spore clusters and sporocarps were covered and counted at 40 $\times$  magnification. For identification, spores were picked under the dissecting microscope with a glass micropipette and subsequently mounted on slides with polyvinyl-lactic acid-glycerol (PVLG) or polyvinyl-lactic acid-glycerol mixed with Melzer's reagent (1:1 (v/v) to get permanent slides for spore observation and identification under a compound microscope at 400 $\times$  magnification. **The spores were identified at the genus level on the basis of size, spore-wall structure, Melzer's reaction, colour and presence or absence of subtending hyphae and compared with descriptions of fungal genera** according to taxonomic criteria (Shenck and Perez, 1990) and information available on the international network for vesicular arbuscular mycorrhizal (IN V A M, 1998) <http://www.invam.caf.wvu.edu/fungi/taxonomy/classification.htm>. The relative abundance, which is the ratio of the number of spores from a particular genus with respect to the total number of spores recovered, was calculated based on percentage per 100 g of dry soil.

### AMF root colonization

The roots of the respective crop species were carefully freed from adhering soil and immediately fixed in 50% ethanol. Roots in ethanol were rinsed thoroughly in tap water, cut into approximately 1 cm segments and cleared in hot KOH solution (10% w/v, at 90°C) for 1 hour. The bleached roots were rinsed to remove excess KOH and stained in acidic glycerol containing methyl blue lacto-glycerol (1:1:1:0.5g) at 90°C for 30 minutes (Phillips and Hayman, 1970). **The stained root segments were**

**mounted on microscopic slides and examined for AMF structure under light microscope to determine percentage root colonization.**

$$\text{Percentage root colonisation} = \frac{\text{Number of root infected}}{\text{Total number of roots}} \times 100 \quad (1)$$

### Statistical analysis

The data collected for spore density and root colonization (%) were subjected to log<sub>e</sub> (x+1) transformation and square root transformation respectively for normalization of the data. Analysis of Variance (ANOVA) was conducted to determine significant differences among the means at 5% probability level. Significant means were separated using Least Significant Difference (LSD). Pearson correlation analysis was used to detect the relationship between spore densities 50 g<sup>-1</sup> soil, percent root colonization and AMF relative abundance using the statistical package Genstat 12<sup>th</sup> Edition.

## RESULTS

### AMF spore density

The spore density of AMF significantly varied between the five field plots examined in the following decreasing order P5 > P2 > P1 > P3 > P4 (Figure 1). The AMF spore density was significantly affected by the cropping practices, with highest spore density in crop rotation field plot (P5) soils at 4, 8 WAP and harvest (141.3, 273.3 and 268 spores/50 g soil respectively) compared to other continuous cropping field plots (P1, P2, P3 and P4). The crops had significant effect on the spore density at 4 and 8 WAP, with higher spore density recorded in maize (P1) and soybean (P2) field plots compared to sesame (P3) and sunflower (P4) field plots (Figure 1).

### Composition and relative abundance of AM

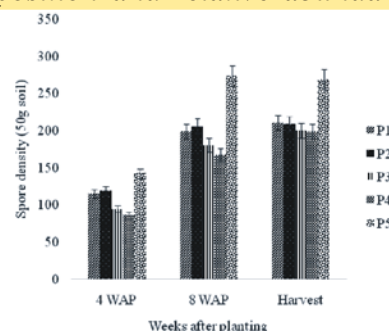


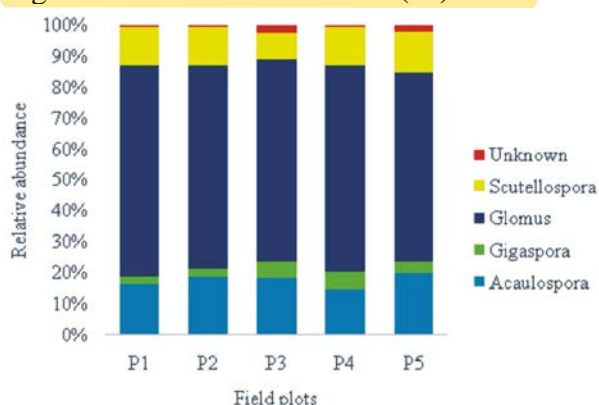
Figure 1. Mean spore density (per 50 g dry soil) of AMF in rhizospheric soil in five different field plots.



**genera**

The identification of the AMF communities based on morphological characterization of the spores showed 4AMF genera and unknown ones in the field soils (Figure 3), namely; *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora*. *Glomus* was the most widespread genus in the five fields analyzed, followed by *Acaulospora* and *Scutellospora* (Figure 3).

**Figure 3. Relative abundance (%) of AM**



**genera from the soil samples. Mean values were calculated from the data obtained in all plots.**

Lowest relative abundance of AM spores was observed for genus *Gigaspora* and unknown. The four most commonly observed species were *Funnelformis mosseae*, *Glomus intraradices*, *Glomus aggregatum*, and *Claroideoglomus etunicatum* (Table 2).

**DISCUSSION**

**Table 2. Distribution of AM species in rhizosphere soil samples**

AM fungi	Plots				
	1	2	3	4	5
<i>Acaulosporascorobiculata</i>	+	+			+
<i>Acaulosporadelicata</i>	+	+	+		
<i>Claroideoglomus claroideum</i>	+	+			+
<i>Claroideoglomus etunicatum</i>	+	+		+	+
<i>Funnelformis mosseae</i>	+	+	+	+	+
<i>Glomus intraradices</i>	+	+	+	+	+
<i>Glomus clalrium</i>	+	+		+	+
<i>Glomus aggregatum</i>	+	+	+	+	+
<i>Glomus fasciculatum</i>	+	+		+	
<i>Funnelformis geosporus</i>	+				+
<i>Gigasporamagarita</i>	+	+			+
<i>Scutellosporapellucida</i>		+			+
<i>Scutellosporanigra</i>	+		+	+	

The present study showed that native AMF successfully forms symbiotic relationships with crops under the different cultural practices in the derived Savannah of Nigeria. Differences were observed between the cropping practices on the spore density and root colonization by AMF. Lower spore density was observed in continuous cropping plots (P1 to P4) compared to the crop rotation field plot (P5). Higher spore density in crop rotation field plot may be due to the presence of mycorrhizal plants in the rotation system. These may have supported AMF development in their roots and also enhanced spore formation in the soil. The low spore density and root colonization under continuous cropping may be as results of rapid sporulation of detrimental AMF species, which leads to a decline in beneficial AMF species and decreases crop performance over time (Johnson et al. 1991). The crop grown had effect on the AMF spore density and root colonization. It had been reported that AMF can differ in their associations with different plants (Rillig, 2004; Douds and Millner, 1999). Maize and soybean plants promotes higher root colonization and spore density. Maize is a very good host and supports AMF development and spore formation, which in turn leads to increased AMF root colonization of the crop (Gavito and Varela 1993). Additionally, soybean is a leguminous crop that requires P for their growth and development, which makes it more dependent on association with AMF.

Based on genus level classifications, five AMF genera were morphologically identified (*Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora*). Unknown or unidentified genera were also found. *Glomus* genus was the most dominant in the soils of the five fields examined. *Glomus* has been reported to be the most common and widespread AMF genus occurring in most tropical soils throughout the world (Snoeck et al. 2010). This may be related to their potential to form a relatively high number of spores within a short period and their adaptability to varying soil and climatic conditions (Pande and Tarafdar, 2004; Oehl et al. 2009). The low sporulation rate of *Scutellospora* and *Gigaspora* may be an inherent character as reported by Bever et al.

(1996). However, the spore density through morphological identification might not give the true representative values for the actual abundance of the AMF in the soil (Clapp et al. 1995). Other methods, such as specific primer quantitative PCR coupled with multiple sampling, are needed might give a reliable quantification of the AMF in the rhizosp hereby targeting soil mycelium network or intraradical colonization structures (Edwards et al. 1997; Jacquot-Plumey et al. 2001).

## CONCLUSION

In conclusion, this present study has investigated the density, root colonization and genera diversity of AMF rhizospheric soil and roots of crops grown under continuous cropping and crop rotation in a derived Savannah of Southwest Nigeria. A wide variety of AMF genera spores and a high colonization rate of roots were identified in the field plots examined. The results study reveals the existence of common AMF genera and a rich taxonomic diversity and contribute to a better understanding of AMF composition as influenced by different cropping practices, providing a primary basis for sustainable crop production.

## REFERENCES

- Alguacil MM, Lumini E, Roldan A, Salinas-Garcia JR, Bonfante P, Bianciotto V. (2008). The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. *Ecol. Appl.* 18, 527–536.
- Auge RM, Stodola AJW, Tims JE, Saxton AM. (2001). Moisture retention properties of a mycorrhizal soil. *Plant Soil* 230, 87–97.
- Bender SF, Wagg C, Van Der Heijden MGA. (2016). An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends Ecol. Evol.* 31, 440–452.
- Berruti A, Lumini E, Balestrini R, Bianciotto V. (2016). Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. *Front. Microbiol.* 6, 1–13.
- Bever JD, Morton JB, Antonovics J, Schultz PA. (1996). Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. *J Ecol* 84:71–82.
- Brundrett MC. (2002). Coevolution of roots and mycorrhizas of land plants. *New Phytol.* 154:275–304.
- Clapp JP, Young JPW, Merryweather JW, Fitter AH. (1995). Diversity of fungal symbionts in arbuscular mycorrhizas from a Natural community. *New Phytol* 130:259–265.
- Douds DD, Millner P. (1999). Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. *Agric. Ecosyst. Environ.* 74, 77–93.
- Edwards SG, Fitter AH, Young JPW. (1997). Quantification of an arbuscular mycorrhizal fungus, *Glomus mosseae*, within plant roots by competitive polymerase chain reaction. *Mycol Res* 101:1440–1444.
- Finlay RD. (2008). Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extra-radical mycelium. *J. Exp. Bot.* 59: 1115–1126.
- Gavito ME, Varela L. (1993). Seasonal dynamics of mycorrhizal associations in maize fields under low-input agriculture. *Agric Ecosyst Environ* 45:275–282.
- Hill JO, Simpson RJ, Ryan MH, Chapman DF. (2010). Root hair morphology and mycorrhizal colonisation of pasture species in response to phosphorus and nitrogen nutrition. *Crop Pasture Sci.* 61, 122–131.
- Jacquot-Plumey E, van Tuinen D, Chatagnier O, Gianinazzi S, Gianinazzi-Pearson V. (2001). 25S rDNA-based molecular monitoring of glomalean fungi in sewage sludge-treated field plots. *Environ Microbiol* 3:525–531.
- Jansa J, Erb A, Oberholzer HR, Smilauer P, Egli S. (2014). Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. *Mol. Ecol.* 23, 2118–2135.
- Johnson NC, Angelard C, Sanders IR, Kiers ET. (2013). Predicting community and ecosystem outcomes of mycorrhizal responses to global change. *Ecol. Lett.* 16, 140–153.
- Lin X, Feng Y, Zhang H, Chen R, Wang J, Zhang J, Chu H. (2012). Long-term balanced fertilization decreases arbuscular mycorrhizal fungal diversity in an arable soil in north China revealed by 454 pyrosequencing. *Environmental Science & Technology* 46, 5764–5771.
- Mosse B. (1986). Mycorrhiza in a sustainable agriculture. *Biol. Agric. Hort.* 3: 191–209.

- Oehl F, Sieverding E, Ineichen K, Mäder P, Wiemken A, Boller T. (2009). Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long-term microcosms. *Agric Ecosyst Environ.* 134:257–268.
- Oehl F, Laczko E, Bogenrieder A, Stahr K, Bosch R, van der Heijden M, Sieverding E. (2010). Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biol. Biochem.* 42: 724–738.
- Pande M, Tarafdar JC. (2004). Arbuscular mycorrhizal fungal diversity in neem-based agroforestry systems in Rajasthan. *Appl. Soil Ecol.* 26:233–241.
- Phillips JM, Hayman DS. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br Mycol Soc.* 55:158–181.
- Rillig MC. (2004). Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecol. Lett.* 7, 740–754.
- Schenck NC, Perez Y, (eds). (1990). *Manual for identification of VA mycorrhizal fungi*. Gainesville (FL): INVAM, University of Florida; pp. 241.
- Schnoor TK, Lekberg Y, Rosendahl S, Olsson PA. (2011). Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland. *Mycorrhiza* 21, 211–220.
- Sikes BA, Cottenie K, Klironomos JN. (2009). Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. *J. Ecol.* 97, 1274–1280.
- Smith SE, Read DJ. (2008). *Mycorrhizal symbiosis*. 3rd ed. New York: Academic Press; pp. 787.
- Snoeck D, Abolo D, Jagoret P. (2010). Temporal changes in VAM fungi in the cocoa agroforestry systems of central Cameroon. *Agroforestry Syst.* 78:323–328.
- Tsiafouli MA, Thebault E, Sgardelis SP, de Ruiter PC, van der Putten WH, Birkhofer K, Hemerik L, de Vries FT, Bardgett RD, Brady MV, Bjornlund L, Jorgensen HB, Christensen S, Hertefeldt TD, Hotes S, Gera Hol WH, Frouz J, Liiri M, Mortimer SR, Setälä H, Tzanopoulos J, Uteseny K, Pizl V, Stary J, Wolters V, Hedlund K. (2015). Intensive agriculture reduces soil biodiversity across Europe. *Global Change Biol.* 21, 973–985.
- van der Heijden MGA, Bardgett RD, van Straalen NM. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* 11, 296–310.
- van der Heijden MGA, Klironomos JN, Ursic M, Moutoglou P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396, 69–72.
- Verbruggen E, Roling WFM, Gamper HA, Kowalchuk GA, Verhoef HA, van der Heijden MGA. (2010). Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytol.* 186, 968–979.
- Wagg C, Jansa J, Stadler M, Schmid B, van der Heijden MGA. (2011). Mycorrhizal fungal identity and diversity relaxes plant–plant competition. *Ecology* 92, 1303–1313.
- Xiang D, Verbruggen E, Hu YJ, Veresoglou SD, Rillig MC, Zhou W, Xu TL, Li H, Hao ZP, Chen YL, Chen BD. (2014). Land use influences arbuscular mycorrhizal fungal communities in the farming–pastoral ecotone of northern China. *New Phytol.* 204, 968–978.