Acta fytotechn zootechn, 22, 2019(3): 84–89
http://www.acta.fapz.uniag.sk

Original Paper

Identification and relative abundance of native arbuscular mycorrhizal fungi associated with oil-seed crops and maize (*Zea mays* L.) in derived savannah of Nigeria

Nurudeen O. Adeyemi[1]*, Muftau O. Atayese[1], Adebanke A. Olubode[2]
[1]*Department of Plant Physiology and Crop Production, Federal University of Agriculture, Abeokuta, P.M.B.2240, Alabata, Ogun State, Nigeria*
[2]*Department of Soil Science and Land Management, Federal University of Agriculture, Abeokuta, P.M.B.2240, Alabata, Ogun State, Nigeria*

Article Details: Received: 2019-07-22 | Accepted: 2019-10-10 | Available online: 2019-09-30

https://doi.org/10.15414/afz.2019.22.03.84-89

A field survey was conducted to assess root colonization, spore densities and relative abundance of native arbuscular mycorrhizal fungi (AMF) based on morphological aspects. Roots and rhizosphere soil samples were collected from established fields of selected oil seed crops [soybean (*Glycine max* L.), sesame (*Sesamum indicum*) and sunflower (*Helianthus annuus*)] and maize (*Zea mays* L.) grown in derived savannah agro-ecology of Southwest Nigeria. The mean percentage of AMF colonization across all crops was 60.8%, ranging from 34% to 87.5%, with highest root colonization observed in soybean. The spore densities retrieved from the different rhizospheres were relatively high, varying from 124 to 298 spores per 50 g dry soil, with highest spore densities observed in maize rhizosphere soils. The spore densities in the soil significantly correlated (r = 0.52, and P <0.05) with the root colonization. A total of 4 morphologically classifiable genera (*Glomus*, *Gigaspora*, *Acaulospora*, and *Scutellospora*) of AMF within the phylum Glomeromycota were detected. The dominant genus was *Glomus* in all the crops with highest relative abundance of 60.9%, followed by *Acaulospora* (21.3%) and *Scutellospora* (12.8%), with lowest relative abundance of AM spores observed for *Gigaspora* (5%). This study could contribute significantly to a better understanding of AMF community structure in derived savannah agro-ecology of Nigeria.

Keywords: k Arbuscular mycorrhizal fungi, community structure, oil-seed crops, root colonization, spore density

1 Introduction

Arbuscular mycorrhizal fungi (AMF) are widespread member of the soil biota and are important components of agricultural ecosystems. AMF form symbiotic relationships with most agricultural crops including maize, sesame, soybean and sunflower (Brundrett 2002; Smith and Read, 2008). AMF belong to the phylum Glomeromycota and about 250 species have been described mostly based on the morphology of their spores with recent molecular study indicating that the real number of AMF species may be much higher, comprising many uncultivated taxa (Schüßler et al., 2001; Ohsowski et al., 2014). They are generally essential for many important ecosystem functions and processes, including nutrient cycling, plant productivity and sustainability (Verbruggen and Kiers, 2010). The bidirectional exchange of nutrients between plants and AMF often results in a nutritional benefit for both partners. The host plant provides the fungus with carbohydrates, while in return the plant obtains rather immobile mineral nutrients such as phosphorus (P) from the fungus (Smith and Read 2008). AMF may also enhance host growth and survival by improving tolerance to drought and to some root pathogens and nematodes (Azcón-Aguilar and Barea, 1997; Yamato et al., 2009). They contribute to soil aggregate stability and may help in reducing salinity effects (Rillig and Mummey, 2006; Evelin et al., 2009). The widespread benefits of AMF may be critical to increasing crop yields and productivity in a sustainable agriculture.

***Corresponding Author:** Nurudeen O. Adeyemi, PMB 2240, Alabata, Abeokuta Nigeria, e-mail: adeyemisworld@gmail.com; Orcid: 0000-0001-6341-775X

AMF have been reported to share a long history of coevolution with plants in various ecosystems, resulting in their adaptation to specific areas (Gosling et al., 2006). The majority of research on AMF symbiosis involves laboratory or greenhouse experiments, in which plants are cultivated in sterilized soil, with particular AMF species. They ignore indigenous AMF species that could alter plant responses or compete with the AMF inoculant (Munkvold et al., 2004). A number of factors have been shown to act as environmental filters, structuring AMF communities, such as host plants, land use, fertilization and soil pH (Lin et al., 2012; Oehl et al., 2010; Peyret-Guzzon et al., 2016). Host preferences have also been demonstrated to exist to a certain extent in AMF (Pivato et al., 2007), but strict host specificity seems to be rare. However, it is well established that individual AM fungal species can differ in their associations with different plants (Rillig, 2004). Most studies on the AMF community structure have been conducted at a small scale, with only a few authors reporting AMF diversity at the regional scale or larger (Hazard et al., 2013). Some AMF taxa have been reported to be surprisingly widespread (Davison et al., 2015), however, many cannot yet be directly linked to a certain set of agricultural practices or environmental conditions. Therefore, the understanding of the geographical distribution of these fungi remains somewhat limited.

Colonization by native AMF species in crops has been reported earlier (Maiti et al., 1995; Sawers et al., 2008; Campos-Soriano et al., 2010; Cosme et al., 2011). Despite its important role, there is little information on the distribution and abundance of the different mycorrhizal fungi species associated with some agricultural crops in derived savannah of tropical soil of southwest Nigeria. The derived savannah of Southwest Nigeria is characterized with tropical soils of low P availability. Under such environmental conditions, symbioses between plants and AMF may be an important factor for plant adaptation and survival. Given the paucity of information of AMF with different oil seed crops and maze in the derived savannah of Nigeria, a field survey was performed to assess the intensity of AMF root colonization, spore densities and relative abundance of native AMF genera in rhizosphere soils of oil-seed crops and maize. The study tested the hypothesis that AMF colonization and community structure would differ among the crops.

2 Material and methods

2.1 Study site

The study area is located in the Teaching and Research Farms, Federal University of Agriculture Abeokuta, Ogun State, Nigeria (Latitude 7º 15′ N, Longitude 3º 28′ E, altitude of 75 m a.s.l.).

2.2 Field sampling

Field soil and root samples were taken from the agricultural fields of the research farms in year 2012. Oil seed crops [soybean (*Glycine max* L.), sesame (*Sesamum indicum*) and sunflower (*Helianthus annuus*)] and maize (*Zea mays* L.) were grown on the fields in a continuous cropping system established in 2008. The crops were cultivated under organic farming system through the application of organic fertilizers (Aleshinloye) with no pesticide application. The samples were collected at 4, 8 and 12 weeks after planting (WAP) from the experimental fields during the growing period of the crops in late cropping season (August – December 2012). The soil samples were collected from depth of 0–20 cm from several points on the fields. At each sampling point, four subsamples (250 g) were collected from each field and mixed to produce composite soil samples. The collected samples were kept in sterilized plastic bags after removing large particles, broken roots and stone and stored in the refrigerator at 4 ºC until processing.

2.3 AMF spore isolation and identification

AMF spore extraction was done in triplicates for individual soil sample. The spores were extracted by the modified wet sieving method of Giovannetti and Mosse (1980). A sample of 25 g of air-dried field soil was mixed with distilled water. The resulting mixture was passed through 250, 150 and 40 µm sieves. The fraction retained in the 500 µm sieve was checked for large spores, spore clusters, sporocarps and organic matter debris. Soil materials retained by the 150 and 40 µm sieves were washed into centrifuge tubes using a small stream of distilled water. Tubes were centrifuged at 4,000 rpm for 2 minutes. The supernatants were decanted and subjected to sucrose centrifugation (70% (w/v)) gradient and centrifuged at 4,000 rpm for 2 minutes. The supernatant was passed through the 40 µm sieve, washed with distilled water and transferred to new Petri dishes. Spores, spore clusters and sporocarps were recovered and counted at 40× 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); Brundrett 2002) to get permanent slides for spore observation and identification under a compound microscope at up to 400× 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). The relative abundance was calculated based on percentage:

$$Relative\ AMF\ abundance = \frac{number\ of\ spores\ of\ a\ genus}{total\ number\ of\ spores\ in\ the\ rhizosphere} \times 100$$

2.4 Staining and estimation of 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 : 0.5 g) 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:

$$Percentage\ root\ colonisation = \frac{number\ of\ root\ infected}{total\ number\ of\ roots} \times 100$$

2.5 Statistical analysis

Spore density and root colonization (%) were subjected to log e $(x+1)$ transformation and square root transformation respectively for normalization of the data. Analysis of Variance 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, percent root colonization and AMF relative abundance using the statistical package Genstat 12th Edition.

3 Results and discussion

3.1 AMF root colonization and spore density in the rhizosphere

All root samples of the selected crops surveyed in this study were colonized by AMF. The percentage of AMF colonization ranged from 34% to 87.5% (Table 1). Soybean plants significantly had the highest AMF root colonization compared to other crops throughout the period of measurements at 4, 8 and 12 weeks after planting (WAP) (44, 87.5 and 85% respectively), but significantly comparable to maize root length colonization (40.5%) at 4 WAP.

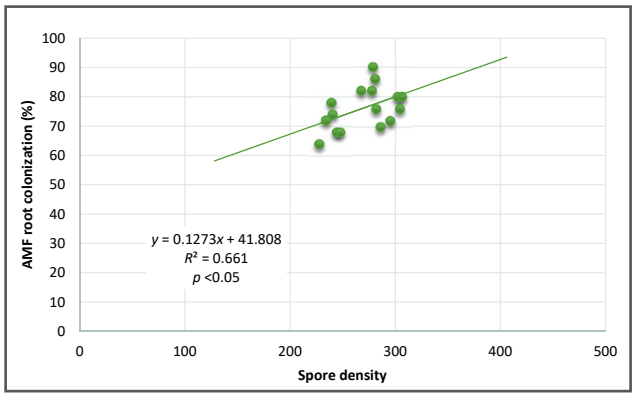

Figure 1 Pearson correlation between root colonization (%) and AMF spore density

Table 1 Percent root colonization of crops by AMF

Treatments	4 WAP	8 WAP	12 WAP
Crops			
Maize	40.5a	78.5b	78.0b
Sunflower	36.0b	69.0c	73.0b
Soybean	44.0a	87.5a	85.0a
Sesame	34.0b	64.5c	68.5c

Different letters within the same column indicate that treatment means are significantly different at $P < 0.05$; WAP = weeks after planting

Table 2 Spore density of AMF (50 g of soil) in rhizosphere of crops

Treatments	4 WAP	8 WAP	12 WAP
Crops			
Maize	164.8a	261.8a	298.5a
Sunflower	128.0c	203.5c	263.5b
Soybean	146.2b	235.0b	276.5ab
Sesame	124.2c	204.2c	239.8c

Different letters within the same column indicate that treatment means are significantly different at $P < 0.05$; WAP = weeks after planting

The spore densities (expressed as per 50 g dry soil) retrieved from different rhizosphere crops were relatively high, varying from 124 in sesame at 4 WAP to 298.5 in maize at 12 WAP (Table 2). AMF spore density was observed to be highest in maize throughout the period of measurement. The spore densities in the soil significantly correlated (r = 0.52, and P <0.05) with the root colonization of the crops (Figure 1).

3.2 AMF relative abundance

A total of 1056 AMF spores were identified and classified from the rhizosphere soil samples at 12 WAP. A total of 4 genera of AMF namely, *Glomus*, *Gigaspora*, *Acaulospora*, and *Scutellospora* were identified from the collected soil samples. Among the four genera of AM fungi observed, the most abundant genus was the *Glomus* with relative abundance of 60.9%, followed by *Acaulospora* (21.3%) and *Scutellospora* (12.8%), while lowest relative abundance of AM spores was observed for genus *Gigaspora* (5%) as shown in Figure 2 and 3.

The spore abundance of *Glomus*, *Acaulospora* and *Scutellospora* were positively correlated with root colonization with significant correlation observed with *Glomus* spore abundance (r^2 = 0.53, and P <0.01) as shown in Figure 4, 5 and 6. The spore abundance of *Gigaspora* was negatively correlated with root colonization (r^2 = 0.18, P >0.05) as shown in Figure 7.

In the present study, the results indicate variation in the spore density and root colonization of AM fungi naturally associated with the selected crops. Moreove, spore densities and root colonization were significantly

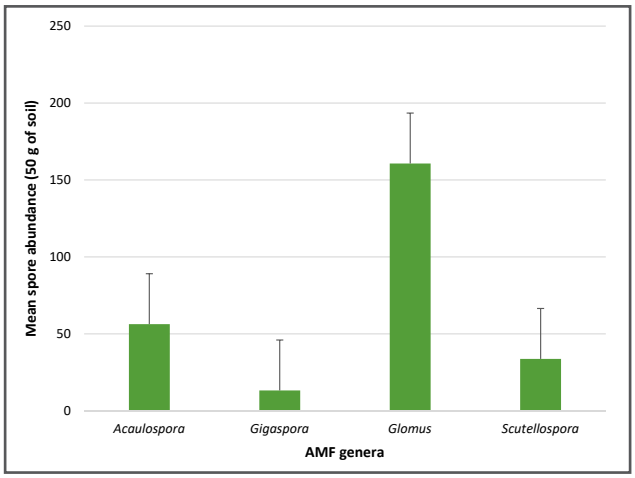

Figure 2 Mean spore abundance of AMF genera in rhizosphere soil of selected crops. Mean values were calculated from the data obtained in all plots. Bars in each column indicate standard error of means (±SE)

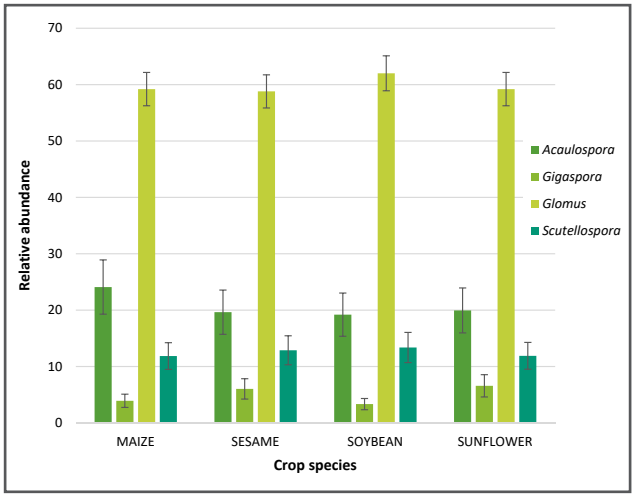

Figure 3 Relative abundance (%) of AM spores identified at genus level from the soil samples. Mean values were calculated from the data obtained in all plots belonging to the particular crop species. Bars in each column indicate standard error of means (±SE)

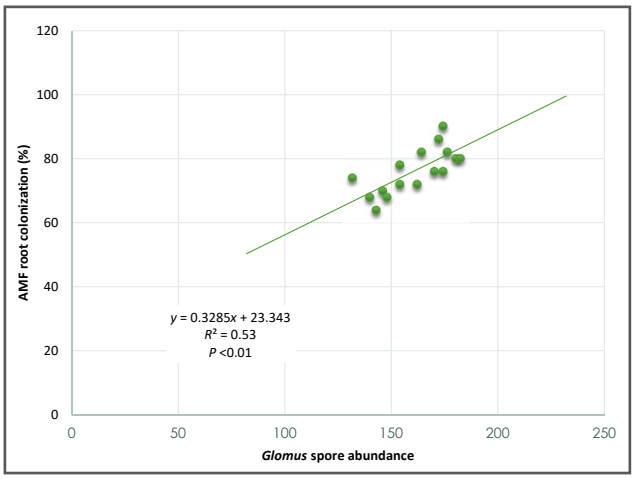

Figure 4 Pearson correlation between root colonization and *Glomus* spore abundance

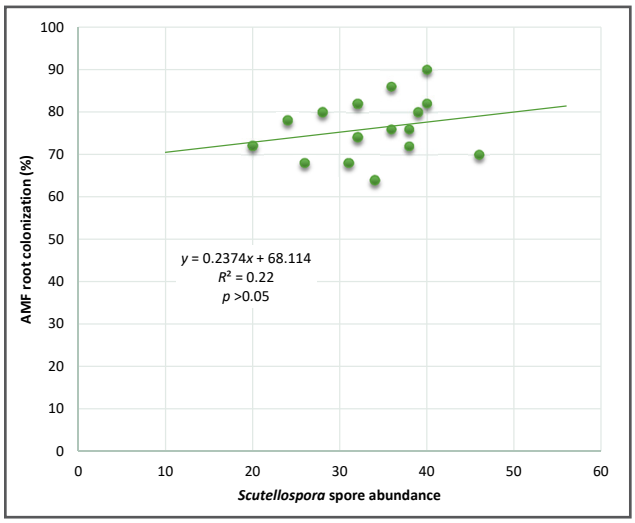

Figure 5 Pearson correlation between root colonization and *Scutellospora* spore abundance

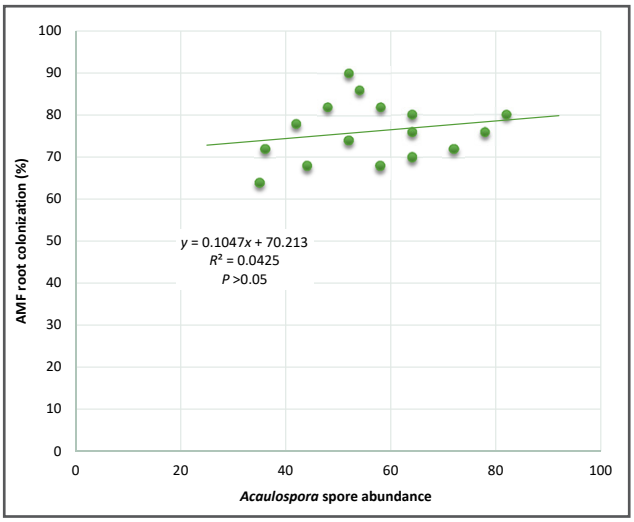

Figure 6 Pearson correlation between root colonization and *Acaulospora* spore abundance

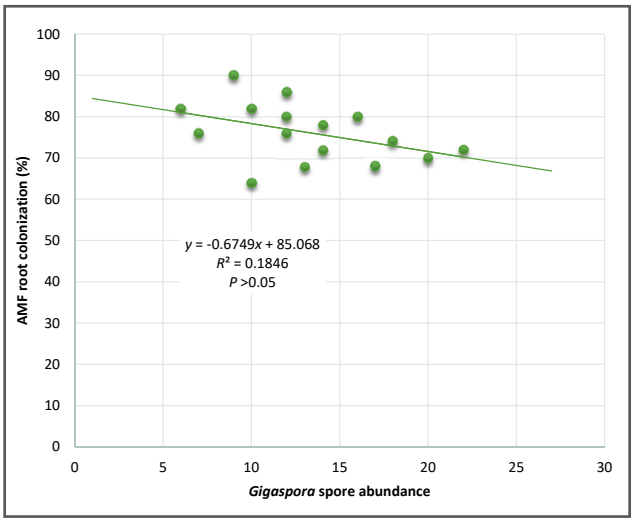

Figure 7 Pearson correlation between root colonization and *Gigaspora* spore abundance

higher in rhizosphere soils of tmaize and soybean. Host preferences have been demonstrated to exist to a certain extent in AMF, but strict host specificity seems to be rare (Pivato et al., 2007). This could possibly be attributed to the differences in rooting habits and nutrients demands of the crops (Douds et al. 2005) or amount of carbon transfer from the hosts to AMF. It has also been reported that AMF community composition depends on host plant species and, therefore, plant species may have varying degrees of selectivity on AMF species that range from selective specialists to non-selective generalists (Oehl et al. 2003; Scheublin et al. 2004). The relatively high root colonization in rhizosphere soil in this study could be due to the relatively low phosphorus in most tropical soils. In general, AMF spore density was found to be positively correlated with root colonization in this study. This could be due to the fact that some AMF species rely more on extensive formation of hyphal networks in roots and survival through spore formation as primary infective propagules in soils (Biermann and Linderman, 1983).

There was a prominent distribution of AMF genera among crop species, with higher relative abundance of *Glomus* genus and lower relative abundance of *Gigaspora* and *Scutellospora*. The genus *Glomus* was the most dominant and widely distributed followed by *Acaulospora* as also confirmed by this study. It has been previously reported that *Glomus* species are the most abundant among the glomeromycotan genera in tropical areas (Snoeck et al., 2010), regardless of the type of hosts and intensity of disturbance in the different ecosystems. Furthermore, *Glomus* species have the abiltiy to produce a relatively high number of spores within a very short period of time (Oehl et al., 2009). The significant reduction in relative spore abundance of *Gigaspora* and *Scutellospora* may be due to soil disturbances due to agricultural practices such as tillage (Boddington and Dodd, 2000). Furthermore, *Gigaspora*ceae have been reported to rely on spores as their primary infective propagules (Biermann and Linderman, 1983). Moreover, soil texture might also play a key role for their occurrence in tropical soils (Lekberg et al., 2007).

4 Conclusions

This study reveals the presence of different AMF genera and a high spore abundance in the rhizospheric soils of soybean, maize, sesame and sunflower grown in derived savannah of Nigeria. The crops regulates the intensity of mycorrhizal colonization and spore density. The information obtained from this study can be used to further investigate the impact of AMF symbiosis on productivity of the crops and could provide a primary basis for sustainable crop production in derived savannah agro-ecology of Nigeria.

5 References

AZCÓN-AGUILAR, C. and BAREA, J.M. (1997) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. In *Mycorrhiza*, vol. 6, pp. 457–464.

BIERMANN, B. and LINDERMAN, R.G. (1983) Use of vesicular-arbuscular mycorrhizal roots, intraradical vesicles and extraradical vesicles as inoculum. In *New Phytolologist*, vol. 95, pp. 97–105.

BODDINGTON, C.L., and DODD, J.C. (2000) The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. I. Field studies in an Indonesian ultisol. In *Plant Soil*, vol. 218, pp. 137–144.

BRUNDRETT, M.C. (2002) Coevolution of roots and mycorrhizas of land plants. In *New Phytologist*, vol. 154, pp. 275–304.

DAVISON, J. et al. (2015) Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. In *Science*, vol. 349, pp. 970- 973.

DOUDS, D.D. Jr, 2005. On-farm production and utilization of arbuscular mycorrhizal fungus inoculum. In *Canadian Journal of Plant Science*, vol. 85, pp. 15–21.

EVELIN, H., KAPOOR, R. and GIRI, B. (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. In *Annals of Botany*, vol. 104, pp.1263–1280.

GIOVANNETTI, M. and MOSSE, B. (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. In *New Phytologist*, vol. 84, pp. 489–500.

HAZARD, C. et al. (2013) The role of local environment and geographical distance in determining community composition of arbuscular mycorrhizal fungi at the landscape scale. In *The ISME Journal*, vol. 7, pp. 498–508.

LEKBERG, Y. et al. (2007) Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. In *Journal of Ecology*, vol. 95, pp. 95–105.

LIN, X. et al. (2012) Long-term balanced fertilization decreases arbuscular mycorrhizal fungal diversity in an arable soil in north China revealed by 454 pyrosequencing. In *Environmental Science & Technology*, vol. 46, pp. 5764–5771.

OEHL, F. et al. (2003) Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. In *Applied Environmental Microbiology*, vol. 69, pp. 2816–2824.

OEHL, F. et al. (2009) Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in longterm microcosms. In *Agric Ecosyst Environ*., vol. 134, pp. 257–268.

OEHL, F. et al. (2010) Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. In *Soil Biology and Biochemistry*, vol. 42, pp. 724–738.

OHSOWSKI, B.M. et al. (2014) Where the wild things are: looking for uncultured Glomeromycota. In *New Phytologist*, no. 204, pp. 171–179.

PEYRET-GUZZON, M. et al. (2016) Arbuscular mycorrhizal fungal communities and Rhizophagus irregularis populations shift in response to short term ploughing and fertilisation in a buffer strip. In *Mycorrhiza*, vol. 26, pp. 33–46.

PHILLIPS, J.M. and HAYMAN, D.S. (1970) Improved procedures for clearing roots and staining parasitic and VA mycorrhizal fungi for rapid assessment of infection. In *Trans Br Mycol Soc.*, vol. 55, no.158–161.

PIVATO, B. et al. (2007) Medicago species affect the community composition of arbuscular myccorhizal fungi associated with roots. In *New Phytologist*, no. 176, pp. 197–210.

RILLIG, M. C. (2004) Arbuscular mycorrhizae, glomalin, and soil aggregation. In *Canadian Journal of Soil Science*, vol. 84, pp. 355–363.

RILLIG, M.C. and Mummey, D.L. (2006) Mycorrhizas and soil structure. In *New Phytologist*, no.171, pp. 41–53

SCHENCK, N.C. and PEREZ, Y. (eds.) (1990) *Manual for identification of VA mycorrhizal fungi*. Gainesville: INVAM, University of Florida. 241 p.

SCHEUBLIN, T.R. et al.(2004) Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. In *Applied Environmental Microbiology*, vol. 70, pp. 6240–6246.

SCHÜBLER, A, SCHWARZOTT, D. and WALKER, C. (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. In *Mycology Research*, vol. 105, pp. 1413–1421.

SMITH, S.E., and READ, D.J. (2008) *Mycorrhizal symbiosis*. 3rd ed. New York: Academic Press. 787 p.

VERBRUGGEN, E., and TOBY KIERS, E. (2010) Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. In *Evolutionary Appl.*, no. 3, pp. 547–560.

YAMATO, M., IKEDA, S., and IWASE, K. (2009) Community of arbuscular mycorrhizal fungi in drought-resistant plants, Moringa spp., in semiarid regions in Madagascar and Uganda. In *Mycoscience*., vol. 50, pp. 100–105.

