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Temporal dynamics of wild herbaceous arbuscular mycorrhizal fungi under crop succession: Rice-bean-amaranth-okra-eggplant

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Abstract

Arbuscular mycorrhizal fungi (AMF) are ubiquitous symbionts that colonize approximately 80% of wild and cultivated plants, with the exception of a few botanical families such as *Amarantaceae*, *Brassicaceae*, *Cyperaceae*, and *Chenopodiaceae*, which mycorrhizae less or not at all. These symbioses are important because they improve the hydro-mineral nutrition of plants. During a succession of crops on a plot, it is possible to observe the diversity of AMF in the soil; this work fits into this framework. The experiment was carried out in pots in a completely randomized block design with 5 crops, including rice-beans-amaranth-okra-eggplant, repeated 5 times. The temporal dynamics of AMF were assessed during crop succession on the same soil. The results show a high species richness for rice, with 23 AMF species compared to 9 AMF species found on amaranth. Spore density and root colonization frequency show the same trend, respectively 5 to 38 spores per 100 g soil for rice compared to 3 to 10 spores per 100 g soil for amaranth. The colonization frequency ranged from 79 to 80% in rice and bean, while amaranth was only colonized to 13%. The introduction of amaranth during crop succession contributes to a decrease in species richness, spore density and AMF colonization frequency, in contrast to other crops.

Keywords: Mycorrhizae-Temporal-Dynamics-Diversity-Crops-Soil.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are the most ubiquitous symbioses, found in about 80% of terrestrial plants in natural ecosystems (Brundrett & Tedersoo, 2018; Trejo *et al.*, 2021). Their presence implies good phosphorus and nitrogen nutrition, enhanced plant growth, good resistance to fungal pathogens as well as water stress in most host plants (Mirzaei *et al.*, 2014; Hawkins *et al.*, 2015; Chen *et al.*, 2018, Zhang *et al.*, 2021).

The majority of crops (95%) such as maize, rice, beans, potatoes, and soybeans also form symbiotic relationships with AMFs, hence their important role in agroecosystems (Hijri, 2016; Zhang *et al.*, 2021). In this way, they enable the efficient use of fertilizers and can thus contribute to the reduction of fertilizer amounts by providing more phosphorus and nitrogen to plants through soil hyphal networks in exchange for carbohydrates derived from host plant photosynthesis (Smith & Read, 2008; Cavagnaro *et al.*, 2015). Thus, among the conditions that favor good activity of these symbioses are a good soil organic carbon content, an acid pH that creates stress conditions to induce their associations with AMF, and a low available phosphorus content in the soil (Tshibangu *et al.*, 2021).

In this case, there are several possible causes for the decline in the diversity of these AMF in the soil, including tillage, which fragments mycelia and reduces spore density, uncontrolled bush fires, cultivation techniques such as: the use of fungicides or high doses of phosphate fertilizers that inhibit the establishment of these symbioses. (Oehl *et al.*, 2008; Marinho *et al.*, 2019; Maquia *et al.*, 2021; Fall *et al.*, 2022), as well as the presence of crops that mycorrhize little or not at all, such as *Cyperaceae*, *Brassicaceae*, *Juncaceae*, *Caryophyllaceae*, *Chenopodiaceae*, as well as *Amarantaceae* in a succession of crops on a plot (Norman *et al.*, 1995; Derkowska *et al.*, 2015; Brundrett & Tedersoo, 2018). The aim of this study is to assess the temporal dynamics of AMFs in a rice-bean-amaranth-okra-eggplant crop succession.

MATERIALS AND METHODS

Crop Varieties in Succession

The varieties that were used in this experiment are listed in table 1 below:

Table 1: Varieties used in crop succession

Crops	Varieties	Crop cycle
Rice	*Supa*	120-160jrs
Bean	*RW2154*	80- 90 days
Amaranth	*Local*	40- 45 days
Okra	*Clemson Spinless*	80-90 days
Eggplant	*African eggplant*	80-90 days

Experimental soil

Soil from wild herbaceous of Kasapa fallow land was selected as the substrate for this experiment as it was the soil with the highest AMF diversity, low pH, high organic carbon content and low available phosphorus content, which would explain the good AMF activity and maintenance of diversity as described by Tshibangu *et al.*, 2020. The physical and chemical characteristics are detailed in Table 2 below:

Table 2: Physical and chemical characterization of experimental soil

Physico-chemical parameters	Properties
Location	**S** 11°36,31'36" E 27°28,36'06"
Color soil	Brown (7.5YR 5/4)
pHeau	4.3 - 4.9
Organic carbon	1.08 – 2.28 (%)
Total nitogen (%)	0.05-0.20 (%)
Total phosphorus (mg Kg^{-1})	112 - 328 (mg Kg^{-1})
Available phosphorus (mg Kg^{-1})	4.39- 8.33 (mg Kg^{-1})
Total potassium (Cmol (+) Kg $soil^{-1}$)	0.92 – 1.25 (%)
Soils types- WRB2014(IUSS Working GroupWRB2015)	Plinthosols

Cmol (+) Kg $soil^{-1}$: Centimol per kilogram soil

Setup Experiment

The experiment was conducted between November 2018 and February 2020 in the shaded net of the Faculty of Agronomic Sciences of the University of Lubumbashi, in vegetation pots using a completely randomized block design with 5 treatments representing the crops (rice-bean-amaranth-okra-eggplant), repeated 5 times. The choice of crops chosen here is justified by the fact that in a succession of crops in a farmer's field in the Lubumbashi region, crops such as maize or rice comes first, followed by *Fabaceae* (beans or soybeans), *Amaranthaceae* or *Brassicaceae*, *Malvaceae* or *Solanaceae*.

Data Collection

AMF spore density was assessed using the method of sieving a moist soil sample on a 45 µm mesh with 1 mm mesh, followed by centrifugation and the addition of sucrose solution (Walker *et al.*, 2016), the identification and classification of spores on slides and coverslips in polyvinyl lactoglycerol (PVLG) observed under the microscope according to their color, presence or absence of pedicels, mode of insertion and membrane ornamentation were based on the methods of Trappe (1982), Morton and Benny (1990); Oehl *et al.*, (2008) and those of the INVAM website (http://invam.caf.wvu.edu). The method of Phillips and Hayman (1970) was used to determine the frequency of root colonization by AMF. In this method, roots are thinned with 10% KOH. Roots are heated in a water bath for 30 min and stained with methylene blue. AMF structures showing colonization are: hyphae, vesicles and arbuscules. The frequency of root colonization was expressed as the number of roots bearing one of these structures, expressed as a percentage of the total number of roots observed.

Data Analyses

Species richness is the only index of AMF diversity that was calculated and presented in a presence/absence cross-tabulation for each crop. Elementary (descriptive) statistics were used to present the density of AMF spores and the changes in species richness over time in the form of quartiles. Relationships between crops and AMF species were established using the Venne diagram. The frequency of AMF root colonization was averaged using an evolutionary trend curve.

RESULTS

Species Richness of AMF

Table 3 below shows that in the initial soil the species richness was 28. During the crop succession, the variation was as follows: a high species richness was observed on rice (23 AMF species) and a low on amaranth (9 AMF species).

Table 3: AMF specific richness with mycorrhizae abundance in initial soil

AMF species list	Initial soil	Rice	Bean	Amaranth	Okra	Eggplant
Acaulospora alpine	+	+	+	-	-	-
Acaulospora cavernata	+	+	+	+	+	+
Acaulospora colossica	+	+	+	+	+	+
Acaulospora delicata	+	+	+	+	+	-
Acaulospora lacunosa	+	+	-	-	-	+
Acaulospora scrobiculata	+	+	+	+	+	+
Acaulospora trappei	+	+	+	-	-	-
Ambispora sp	+	+	+	+	+	+
Entrophospora infrequens	+	-	-	-	-	-
Entrophospora schenkii	+	-	-	-	-	-
Entrophospora sp	+	+	+	-	-	-
Funneliformis sp	+	+	+	+	+	+
Gigaspora albida	+	+	+	-	-	-
Gigaspora calospora	+	+	+	-	-	+
Gigaspora gigantea	+	+	+	-	-	-
Gigaspora margarita	+	+	+	-	-	-
Gigaspora rosea	+	+	+	+	-	+
Gigaspora sp	+	+	+	+	+	+
Glomus caledonicum	+	-	-	-	-	-
Glomus constrictum	+	+	+	+	+	+
Glomus mosseae	+	+	+	-	-	+
Glomus pubescens	+	+	+	-	+	+
Glomus sp	+	+	+	-	+	-
Racocetra gregraria	+	-	-	-	-	-
Rhizophagus sp	+	+	+	-	-	-
Scutellospora calospora	+	+	+	-	-	-
Scutellospora pellucida	+	-	-	-	-	-
Scutellospora scutata	+	+	-	-	-	-
AMF species number	**28**	**23**	**21**	**9**	**10**	**12**

Spore density and evolution of AMF specific richness under crops

The results in figure 1 (A) above show that the spore density ranged from 5 to 38 spores in 100g of soil on the rice crop, with 1/4 of the rice plants showing a density of less than or equal to 8, while half of the rice plants showed a density of less than or equal to 12 and 3/4 of the rice plants showed a density of less than or equal to 22 in 100g of soil; while in the amaranth crop the spore density varied from 3 to 10 spores, with 1/4 of the amaranth plants showing a density of 3 or less, half of the amaranth plants showing a density of 4 or less, and 3/4 showing 7 spores in 100g of soil. Figure 1B shows a decrease in the specific richness of AMF species observed across the crop succession, with the highest value observed on rice (23 species) and the lowest on amaranth (9 species), while the other species show intermediate specific richness.

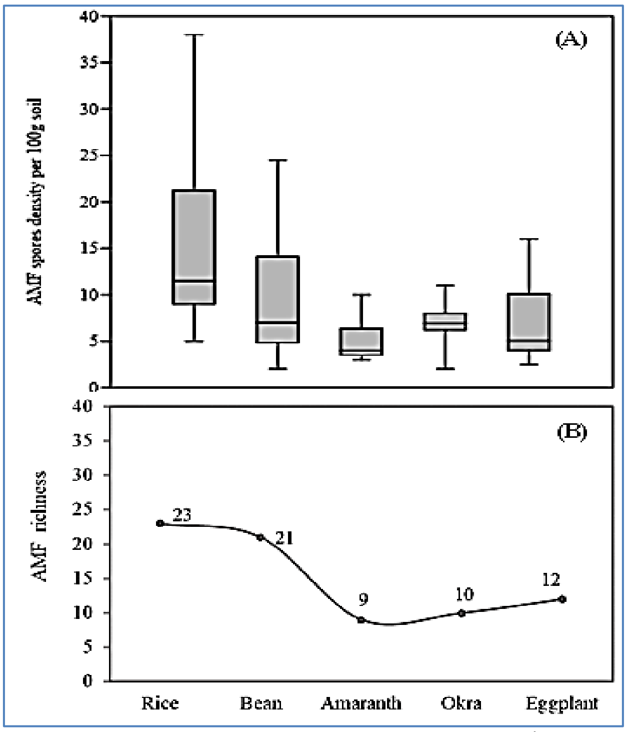

Figure 1: AMF spore density (A) in soil across successive crops (100g soil^{-1}) and (B) Specific richness of AMF species under a succession of crops.

AMF Species Identified Under Crop Succession

The inventory of common and specific AMF species, shown in figure 2, shows that 7 AMF species that were identified among the wild herbaceous plants are colonizing all the crops: *Acaulospora cavernata*, *Acaulospora colossica*, *Acaulospora scrobiculata*, *Ambispora* sp, *Funneliformis* sp, *Gigaspora* sp and *Glomus constrictum*. Amaranth, which is known as a crop that is less susceptible to mycorrhization, has 2 types of AMF in common with beans, rice, and okra, including *Acaulospora colossica* and *Gigaspora rosea*. The rice plants showed specificity by having a single AMF species "*Scutellospora scutata"*.

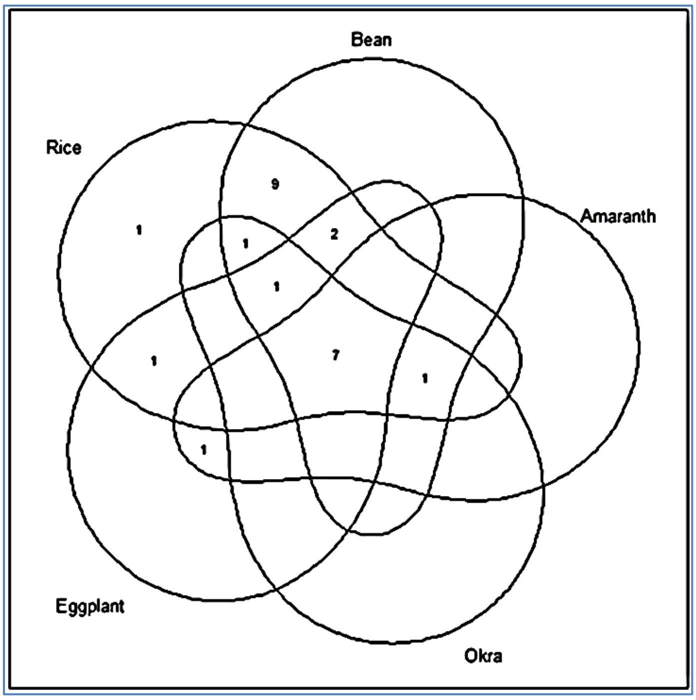

Figure 2: Venn diagram illustrating exclusive and share AMF species in crops succession.

Root Colonization Frequency

The results obtained in figure 3 below show that the majority of crops show a root colonization frequency of 45-80% by AMF, while a decrease in root colonization frequency is observed in the amaranth crop (15%).

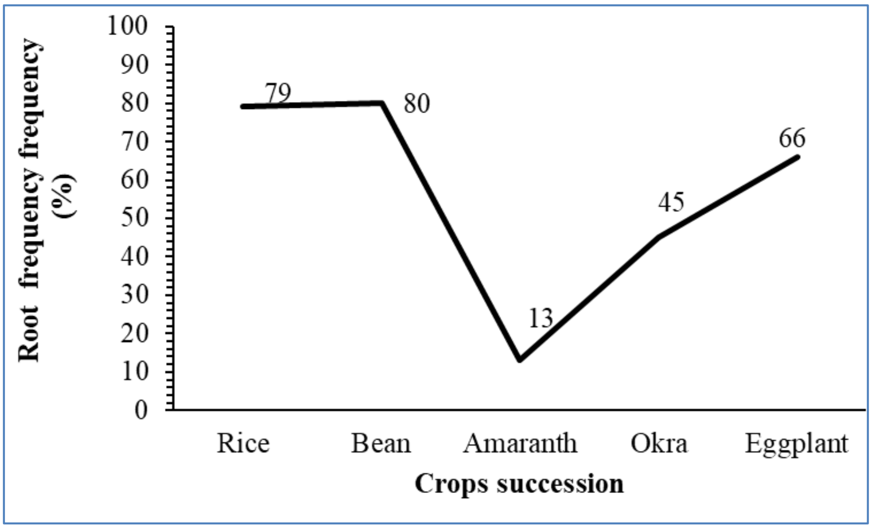

Figure 3: Root colonization frequency by the AMF on crop succession soil.

DISCUSSION

AMF Specific Richness and Spore AMF Density

During the crop succession, a variation in the specific richness of AMF species was observed in the rice crop (23 species) and a lower one in the amaranth crop (9 species), while the other species showed an intermediate specific richness. This would be due to the fact that the other botanical families are among the 80% of plant species and/or botanical families that perform these symbiotic relationships with AMF (Brundrett & Tedersoo, 2018; Trejo *et al*., 2021), with the exception of botanical families that mycorrhize very little or almost not at all, including the *Amarantaceae* (Derkowska *et al.*, 2015; Brundrett & Tedersoo, 2018). Spore density was highest in rice, ranging from 5 to 38 spores per 100 g of soil, compared to 3 to 10 spores observed on amaranth. The results of Muneer *et al.,* (2020) and Kuila & Ghosh (2022) show that the installation of a non-mycorrhizal crop in a rotation severely affects the AMF community through a decrease in spore density and mycorrhizal propagule activity, with a consequent negative effect on the yield of successive crops (Higo *et al.*, 2015).

AMF species identified on crop succession soil

Of the 28 AMF species identified in the soil of post-cultivation fallow land under wild herbaceous plants, 7 AMF species identified show a ubiquitous character by colonising all crops in succession: *Acaulospora cavernata, Acaulospora colossica, Acaulospora scrobiculata, Ambispora sp, Funneliformis sp, Gigaspora sp and Glomus constrictum*; this demonstrate the ubiquitous nature and ability that these AMF have to colonize most vegetable crops (Van der Heidjen, 1998; Brundrett & Tedersoo, 2018; Trejo *et al.*, 2021). The crop rotation can positively influence the function of AMF with a strongly mycotrophic crop of the grass or Fabaceae family, as the composition of AMF species can vary depending on the plant species and may take some time to be replaced (Higo *et al.*, 2015; Higo et *al.*, 2016). Unlike amaranth, which is known to be a less mycorrhizal crop, AMF shares 2 species with beans, rice and okra (*Acaulospora colossica*) et (*Gigaspora rosea*) This contradicts research by Njeru *et al.*, (2014) and Dada *et al.*, (2017), who showed that *Amaranthaceae* do not mycorrhize and that using AMF for amaranth cultivation is not a good option, yet it shares two species with mycotrophic crops.

Root Colonization Frequency

In crop rotations, colonized roots and hyphae are an important source of inoculum for the following crop (Brito *et al.*, 2012; Muneer *et al.*, 2020), as they take up nutrients such as phosphorus from the soil. However, the effect of introducing a non-mycotrophic crop such as *Amantaceae* reduces this root colonization by AMF from 80% to 13%, which would slow the development of AMF spores to infect the roots of the previous crop (Kuila & Ghosh, 2022). On the other hand, non-mycotrophic crops such as *Brassicaceae* and *Amaranthaceae* are known to lack the root architecture necessary to access sufficient phosphorus and form symbioses with AMF, which may explain this low root colonization rate (Berruti *et al.*, 2016).

CONCLUSIONS

The objective of this study was to investigate the temporal dynamics of arbuscular mycorrhizal fungi in a series of crops, some of which are more symbiotic and others less so. The selected crops were rice, beans, amaranth, okra and eggplant. The results show a high specific richness, with 23 AMF species colonizing the rice crop, in contrast to the amaranth, which had fewer (9 AMF species); the same trend was observed for spore density, with 5 to 38 spores in 100g of soil for the rice crop, compared to 5 to 10 spores in 100g of soil for the amaranth. The root colonization frequency by AMF was also higher on rice roots (80%), while it was only 13% on amaranth. Rice (family *Graminaceae*) and beans (family *Fabaceae*) were found to be most colonized by AMF, and the introduction of amaranth (*Amarantaceae*) into the crop succession chain contributed to a decrease in this colonization. The trend towards the resumption of mycorrhizal symbioses was observed in okra (*Malvaceae*), followed by eggplant (*Solanaceae*).

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