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Diversity of Arbuscular Mycorrhizal Fungi Species Associated with Soybean (*Glycine max* L. Merill) in Benin

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Abstract

Arbuscular Mycorrhizal Fungi (AMFs) could be used to sustainably improve crop yields. The present study evaluated the diversity of AMF species associated with soybean (*Glycine max* L. Merill) in main soybean-producing areas in Benin. Composite soil samples from 13 production areas at a rate of 04 villages per production areas were collected. A spore trapping device was set up to reveal the diversity of spores. The physical and chemical properties of the soils, the frequency and intensity of mycorrhization of roots, and the diversity of AMF spores were determined in the soil samples following trapping. As result, eight morphotypes belonging to four genera: *Glomus*, *Acaulospora Gigaspora* and *Disversispora* and three families: *Diversisporales*, *Glomérales and Paraglomérales* were observed. An important variability of spore densities was observed from one production areas to another with a higher abundance in the production areas of Copargo estimated at 3584 spores/100g soil. The biological diversity indexes as Shannon (0.0311), Simpson (0.0204) and Hill (0.0235), varied significantly ($p < 0.05$) from one production areas to another. There was significant correlation between the parameters studied, particularly between the physico-chemical parameters of the soils and between the physico-chemical parameters and the biological diversity indexes.

For the mycorrhization parameters, the mycorhization frequencies did not vary from one production areas to another, unlike the intensities, which significantly varied from one production areas to another (2.31% to 24.62%). Finally, this study revealed that the physico-chemical parameters of the soils had an influence on the other parameters studied. Moreover, there were an abundance and a significant diversification of AMFs associated with soybean in the different production areas, which are influenced by certain physico-chemical soil parameters.

Keywords

Arbuscular Mycorrhizal Fungi (AMFs), Soybean, Spore, Density, Diversity

1. Introduction

In Africa, one of the main constraints in agriculture is the constant decline in the level of soil fertility [1] which leads to crop yield losses. Farmers often rely on the use of chemical fertilizers whether registered or not, so as to increase crop productivity. This increased application of chemical fertilizer leads to increased crop yields, while having negative environmental impacts [2] and constitutes a major concern [3].

An alternative to application of chemical fertilizer could be the use of legumes as they are able to symbiose with nodule bacteria (rhizobia) present in most, if not all, tropical soils [4] and these rhizobia possess the nitrogenase complex, an enzyme capable of reducing atmospheric nitrogen (N2) into compounds assimilable by the host plant [4]. This attribute then fertilizes the soil allowing adequate yield in N-deficient soils where non-nodulated crops such as cereals fail. Maximizing N2 fixation is an economical way to cope with the shortage of expensive nitrogenous fertilizers in tropical countries and this biological nitrogen fixation by legume crops is even beneficial to subsequent cereal crops in rotation or association with the [4].

As a food legume with many agronomic and nutritional benefits [5], soybean (*Glycine max* L. Merill) is, sometimes considered as a miracle plant [6] and has progressively been adopted by several farmers, especially in Benin. The majority of the soybean farmers do not use fertilizers to improve the soil fertility level claiming that the crop does not need fertilizers [7]. This situation, despite the attribute of soybean plant able to fertilize soils, leads to deficiency in nitrogen, phosphorus and potassium in the soybean-producing soils, reducing the yield worryingly [8] [9].

In Benin, the importance of soybean cultivation is thus reflected in the different sectors of the economy and it appears beneficial to the whole society [10]. However, yields remain low and fluctuate around 1100 kg/ha [11] compared to the potential yield of 4000 kg per hectare [12]. This is said to result from poor agricultural practices and soil erosion leading to deficiency of nitrogen and as-

similable phosphorus and consequently to decline in soil fertility [7]. It is then crucial to identify the best practices able to increase soybean productivity without degrading soil and environment. One of these practices could be application of arbuscular mycorrhizal fungi (AMF) known to increase crop capacity in soil minerals absorption in general, and phosphorus in particular [13]. [14] Indicated that AMF increase crop absorption of phosphorus in two ways: first, by mineralizing organic phosphorus through activity of phosphatases located in mature arbuscular and intercellular hyphae, and second, by solubilizing insoluble phosphorus (tricalcium phosphate, rock powder) through acid production.

Mycorrhizal fungi are microorganisms naturally inhabiting crop rhizosphere, but the species richness depends on the crop species. The objective of the current study was to evaluate the diversity and density of arbuscular mycorrhizal fungi associated with soybean (*Glycine max* L. Merrill) cultivation in the major production areas in Benin.

2. Materials and Methods

2.1. Study Area

The study was carried out in the major soybean production areas of Benin. It covered 13 production areas, falling into 4 agroecological zones. Soil sampling was carried out during the rainy season in September 2017. Four sites were selected in each production areas resulting in a total of 52 samples for the study. Survey was conducted at each site in the soybean rhizosphere considering producers with at least 1 ha of cultivated area, farming experience (time) and land cultivation history (**Figure 1**).

2.2. Plant Material

The plant material used was the soybean variety TGX 1910-14F developed by the International Institute of Tropical Agriculture (IITA). This variety has good agronomic and morphological characteristics [7].

2.3. Trapping Device of AMF

In order to reveal the diversity of AMF in the sampled soils, a trapping device was set up using the trapping method of [15]. It consisted of growing soybean in pots containing the sampled soils. There were four replicates per soil sample. A total of 52 pots with 2 kg soil were displayed. The trial was daily watered until five days before harvest to create water stress and facilitate the multiplication of mycorrhizal fungi.

2.4. Evaluation of Mycorrhization Parameters

Roots were sampled, carefully washed and stained using modified method [16]. The youngest roots were sampled and cut into 1 - 2 cm length, immersed into a KOH solution (10%) and heated in a 90˚C water bath for about 45 - 60 min. The roots were then rinsed with water and immersed into a 0.5% Trypan Blue solution.

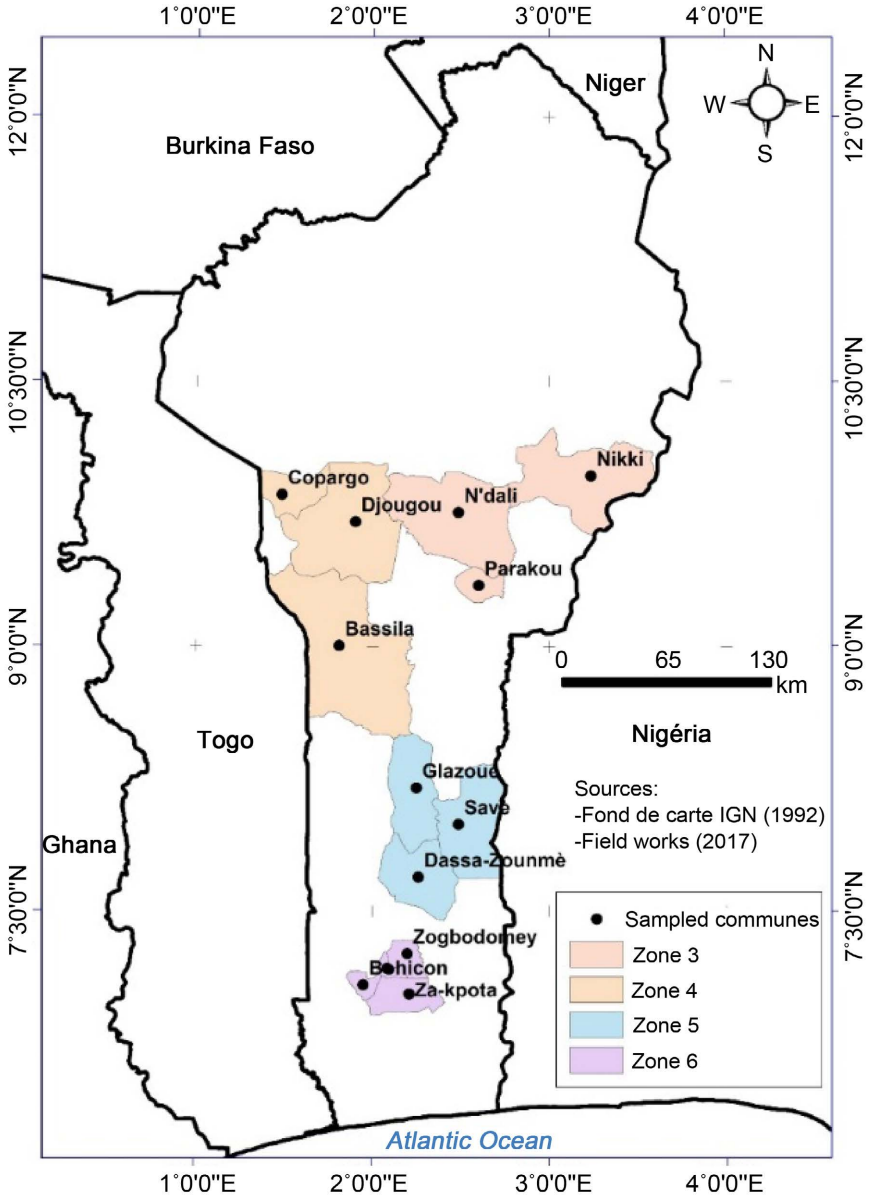

Figure 1. Map of soybean main production areas and soil sampling sites. Zone 3: South Borgou food-producing agro-ecological zone; Zone 4: West Atacora agro-ecological zone; Zone 5: Central cotton agro-ecological zone; Zone 6: Bar land agro-ecological zone (IRD, 2020).

The contents were placed in the water bath for 45 min at 90˚C again. The frequency and intensity of mycorrhization were assessed under a light microscope as described [17].

2.5. Spores Extraction

Spores were extracted by the wet sieving method described [18]. Dry soil sample of 100 g was suspended in 500 ml of tap water and stirred. After 30 seconds, the suspension was poured onto a series of four superimposed sieves of decreasing mesh size (500-200-100-50 µm). This was repeated three times in order to re-

cover maximum number of spores. For each sieve, the resulting mixture was suspended in tap water and then distributed into centrifuge tubes (Corex tubes). A viscosity gradient is created by carefully injecting at the bottom of each tube, using a pipette, firstly 5 ml of a 20% sucrose solution and secondly 5 ml of a 60% sucrose solution. The corex tubes were then centrifuged for 3 min at 3000 rpm and at 4˚C. The spores were finally recovered with a Pasteur pipette, rinsed with water through a 50-µm sieve and deposited in a petri dish for observation and enumeration.

2.6. Spores Enumeration and Identification

Spores abundance was assessed under a binocular magnifying glass using a gridded Petri dish (5 cm diameter). Spores were counted and classified into different morphotypes according to their size, color, shape, cluster shape and mode of attachment of the suspensory hyphae. They were then photographed using a binocular magnifying glass with a camera connected to a computer. Spore identification was carried out based on their morpho-anatomical characteristics. Biological diversity indices were then calculated.

For microscopic identification, healthy spores were mounted on glass slides and stained with polyvinyl lactic acid glycerol (PVLG) mixed with Melzer's reagent (1:1 vol/vol) as reported [19]. They were then left for 72 h in the dark. The spores were examined under a binocular microscope (G x 40) and photographed. Spore identification was done using INVAM (International culture collection of vesicular/arbuscular mycorrhizal fungi) database according to their morphological characteristics.

2.7. Biological Diversity Indexes

Different diversity indexes were used to assess the structure of the mycorrhizal fungi population referring or not to a concrete spatio-temporal framework.

2.7.1. The Shannon Diversity Index (*H'*)

The [20] diversity index is the most commonly used in the literature. It is defined as:

$$H' = \sum_{i=1}^{s} p_i \left(\ln p_i \right)$$

where

p_i = the proportional abundance or percent abundance of a species present ($p_i = n_i/N$).

n_i = the number of individuals counted for a species present.

N = the total number of individuals counted, all species combined.

S = the total or cardinal number of the list of species present.

2.7.2. Simpson's Diversity Index (1-*D*)

The Simpson index measures the probability that two randomly selected individuals belong to the same species [21]. It is defined as:

$$D = \frac{\sum N_i \left(N_i - 1\right)}{N\left(N - 1\right)}$$

where

N_i = number of individuals of species *i*.

N = total number of individuals.

2.7.3. Hill's Diversity Index (1-Hill)

It is a measure of proportional abundance, allowing the Shannon-Weaver and Simpson indices to be combined. It is defined by:

$$\text{Hill} = \left(1/\lambda\right)e^{H'}$$

where H' is Shannon diversity index.

2.7.4. Analysis of Soil Parameters

The physico-chemical analyses of the collected soil samples were carried out at the Laboratory of Soil Microbiology and Microbial Ecology (LMSEM) of the Faculty of Agricultural Sciences of the University of Abomey-Calavi in Benin. Granulometry was determined with Robinson pipette method; organic carbon was measured using [22]; available phosphorus was determined with [23] whilst pH_{water} and pHKCl were determined by potentiometric method and Cationic Exchange Capacity (CEC) [23].

2.8. Statistical Analysis

Statistical analyses were performed under R software version 4.1.0. After checking the normality and equality of variances, collected data and calculated parameters were subjected to an analysis of variance (ANOVA) at a threshold of 5%. The physical and chemical parameters of soil were used to assess the relationship among them and the diversity of AMFs using correlation tests (Pearson). Factorial Component Analysis (FCA) was carried out to obtain the characterization of the AMF genera in the different study sites.

3. Results

3.1. Density and Diversity of AMF Spores

AMF Spore Density in Soils

The results showed that the AMFs spore density significantly ($p < 0.0001$) varied from one site to another. Thus, spore densities are higher in Copargo soils (3584 spores/100g soil) and lower in Dassa soils (1146 spores/100g soil) (**Figure 2**).

3.2. Diversity of AMF Spores in Soils after Trapping

3.2.1. Biological Diversity Indices of AMFs in Sampled Soils

There was a significant difference ($p < 0.05$) among the biological diversity indices from the different studied production areas. The values of the Shannon diversity index vary from 1.58 to 1.97; those of the Simpson index ranged between 0.69 and 0.85. Thus, the production areas of Copargo recorded the highest

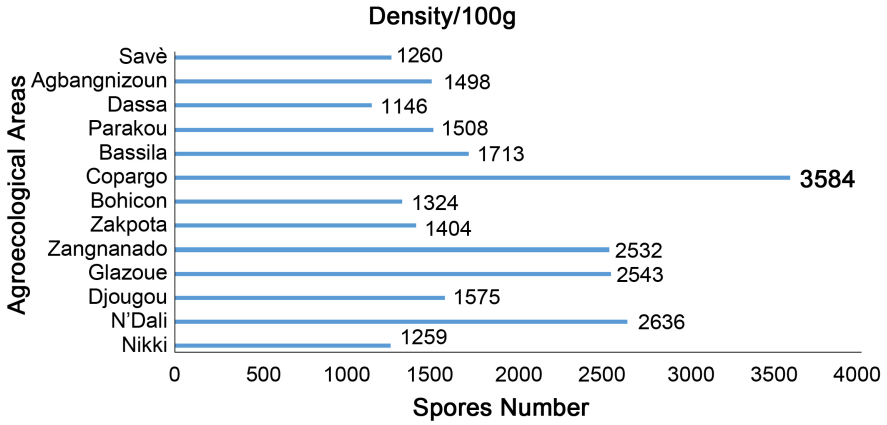

Figure 2. Density of AM fungal spores in soils from different studied areas. Y axis is studied production areas while X axis is AMF spores number per 100 g soil.

diversity indexes whereas the production areas of Zakpota recorded the lowest. As for the Hill index, it varied from 0.70 to 0.4. The diversity of AMFs therefore differs significantly from one production areas to another (**Table 1**).

3.2.2. Distribution of Morphotypes Inventoried in the Sampled Soils

Eight species were identified in all the soils sampled. The identified species were totally eight with two belonging to Glomus genus, three to Gigaspora genus, two to Acaulospora genus and one species to Diversispora genus. The results of the spore enumeration are as shown in **Table 2**. The eight (08) species identified were found in all the sampled soils with the exception of the species "*Scutellospora savannicola*" absent in the soils sampled in the production areas of Agbangnizoun.

3.2.3. Relative Abundance of Different Spores

All identified species were recorded in all sampled soils except for "*Scutellospora savannicola*" species absent in the soils sampled in the production areas of Agbangnizoun. The species with high dominance in all soils were "*Rhizophagus partial*" followed by "*Acaulospora denticulate*". The species with low dominance in sampled soils were "*Scutellospora savannicola*" and "*Racocetra crispa*".

3.3. Typology of Studied Communities According to AMF Species

The first two components present 80% of the information that is taken into account. The results of the data analysis can be validly exploited as they exceed 50% (**Figure 3**).

The results of the Factorial Component Analysis showed that the production areas of Dassa, Djougou, Savè, Bohicon, Zakpota and Bassila are negatively correlated by the dominance of *Rhizophagus partial*. *Diversispora sp* and *Scutellospora savanicola* are very well represented on axis 2 and characterize the production areas of Nikki. The production areas of Zangnanado and Glazoué are characterized by the species *Acaulospora denticulate* and *Paraglomus occultum*. As for the production areas of N'dali, Parakou and Copargo, they are positively

Table 1. Biological diversity indexes obtained from the sampled soils.

Production areas	Biodiversity indexes		
	Shannon	Simpson	Hill
Nikki	1.915	0.831	0.823
N'Dali	1.944	0.837	0.829
Djougou	1.878	0.811	0.811
Glazoué	1.777	0.790	0.786
Zangnanado	1.712	0.781	0.769
Zakpota	1.578	0.689	0.701
Bohicon	1.776	0.771	0.781
Copargo	1.972	0.845	0.835
Bassila	1.762	0.776	0.779
Parakou	1.945	0.834	0.829
Dassa	1.788	0.776	0.784
Agbangnizoun	1.810	0.821	0.801
Savè	1.824	0.794	0.797
p-value	0.0311*	0.0204*	0.0235*

*: Significant at the 5% level.

Table 2. Inventory of spores species in the sampled soils from different soybean-producing production areas in Benin.

Genres	Diversisporaceae	Glomeraceae		Acaulosporaceae		Gigasporaceae		
Species	*Diversispora* (*Unc*)	*Rhizophagus Partial*	*Paraglomus occultum*	*Acaulospora denticulate*	*Accaulospora sp*	*Gigaspora magarita*	*Racocetra crispa*	*Scutellospora savannicola*
Copargo	102.93b	242.97a	77.61abc	152.65bc	68.10c	98.99bc	61.72b	90.98d
N'Dali	67.63ab	191.88a	38.63abc	83.94ab	57.50bc	107.88c	42.31ab	69.13cd
Glazoue	34.44a	208.31a	78.88bc	170.94c	23.00a	34.25a	45.88ab	40.00abc
Zangnanado	18.97a	179.33a	84.51c	193.89c	47.72abc	61.44abc	20.53a	14.14ab
Djougou	51.31a	140.86a	35.25abc	50.94a	28.00ab	33.00a	24.72a	29.31abc
Parakou	40.81a	118.81a	36.69abc	44.19a	34.94ab	45.19ab	28.44a	28.00abc
Bassila	49.09a	173.16a	20.09ab	37.47a	14.41a	56.34abc	18.66a	58.94bcd
Agbangnizoun	25.06a	98.06a	42.25abc	88.75ab	49.07abc	53.06abc	18.25a	0.00a
Nikki	67.81ab	90.69a	23.31abc	24.50a	18.44a	35.06a	23,.63a	31.38abc
Bohicon	46.13a	139.69a	25.50abc	31.31a	20.50a	19.75a	18.00a	30.13abc
Zakpota	39.06a	184.13a	16.00a	30.81a	13.56a	29.88a	11.88a	25.63abc
Dassa	38.06a	119.06a	26.81abc	28.75a	15.88a	24.06a	15.75a	18.00abc
p-value	0.0159*	0.5015	0.0052*	0.0001*	0.0001*	0.0063*	0.0097*	0.0026*

Different letters indicate significant differences of the post hoc SNK test performed in case effects if the model were significant ($p \leq 0.05$).

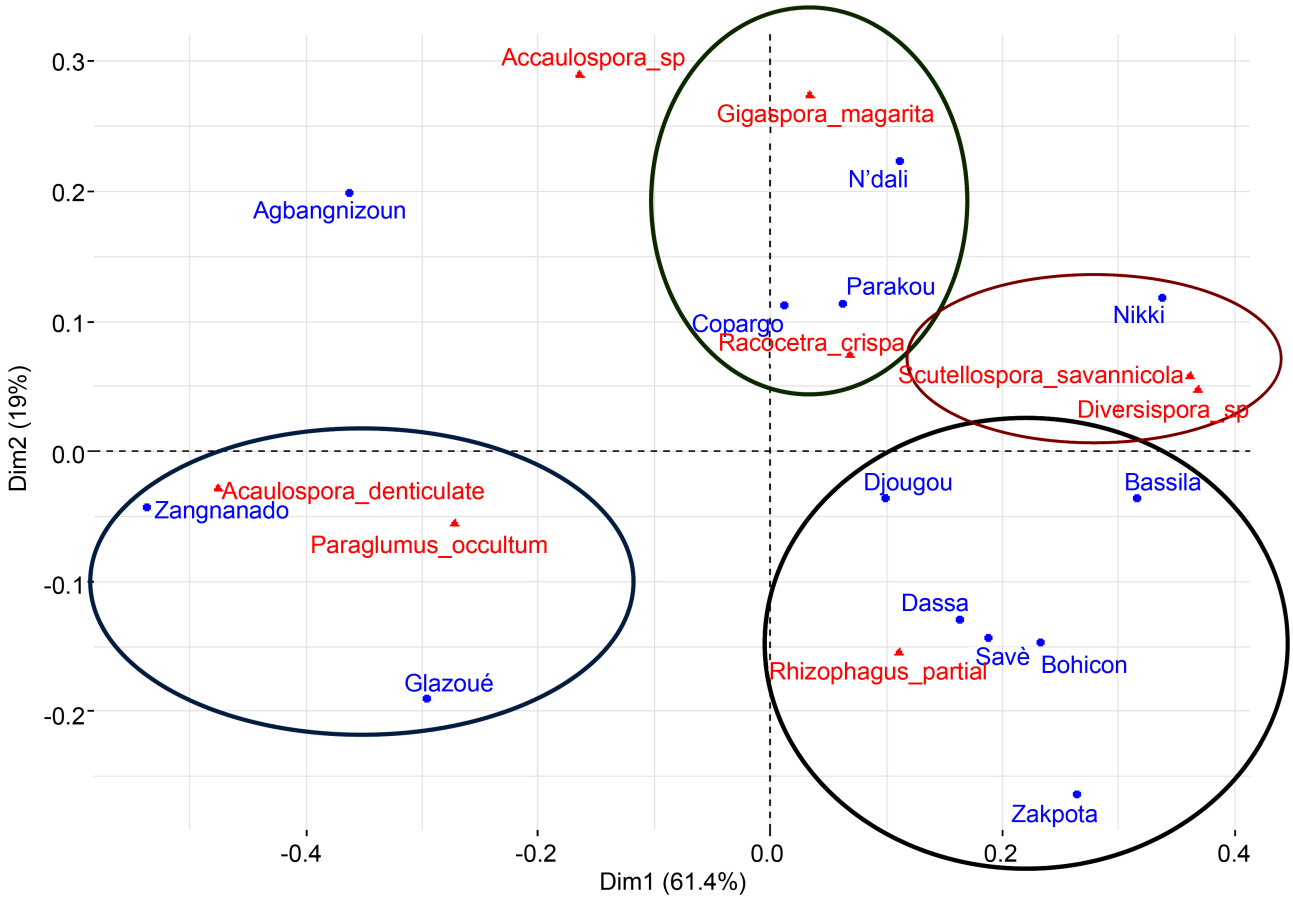

Figure 3. Characterization of AMF species by production areas.

correlated by the species *Gigaspora margarita* and *Racocetra crispa* well represented on axis 1.

3.4. Evaluation of the Relationship of the Studied Parameters

In general, in the study areas, there was a significant ($p < 0.05$) correlation among available phosphorus and Cationic Exchange Capacity; organic carbon and sodium; sodium, calcium, Shannon index and Hill index; potassium and magnesium; mycorrhization intensity and magnesium; sodium and Shannon intensity, Hill intensity. There was also a highly significant correlation ($p < 0.01$) between sodium and potassium. Significant differences ($p < 0.01$) are noted between calcium and magnesium; mycorrhization frequency and mycorrhization intensity and finally among all biological diversity indices (Shannon, Simpson and Hill) (**Table 3**).

4. Discussion

The results of the current study reveal a high level of spore density for all the studied production areas (approximately 23,980 spores/100g soil). The density of these spores under soybean cropping habitat thus varies from 1146 to 3584 spores per 100 g of soil. These recorded data are lower than those obtained by

Table 3. Correlation matrix (Pearson) between the studied parameters.

	pH	Pass	COrga	Na	K	Ca	Mg	CEC	F	I	Simpson	Shannon	Hill
pH	-	Ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Pass	ns	-	ns	ns	ns	ns	ns	**−0.3166***	ns	ns	ns	ns	ns
COrga	ns	ns	-	**0.3138***	ns	ns	ns	ns	ns	ns	ns	ns	ns
Na	ns	ns	**0.3138***	-	**0.4714****	**−0.2802***	ns	ns	ns	ns	ns	**0.3270***	**0.2930***
K	ns	ns	ns	**0.4714****	-	ns	**0.3120***	ns	ns	ns	ns	ns	ns
Ca	ns	ns	ns	**−0.2802***	ns	-	**0.6166*****	ns	ns	ns	ns	ns	ns
Mg	ns	ns	ns	ns	**0.3120***	**0.6166*****	-	ns	ns	**0.2935***	ns	ns	ns
CEC	ns	**−0.3166***	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
F	ns	ns	ns	ns	ns	ns	ns	ns	-	**0.6356*****	ns	ns	ns
I	ns	ns	ns	ns	ns	ns	**0.2935***	ns	**0.6356*****	-	ns	ns	ns
Simpson	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	**0.9621*****	**0.9873*****
Shannon	ns	ns	ns	**0.3270***	ns	ns	ns	ns	ns	ns	**0.9621*****	-	**0.9822*****
Hill	ns	ns	ns	**0.2930***	ns	ns	ns	ns	ns	ns	**0.9873*****	**0.9822*****	-

: Highly significant ($p < 0.01$); *: Very highly significant ($p < 0.001$) ns: Not significant at the 5% level; *: Significant at the 5% level; Pass: Available Phosphorus; COrga: Organic carbon; Na: Sodium; K: Potassium; Ca: Calcium; Mg: Magnesium; CEC: Cation exchange capacity; F: Frenquency; I: Intensity.

[24] under maize (*Zea mays* L.) (6260 spores per 100 g soil) but higher than the results reported by [25] under cowpea (*Vigna unguiculata* (L.) Walp.) (202 ± 42 per 100 g soil in different agro-ecological zones of Benin) [26], under cashew (*Anacardium occidentale* L.) plantation in central Benin [27], in the Wari-Maro classified forest in northern Benin under *Isoberlinia doka* (237 to 258 spores per 100 g soil). This difference in spore density levels could be due to the plant species itself. Indeed, a plant species can directly influence the abundance and composition of mycorrhizal fungi spores [28] [29]. According to [30], the presence and natural distribution of glomerales is a function of floristic composition as well as environmental conditions. [31] showed that legumes grow better on poor soils, partly because of the symbiotic microorganisms that colonize their root system such as mycorrhizal fungi and rhizobia. They have the ability to promote the development of fungal propagules (mycelial hyphae, spores) by releasing exudates into their rhizosphere that promote the development of microorganisms, including mycorrhizal fungi.

In early studies, [25] showed no significant difference between the different Agro-Ecological Zones of Benin (AEZ) regarding the spore density of *Glomeromycota* associated with cowpea cropping. These results are contrary to those from the current study showing a significant difference among production areas in terms of number of spores recorded with Copargo showing the highest. It should be noted that this production areas is located in one of the cotton growing areas and food crops and therefore in perpetual use, which favors the development of microorganisms. In addition, the fields constitute an environment of

continuous crop rotation which, in the long term, favors high abundance of spores [27]. In the current study, the soil samples were taken at full bloom, but also under water stress. It is said that period from sowing to flowering associated with favorable environmental conditions (moisture and presence of plant roots) would have allowed an activation and multiplication of spores and this could, indeed, explain the high densities recorded. According to [32] [33], the number of spores is higher in the soil after being subjected to water stress conditions. This is consistent with the results from this current study.

The eight species collected and identified on the basis of morphological characters under soybean cultivation in all studied production areas in Benin belonged to four genera: *Glomus*, *Acaulospora Diversispora and Gigaspora*. This specific richness obtained is lower than that obtained by [24] under maize in Benin (12 species divided into 04 genera: *Glomus*, *Acaulospora*, *Gigaspora and Scutellospora*), than that by [25] under cowpea cultivation in all Benin's AEZs (15 species divided into 04 genera: *Glomeraceae*, *Acaulosporaceae*, *Gigasporaceae* and *Claroideoglomeraceae*); by [34] in Senegal (15 species) and [35] under voandzou (*Vigna subterranea* (L.) Verdcourt) in different agro-ecological zones of Benin (14 species; 05 genera). On the other hand, this species richness is higher than that obtained in Benin [26] (07 species distributed in 03 genera: *Glomeraceae*, *Acaulosporaceae and Gigasporaceae*) and [27] (06 species distributed in 02 genera: *Glomeraceae*, *Gigasporaceae*).

The results in the current study showed that the diversity of fungal spores varies from one production areas to another with a dominance of the genus *Glomus* in almost all the production areas studied. Indeed, this dominance of *Glomus* has also been reported in AMF morphotypes in various tropical soils [27] [36] and in agricultural soils in temperate zones [28] [37]. According to [38], the predominance of species of the genus *Glomus* in most ecosystems suggests a better adaptation of this genus either to the most hostile conditions such as drought, salinity and other environmental stresses, or to a wide range of ecological niches [27]. Furthermore, according to [39] the genera *Glomus* would spread much more by spores which are forms of resistance of AMF to harsh conditions while the genera *Gigaspora* and *Scutellospora* would spread more with other types of propagules such as hyphae, extra root mycelial fragments. Determination of the indices of biological diversity indicates that there are significant differences between production areas on all indices (Shannon-Weiner, Simpson and Hill). The AMF community is very diverse in all communities.

The results from this study show positive and negative correlations among different parameters evaluated. Indeed, there is a positive correlation between the assimilable phosphorus and the biological diversity indices (case of the Savè production areas). The major role of AMFs is in the mobilization for the plant nutrients that are not very mobile in the soil, mainly phosphorus [24] [40]. Depending on the soil pH, this element is mostly trapped by iron, aluminum or calcium in forms that are difficult for plants to mobilize [41]. Moreover, according to the studies by [25] [42] [43] [44], there is a broad and diverse influ-

ence of soil properties on AMFs.

In general in the study communities, no significant correlation was found between physico-chemical parameters namely pH and mycorhization frequencies. These results are contrary to those [27], who reported a negative correlation between soil phosphorus, nitrogen and carbon with spore density, but also between mycorrhizal frequency and spore density. Subramanian *et al*. (2006) also showed that phosphorus application can positively or negatively influence spore production. According [45], phosphorus can be a limiting factor for spore abundance, either at too high or too low concentrations [46]. This could also be explained by the fact that sampling was not carried out in the same area and under the same conditions, but also that the specific diversity of the different samples is not the same and varies according to the production areas. It is therefore important to note that in order to optimize soybean production, it will be necessary to determine the right concentration of mycorrhizal inoculum to apply to get good yield.

5. Conclusion

The overall objective of this study was to evaluate the diversity of arbuscular mycorrhizal fungi (AMF) associated with soybean cultivation in the main soybean production areas of Benin. This study showed that there are mycorrhizal flora of significant spore density associated with soybean and which differs from one production areas to another. From the results obtained, it appeared that soil parameters had an influence on the diversity of AMF. Variability in spore densities was also observed from one production areas to another but with high levels of diversity not necessarily stipulating a strong mycorrhizal symbiosis. A total of eight species belonging to three genera and three families were obtained in the studied production areas. The genera are *Glomus*, *Acaulospora* and *Gigaspora* in all the production areas with an abundance of the genus *Glomus*. These species belong to the following families: *Diversisporales*, *Glomérales and Paraglomérales*. Finally, the current study revealed that there was an abundance and diversity of AMF associated with soybean cultivation in the different soybean-producing production areas in the Republic of Benin. It will be necessary to produce inoculums based on these local species and test their response on soybean crop to determine the best way to achieve good soybean productivity in Benin.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Saïdou, A., Kossou, D., Acakpo, C., Richards, P. and Kuyper, T.W. (2012) Effects of Farmers' Practices of Fertilizer Application and Land Use Types on Subsequent

Maize Yield and Nutrient Uptake in Central Benin. *International Journal of Biological and Chemical Sciences*, **6**, 365-378. https://doi.org/10.4314/ijbcs.v6i1.32

[2] Meena, R.S., Vijayakumar, V., Yadav, G.S. and Mitran, T. (2017) Response and Interaction of *Bradyrhizobium japonicum* and Arbuscular Mycorrhizal Fungi in the Soybean Rhizosphere. *Plant Growth Regulation*, **84**, 207-223. https://doi.org/10.1007/s10725-017-0334-8

[3] Ashoka, P., Meena RS. and Kumar, S. (2017) Green Nanotechnology Is a Key for Eco-Friendly Agriculture. *Journal of Cleaner Production,* **142**, 4440–4441. https://doi.org/10.1016/j.jclepro.2016.11.117

[4] Singh, S.R. and Rachie, K.O. (1985) Cowpea Research, Production and Utilisation. International Institute of Tropical Agriculture (IITA). John Wiley and Son, Chichester, 460.

[5] Ogumniyi, S., Johan, W., Andries, J. and Roem, W. (2012) Bacterial Translocation and *in Vivo* Assessment of Intestinal Barrier Permeability in Rainbow Trout (*Oncorhynchus mykiss*) with and without Soyabean Meal-Induced Inflammation. *Journal of Nutritional Science*, **5**, Article No. e26. https://doi.org/10.1017/jns.2016.7

[6] Aganze, M.V. (2014) Réponse de trois variétés de soja a l'inoculation par *Bradyrhizobium japonicum* sans limitation de phosphore et de potassium en territoire de kabare (République Démocratique du Congo). Mémoire présenté en vue de l'obtention du diplôme d'ingénieur agronome, 50 p.

[7] Zoundji, C.C., Houngnandan, P., Dedehouanou, H. and Toukourou, F. (2015) Determinants of Soybean [*Glycine max* (L.) Merrill] Production System in Bénin. *Journal of Experimental Biology and Agricultural Sciences*, **3**, 430-439. https://doi.org/10.18006/2015.3(5).430.439

[8] Javaheri, F. and Baudouin, J.P. (2001) Soja (*Glycine max* (L.) Merrill.). *Agriculture en Afrique Tropicale*, No. 1634, 660-883.

[9] Houngnandan, P., Sanginga, N., Woomer, P., Vanlauwe, B. and Van Cleemput, O. (2000) Response of *Mucuna pruriens* to Symbiotic Nitrogen Fixation by Rhizobia Following Inoculation in Farmers' Fields in the Derived Savana of Benin. *Biology and Fertility of Soils,* **30**, 558-565.

[10] Kpenavoun C.S., Okry, F., Santos, F. and Hounhouigan, D.J. (2018) Efficacité technique des producteurs de soja du Bénin. *Annales des sciences agronomiques,* **22**, 93-110.

[11] Agence internationale de l'énergie atomique (2020, May 20) Le Bénin augmente sa production et ses exportations de soja au moyen d'engrais biologiques et de la technologie isotopique. https://www.iaea.org/fr/newscenter/news/le-benin-augmente-sa-production-et-ses-exporta-tions-de-soja-au-moyen-dengrais-biologiques-et-de-la-technologie-isotopique

[12] Batamoussi, M.H., Boulga, J., Yolou, I., Tokore, J.S.B.O.M., Lafia, K. and Issa, A. (2016) Analysis of Peasant Practices for Soy Production (*Glycine max*) in the District of Kalale (Northern-Bénin): Implication for Their Improvement. *International Journal of Innovation and Applied Studies*, **25**, 501-509.

[13] Vestberg, M. and Estaun, V. (1994) Micropropagated Plants, an Opportunity to Positively Manage Mycorrhizal Activities. In: Gianinazzi, S., Schuepp, H., Eds., *Impact of arbuscular mycorrhizas on Sustainable Agriculture and Natural Ecosystems*, Birkhäuser, Basel, 217-226. https://doi.org/10.1007/978-3-0348-8504-1_17

[14] Hernandez-Sebastia, C., Samson, G., Bernier, P.Y., Piché, Y. and Desjardins, Y. (2000) *Glomus intraradices* provoque des changements différentiels dans les con-

centrations d'acides aminés et d'amidon de fraises *in vitro* soumises à un stress hydrique. *New Phytologist*, **148**, 177-186. https://doi.org/10.1046/j.1469-8137.2000.00744.x

[15] Morton, J.B. and Benny, G.L., (1990) Revised Classification of Arbuscular Mycorrhizal Fungi (*Zygomycetes*): A New Order Glomales and Gigasporineae and Two New Families Acaulosporaceae and Gigasporaceae with an Emendation of Glomaceae. *Mycotaxon,* **37**, 471-491.

[16] Phillips, J.M. and Hayman, D.S. (1970) Improved Procedures for Clearing Roots and Staining Parasitic and Vesicular Arbuscular Mycorrhizal Fungi for Rapid Assessment of Infection. *Transactions of the British Mycological Society*, **55**, 158-161, IN16-IN18. https://doi.org/10.1016/S0007-1536(70)80110-3

[17] Trouvelot, A., Fardeau, J.C., Plenchette, C., Gianinazzi, S. and Gianinazzi-Pearson, V. (1986) Nutritional Balance and Symbiotic Expression in Mycorrhizal Wheat. *Physiologie Végétale*, **24**, 300.

[18] Gerdemann, J.W. and Nicolson, T.H. (1963) Spores of Mycorrhizal Endogone Species Extracted from Soil by Wet Sieving and Decanting. *Transactions of the British Mycological Society*, **46**, 235-244. https://doi.org/10.1016/S0007-1536(63)80079-0

[19] Brundett, M.C., Melville, L. and Peterson, R.L. (1994) Practical Methods in Mycorrhiza Research. Mycologue Publications, Sidney, British Columbia, Canada.

[20] Shannon, C.E. and Weaver, W. (1963) A Mathematical Theory of Communication. Paperback Edition, University of Illinois Press, Urbana.

[21] Simpson, E.H. (1949) Measurement of Diversity. *Nature*, **163**, 688. https://doi.org/10.1038/163688a0

[22] Walkley, A. and Black, I.A. (1934) An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method. *Soil Science*, **37**, 29-37. https://doi.org/10.1097/00010694-193401000-00003

[23] Bray, R.H. and Kurtz, L.T. (1945) Determination of Total, Organic, and Available Forms of Phosphorus in Soils. *Soil Science*, **59**, 39-45. https://doi.org/10.1097/00010694-194501000-00006

[24] Bossou, L.-D.R., Houngnandan, H.B., Adandonon, A., Zoundji, C. and Houngnandan, P. (2019) Diversité des champignons mycorhiziens arbusculaires associés à la culture du maïs (*Zea mays* L.) au Bénin. *International Journal of Biological and Chemical Sciences*, **13**, 597-609. https://doi.org/10.4314/ijbcs.v13i2.2

[25] Johnson, J.-M., Houngnandan, P., Kane, A., Sanon, K.B. and Neyra, M. (2013) Diversity Patterns of Indigenous Arbuscular Mycorrhizal Fungi Associated with Rhizosphere of Cowpea (*Vigna unguiculata* (L.) Walp.) in Benin, West Africa. *Pedobiologia*, **56**, 121-128. https://doi.org/10.1016/j.pedobi.2013.03.003

[26] Balogoun, I., Saïdou, A., Kindohoundé, N.S., Ahoton, E.L, Amadji, G.L., Ahohuendo, B.C., Babatoundé, S., Chougourou, D., Baba-Moussa, L. and Ahanchédé, A. (2015) Soil Fertility and Biodiversity of Arbuscular Mycorrhizal Fungi Associated with Cashew's *Anacardium occidentale,* L. Cultivars Characteristics in Benin (West Africa). *International Journal of Plant and Soil Science,* **51**, 50-63. https://doi.org/10.9734/IJPSS/2015/13817

[27] Houngnandan, P., Yemadje, R.G.H., Kane, A., Boeckx, P. and Van Cleemput, O. (2009) Les glomales indigènes de la forêt claire à *Isoberlinia doka* (Craibet Stapf) à Wari-Maro au centre du Bénin. *Tropicultura,* **27**, 83-87.

[28] Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T. and Wiemken, A. (2003) Impact of Land Use Intensity on the Species Diversity of Arbuscular Mycorrhizal

fungi in Agroecosystems of Central Europe. *Applied and Environmental Microbiology,* **69**, 2816-2824. https://doi.org/10.1128/AEM.69.5.2816-2824.2003

[29] Lovelock, C.E., Wright, S.F., Clark, D.A., Ruess, R.W. (2004) Soil Stocks of Glomalin Produced by Arbuscular Mycorrhizal Fungi across a Tropical Rain Forest Landscape. *Journal of Ecology*, **92**, 278-287. https://doi.org/10.1111/j.0022-0477.2004.00855.x

[30] Brundrett, M. (2009) Mycorrhizal Associations and other Means of Nutrition of Vascular Plants: Understanding the Global Diversity of Host Plants by Resolving Conflicting Information and Developing Reliable Means of Diagnosis. *Plant and Soil*, **320**, 37-77. https://doi.org/10.1007/s11104-008-9877-9

[31] De la Cruz, R.E. & Garcia, M.U. (1992) Nitrogen Fixation and Mycorrhizae in Acacias on Degraded Grasslands. In: Kamis, A. and Taylor, D.A., Eds., *Tropical Acacias in East Asia and the Pacific*, Winrock International, Bangkok, 59-71.

[32] Jacobson, K.M. (1997) Moisture and Substrate Stability Determine VA-Mycorrhizal Fungal Community Distribution and Structure in Arid Grassland. *Journal of Arid Environments*, **35**, 59-76. https://doi.org/10.1006/jare.1995.0140

[33] Bohrer, G., Kangan-Zur, V., Roth-Bejerano, N., Ward, D., Beck, G. and Di Bonifacio, E. (2003) Effect of Different Kalahari-Desert VA Mycorrhizal Communities on Mineral Acquisition and Depletion from Soil by Hast Plants. *Journal of Arid Environment,* **55**, 193-208. https://doi.org/10.1016/S0140-1963(03)00047-8

[34] Diop, T.A., Gueye, M., Dreyfus, B.L., Plenchette, C. and Strullu, D.G. (1994) Indigenous Arbuscular Mycorrhizal Fungi Associated with *Acacia albida* Del. in Different Areas of Senegal. *Applied et Environmental Microbiology*, **60**, 3433-3436. https://doi.org/10.1128/aem.60.9.3433-3436.1994

[35] Mama, F. (2018) Distribution et diversité des champignons mycorhiziens arbusculaires associés au Voandzou *(Vigna subterranea* (L.) Verdcourt) dans différentes zones agro-écologiques du Bénin. Mémoire pour l'obtention du diplôme de master, Université Nationale d'Agriculture, Bénin, 82 p.

[36] Tchabi, A., Coyne, D., Hountondji, F., Lawouin, L., Wiemken, A. and Oehl, F. (2008) Arbuscular Mycorrhizal Fungal Communities in Sub-Saharan Savannas of Benin, West Africa, as Affected by Agricultural Land Use Intensity and Ecological Zone. *Mycorrhiza*, **18**, 181-195. https://doi.org/10.1007/s00572-008-0171-8

[37] Mathimaran, N., Ruh, R., Vullioud, P., Frossard, E. and Jansa, J. (2005) *Glomus intraradices* Dominates Arbuscular Mycorrhizal Communities in a Heavy textured Agricultural Soil. *Mycorrhiza*, **16**, 61-66. https://doi.org/10.1007/s00572-005-0014-9

[38] Błaszkowski, J., Tadych, M. and Madej, T. (2001) Glomus Arenarium, a New Species in Glomales (*Zygomycetes*). *Acta Societatis Botanicorum Poloniae*, **70**, 97-101. https://doi.org/10.5586/asbp.2001.013

[39] Brito, I., Goss, M.J. and De Carvalho, M. (2012) Effect of Tillage and Crop on Arbuscular Mycorrhiza Colonization of Winter Wheat and Triticale under Mediterranean Conditions. *Soil Use and Management*, **28**, 202-208. https://doi.org/10.1111/j.1475-2743.2012.00404.x

[40] Duponnois, R., Sanon, A., Hafidi, M., Ndoye, I. and Bâ, A.M. (2013) Généralités sur la symbiose mycorhizienne. In: Duponnois, R., Hafidi, M., Ndoye, I., Ramanankierana, H. and Bâ, A.M., Eds., *Des Champignons Symbiotiques Contre La Desertification Ecosystemes Mediterraneens, Tropicaux et Insulaires*, IRD Editions, Marseille, 20-55.

[41] Hinsinger, P. (2001) Bioavailability of Soil Inorganic P in the Rhizosphere as Affected by Root Induced Chemical Changes: A Review. *Plant & Soil*, **237**, 173-195.

https://doi.org/10.1023/A:1013351617532

[42] Sano, S.M., Abbott, L.K., Solaiman, M.Z. and Robson, A.D. (2002) Influence of Liming, Inoculum Level and Inoculum Placement on Root Colonization of Subterranean Clover. *Mycorrhiza,* **12**, 285-290. https://doi.org/10.1007/s00572-002-0185-6

[43] Mechri, B., Mariem, F.B., Baham, M., Elhadj, S.B. and Hammami, M. (2008) Change in Soil Properties and the Soil Microbial Community Following land Spreading of Olive Mill Wasterwater Affects Olive Trees Key Physiological Parameters and the Abundance of Arbuscular Mycorrhizal Fungi. *Soil Biology and Biochemistry*, **40**, 152-161. https://doi.org/10.1016/j.soilbio.2007.07.020

[44] Gryndler, M., Hrselová, H., Cajthaml, T., Havránková, M., Rezáčová, V., Gryndlerová, H. and Larsen, J. (2009) Influence of Soil Organic Matter Decomposition on Arbuscular Mycorrhizal Fungi in Terms of Asymbiotic Hyphal Growth and Root Colonization. *Mycorrhiza*, **19**, 255-266. https://doi.org/10.1007/s00572-008-0217-y

[45] Amijee, F., Tinker, P.B. and Stribley, D.P. (1989) The Development of Endomycorrhizal root Systems. VII. A Detailed Study of Effects of Soil Phosphorus on Colonization. *New Phytologist*, **111**, 435-446. https://doi.org/10.1111/j.1469-8137.1989.tb00706.x

[46] Lagrange, A. (2009) Etudes écologiques et microbiologiques des espèces du genre Costularia (Cyperaceae) pionnières des sols ultramafiques de Nouvelle-Calédonie: applications à la restauration écologique, Nouméa, Nouvelle-Calédoni.

Appendix

Webographie

http://www.fao.org/faostat/fr/#data/QC/visualize

http://www.universalis.fr/encyclopedie/soja/

Mycological publication, Waterloo, Canada, 161p.