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Soil Fertility and Biodiversity of Arbuscular Mycorrhizal Fungi Associated with Cashew's (*Anacardium occidentale* L.) Cultivars Characteristics in Benin (West Africa)

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Authors' contributions

This work was carried out in collaboration between all authors. Author NSK conducted the study under the supervision of the authors IB and AS. Authors AS and ELA designed the study. Author IB wrote the protocol and the first draft of the manuscript under the supervision of author AS. GLA, BCA, SB and DC managed the literature searches. All authors collaborated to the revision and improvement of the initial draft submitted. All authors read and approved the final manuscript.

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ABSTRACT

The present study aims to assess the biodiversity of arbuscular mycorrhizal fungi (AMF) associated with cashew (*Anacardium occidentale* L.) cultivars characteristics in Benin (West Africa). 108 soil samples were collected at 0-20, 20-40 and 40-60 cm depth under cashew trees at Glazoué in the centre Benin. Cashew's cultivars characteristics regarding nut and apple size, apple colors and classes of Diameter at Breast Height (DBH) were the treatments. Soil chemical properties, AMF spores' density and the diversity of AMF in the soil were assessed. Plantation of cashew improved the stock of soil organic carbon (11.24±0.63 and 10.93±0.71 g/kg for 0-20 cm soil depth respectively for cultivars producing big nuts and big apples and cultivars producing small nuts and small apples) and total nitrogen content (1.29±0.11 and 1.21±0.12 g/kg for 0-20 cm soil depth respectively for cultivars producing big nuts and big apples and cultivars producing small nuts and small apples) but it depletes available phosphorus (36.86±6.53 and 41.42±9.22 mg/kg for 0-20 cm soil depth respectively for cultivars producing big nuts and big apples and cultivars producing small nuts and small apples) and exchangeable potassium (0.93±0.10 and 0.79±0.04 cmol/kg for 0-20 cm soil depth respectively for cultivars producing big nuts and big apples and cultivars producing small nuts and small apples). Seven species of AMF (*Scutelospora gregarine*, *Acaulospora colossica*, *Acaulospora lacunosa*, *Enthrophospora infrequens*, *Glomus hoi*, *Glomus geosporum* and *Glomus sp.*) were identified. They belong to three families and four genera. The species belonging to Glomeracea family were dominants (91.8%) against 5.5% and 2.7% respectively for the Gigasporaceae and Acaulosporaceae. In general, the average numbers of AMF spores were 5.63±0.26; 3.54±0.19 and 1.94±0.15 per gramme of dry soil respectively for 0-20, 20-40 and 40-60 cm depth. The community of AMF was fairly diversified in the soil under cashew's trees. There was an equitable distribution of AMF genera associated with cashew's cultivars characteristics tree.

Keywords: Cashew plantation; Shannon-Wiener diversity index; Pielou index; land-use system; tropical ferruginous soils.

1. INTRODUCTION

The low productivity followed by the instability of the cashew tree's yields recorded during these past years could be justified by some constraints especially climate change and the chronic soil fertility decline marked by nitrogen and phosphorus deficiency [1]. Thus, in many African countries, especially in West Africa, for a sustainable land-use management, there is a need for better knowledge on the processes and factors that govern the bioavailability of soil nutrients to plants, including the root–soil interactions of microorganisms in the rhizosphere [2,3].

On the tropical and subtropical soils especially the tropical ferruginuous soil, the supply of phosphorus for most crops is very important because of its low availability [4,3]. Phosphorus deficiencies are often corrected using rock phosphate or inorganic fertilizers and often, the long-term application of these fertilizers leads to the decrease in pH and exchangeable bases and in turn causes the drop in crop productivity [5]. This decline in crop productivity is due to the fact that, the soil is acidified and becomes unsuitable for the development and growth of plants. In addition, the limited financial resources of smallholder farmers, and the unavailability of these fertilizers justify the weak adoption of fertilization practices to sustain cashew trees' productivity in Benin [1].

For these reasons, the management of symbiotic fungal populations would become an alternative to improve plant nutrition, water use efficiency and plant health result of sustainability in the production [3]. In most ecosystems, organisms living in the rhizosphere support plant growth and productivity in several ways [3]. In this context, mutually beneficial associations between these organisms and the plants may play an important role in the ecology of natural ecosystems, but also in terms of management of sustainable agriculture.

It appears from the study carried out by [1] in Benin that, cashew tree contributes to nutrient cycling under plantation by pumping nutrient in the deep layer, stocking them in the leaves that are recycled during leaves litter decomposition. Soil quality improvement could also be attributed to intensive activity of the arbuscular mycorrhizal fungi (AMF) associated with the plant fine roots [6,7,8]. The potential of AMF in increasing plant productivity has been recognized [9,6,4].

AMF can offer considerable benefits to plants as a result of their ability to increase the uptake of poorly mobile soil nutrients especially P [3,10,11]. Furthermore, they increase tolerance to water stress, induce greater resistance to pathogens and reduce sensitivity to toxic substances present in the soil, formation and maintenance of soil structure and increase C input to soils, crop growth and in some case yield [10,12]. AMF symbiosis in agriculture is a reasonable point to initiate the evaluation of mycorrhizal functioning in a system that approaches ideal management of low input sustainable agriculture [3]. Therefore, AMF take role in maintaining ecosystem processes by promoting plants fitness through a range of mechanisms; protecting plant host from soil pathogens and improving soil structure, enhancing water and nutrient uptake [13], increase the efficiency of fertilizer use and plant growth.

The presence of perennial mycorrhizal tree species with deep roots increases the volume of soil to be exploited and there, improves the efficiency of P cycling by AMF [10,14]. In agricultural systems under tropical climate, AMF are essential for maximizing the utilization of P and other soil nutrients [10] therefore. The development of AMF varies with host species, plant life history stage, resource availability and abiotic conditions, soil type, depth and season [15]. High spore and AM populations are found during the dry season, under low input agriculture, low tillage agricultural systems and plant phenology [3,15]. Knowledge on the number of AM fungal species associated with respective tree species under natural ecosystems, and the influence of environmental factors on AM fungal spore density, their diversity, distribution, establishment and survival over the time are of prime importance in identifying and utilizing the most suitable mycorrhizal species for large scale inoculation programs [6,3,8].

Recent works have been done on improving the productivity of cashew through inoculation with AMF [5]. These researches showed positive effect of arbuscular mycorrhizal fungi inoculum on the growth of the tree nursery and also, showed that the roots of the cashew are infected with strains of fungi used. Furthermore, it has been proved that, cashew trees develop symbiotic association with AMF [8,16]. Five genera of AMF (*Acaulospora*, *Gigaspora*, *Scutellospora*, *Entrophospora* and *Glomus*) associated with cashew trees were identified and, the genus *Glomus* is the most predominant.

In Benin, there is a lack of knowledge concerning the diversity and efficient species contributing in the improvement of cashew trees nutrition. Furthermore, few studies have been carried out on the symbiosis between AMF and cashew trees in the cropping systems in Benin. The purpose of this work was to study the biodiversity of AMF associated with different cultivars characteristics of cashew trees in Benin for their valorization as mycorrhizal inoculum to improve the trees' productivity. Specifically, it aims to 1) study the effect of cultivars characteristics and Diameter at Breast Height of cashew trees on soil chemical properties at different depths; and 2) study the diversify and the density of AMF species in different soil depths regarding the cultivars characteristics and classes of Diameter at Breast Height of the cashew trees.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out on cashew (*Anacardium occidentale* L.) plantation in Adourékoman village, district of Glazoué in the centre of Benin around 234 km from Cotonou, economic capital. It is located between 7º91'58''N and 2º27'30''E and 152 masl. The study area has a transition climate between sub-equatorial in south and Sudanese in north. The average yearly rainfall varies from 960 to 1256 mm and the average yearly temperature varies between 24 and 29ºC. The soil is essentially dominated by tropical ferruginous soils from Precambrian crystalline rocks (granite and gneiss), classified as Ferric Lixisol [17].

2.2 Study Plant Selection

The cashew (*Anacardium occidentale* L.) trees were identified based on a forest inventory carried out from June to July 2013. These cashew trees were selected regarding the two cultivars characteristics (Table 1) identified from a study on the agro-morphological characterization of the cultivars of cashew trees in Benin [18].

In addition to these two cashew's cultivars characteristics (nuts and apples size), the trees were selected according to the apples colors (red and yellow) and three classes of Diameter at Breast Height (DBH): 0-20, 20-40 and 40-60 cm. Three trees were considered as replications. In total, we had 2 cashew's cultivars characteristics x 2 apples colors x 3 classes of DBH x 3 replications = 36 trees. The nuts and apples sizes were appreciated based on visual observation, therefore, no measurement were done.

Table 1. Characteristics of the two cultivars of cashew tree identified

	Cashew nuts and apples shape	
	Apples	**Nuts**
Cultivars 1	Very large and long apples with round base and round apex.	Very large nuts with oblong shape and round apex.
Cultivars 2	Small apples	Small nuts having kidney form with round base

2.3 Soil Sampling and Preparation for the Laboratory Analysis

Under each cashew tree identified, the soils were sampled at 0-20, 20-40 and 40-60 cm depth considering the North, South, West and East and the samples were mixed to obtain a representative composite sample per tree and per depth. In total, 108 soils samples were collected (36 trees x 3 depths). In laboratory, the soils samples were air dried and sieved at 2 mm in order to remove the rough materials. These samples were used for the chemical analysis and for the AMF spores extraction.

2.4 Soil Chemical Analyses

Soil chemical analyses were performed in the Laboratory of Soil Sciences of the Faculty of Agronomic Sciences, University of Abomey-Calavi and in the Laboratory of Soil Sciences, Water and Environment of Benin National Research Institute (LSSE/INRAB) following procedures developed by [18]. Soil analyses were carried out on pH(water) and pH(KCl) (using a glass electrode in 1:2.5 v/v soil solution), total N (Kjeldahl digestion in a mixture of H_2SO_4-Selenium followed by distillation and titration), available P (Bray 1 method), exchangeable potassium (1 N ammonium acetate at pH 7), organic carbon (Walkley & Black method).

2.5 AMF Spores Extraction from Soil and Morphological Identification

The AMF' spores extraction and counting were assessed according to method described by [19]. Thus, after air drying and sieving, a soil sample of 100 g was suspended in 900 ml of tap water. After a strict agitation and decantation, the floating was versed through four sieves superposed: 425 µm, 300 µm, 125 µm and 32 µm, under following tap water to separate the AMF spores according to their size. This operation was repeated four times with each soil sample because in heavy clay soils some spores may be buried in the soil micro aggregates or encircled by clay particles. The content of sieves 125 µm and 32 µm were collected and centrifuged at 2000 rpm for 5 min. This first floating was collected and a water sucrose solution at 50% was versed on the depositing in the cup then vigorously agitated, centrifuged at 2000 rpm for 1 min.

After an abundant rinsing in tap water, the AMF spores were collected in Petri dish then, deposited on gridline millimeter paper in order to make easy the counting of spores according to the method describes by [19]. The spores' density was estimated under a stereomicroscope at x 40 (Stemi DRC Zeiss) and grouped according to their morphological characteristics (spore size, color, and hypha attachment). Only healthy spores, meaning those showing the nucleus were counted [20]. The number of AMF spores was expressed per gramme dry soil.

The relative abundance of spores (RAS) per cashew's cultivar characteristic was determined by [21] formula such as:

$$\mathrm{RAS} = \frac{\text{Total number of spores observed for one specie}}{\text{Total number of spores observed for all species}}$$

The spores were identified using information from the International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi website (http//www.invam.caf.wdu.edu). They were named according to the current valid taxonomy [22,6,23,8].

2.6 AMF Diversity Assessment

The diversity of AMF per cashew's cultivar characteristic was analyzed using the specific

richness (S), the Shannon-Wiener diversity index (H) and the Pielou index of evenness (E).
The specific richness (S) was calculated as a number of genus recorded in each cashew's cultivar characteristic.

The Shannon-Wiener diversity index [24] is a mathematical measure of each species distribution in a community which takes into account number of species regarding their morphological characteristic and their abundance. A low value of H generally suggests few dominant species, while a high H value suggests considerably more species.

The Shannon-Wiener diversity index (H) was estimated by a following formula:

$$H = -\sum \left(P_i \mathrm{Log}_2 P_i\right)$$

Pi is a relative frequency of species i and it was calculated by:

$$P_i = \frac{\text{Number of spores for specie i}}{\text{Total number of spores}}$$

The Pielou's evenness index [25] varies from 0 to 1. It denotes an equitable distribution of genus in the cashew's cultivars characteristics when it approaches 1. However, when it is close to 0, it indicates that, some genera are prevailing.

The Pielou's evenness index (E) was calculated using the following formula:

$$E = \frac{H}{\mathrm{Log}_2(S)}$$

H is the Shannon-Wiener diversity index and S is the specific richness.

2.7 Statistical Analysis

The statistical analyses were performed using the Statistical Analysis System (SAS v 9.2) package. Soil chemical properties and the spore numbers of AMF were subjected to a two-way analysis of variance (ANOVA) with cashew's cultivars characteristics and class of Diameter at Breast Height (DBH) as factors. Before running the ANOVA, variance homogeneity was tested. Therefore no data transformation especially number of AMF spores counted were needed. The Student Newman-Keuls test was performed to compare differences in means among treatments at 5%.

3. RESULTS

3.1 Effect of the Cultivars Characteristics and DBH Classes of the Cashew Trees on Soil Chemical Properties

The results of each pH(water) and pH(KCl) shown similar values for both cashew's cultivars characteristics. Whatever the cultivars characteristics of the cashew tree, the pH(KCl) values ranged between 5 and 6 which leads to weakly acid (Table 2). Only the DBH classes of the cashew trees influenced significantly (*$P < 0.01$*) the pH(KCl). The higher soil organic carbon values were obtained under cashew's cultivars producing big nuts and big apples.

Soil total N content under cashew's cultivars producing big nuts and big apples was higher than those producing small nuts and small apples (Table 3). Furthermore, P (Bray 1) content increased from soil top layer to the deeper layer. Exchangeable K^+ content was higher under cashew's cultivars producing big nuts and big apples compared to those producing small nuts and small apples. The results of the analysis of variance revealed that, cultivars characteristics and the DBH classes of cashew trees had significant ($P < 0.01$) effect on soil exchangeable K^+ (Table 3). In general, it appeared that, under cashew's cultivars producing big nuts and big apples, the soils presented the highest values for P(Bray 1), total-N and exchangeable K^+.

3.2 AMF Species Developing Mutualistic Relationship with Cashew Trees

The species of AMF associated with the cashew's cultivars were presented in Table 4. These results revealed that, the AMF spores counted belong to the branch of Glomeromycota. In total, the seven species identified belong to four genera (*Acaulospora*, *Scutelospora*, *Enthrophospora* and *Glomus*) and three families (Glomeraceae, Acaulosporaceae and Gigasporaceae).

The results presented in Table 4 show that, in general, for the two cashew's cultivars, the Glomeraceae family was dominant (its relative abundance was 91.2% compared to 5.5% for

Gigasporaceae and 2.7% for Acaulosporaceae family). Furthermore, *Glomus sp* was more abundant (59.37%) in the soils under the different cashew's cultivars while, *Acaulospora colossica* (0.36%) and *Acaulospora lacunosa* (0.11%) were almost absent.

3.3 Dynamics of AMF Spores Associated With Cashew Trees

The analysis of variance revealed that, the cultivars characteristics had significant effect ($P < 0.05$) only on *Acaulospora colossica* distribution considering soil layers. Soil depth had significant effect ($P < 0.05$) on all of the species distribution except *Acaulospora lacunosa*. The interaction between cultivars characteristics and soil depth was not significant ($P > 0.05$) on these species of AMF identified.

Table 5 presents the average distribution of the AMF spores regarding species identified per cultivars characteristics and soil depth. All species identified were noticed in the different soil layers except *Acaulospora colossica* and *Acaulospora lacunosa* whose spores were not obtained respectively at 20-40 cm for cultivars producing big nuts and big apples and 0-20 cm for cultivars producing small nuts and small apples.

The total number of AMF spores regarding soil layers and cultivars characteristics is reported in Fig. 1. These results show significant difference ($P < 0.05$) between soil depths for total number of spores regarding the cultivars characteristics. Thus, total number of AMF spores per gramme of dry soil decreased significantly ($P < 0.05$) from the superficial layer to the deeper layer regarding cashew's cultivars characteristics. Soils under cultivars producing small nuts and small apples shown high values regarding soil depth. In general, the average numbers of AMF spores per gramme of dry soil are 5.63±0.26; 3.54±0.19 and 1.94±0.15 respectively for 0-20, 20-40 and 40-60 cm of soil depth.

3.4 Diversity of AMF Associated with the Cashew's Cultivars Characteristics

The results of the diversity of the AMF using the specific richness (S), Shannon-Wiener index (H) and Pielou index of evenness (E) are presented in Table 6. It was observed that, the specific richness and Pielou index of evenness varied according to soil depth while Shannon-Wiener diversity index was not affected by soil depth.

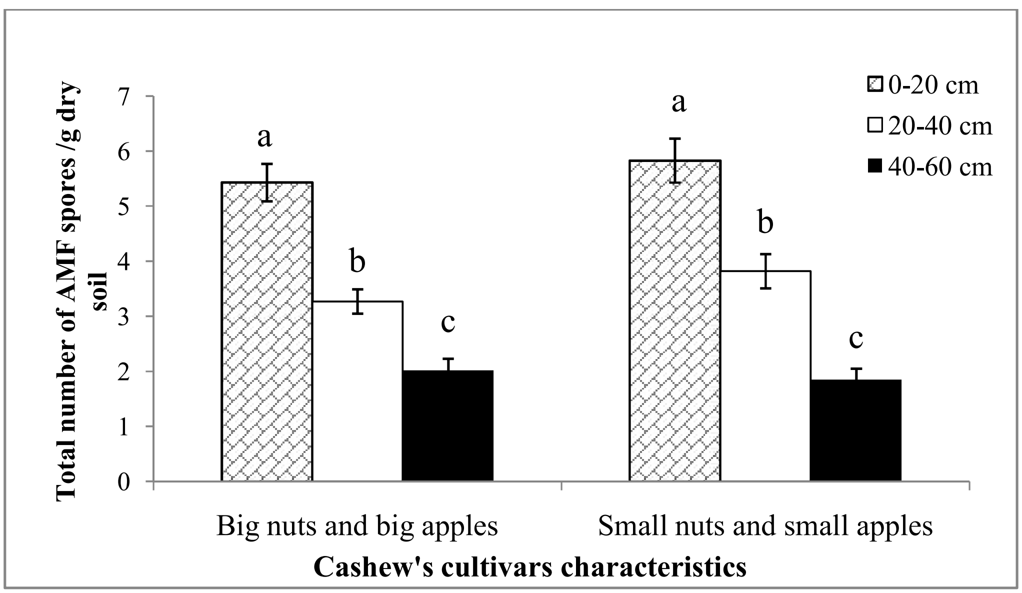

Fig. 1. Total number of arbuscular mycorrhizal fungi (AMF) spores regarding soil depth and cashew's cultivars characteristics

Vertical bars denote standard errors. Bars of the same types labeled with the same letter are not significantly different (P > 0.05) according to Student Newman-Keuls test

Table 2. Soil pH(water), pH(KCl) and organic carbon (mean values ± standard errors) regarding cultivars characteristics, DBH classes of cashew trees and soil sampling depth

Cultivars characteristics	Class of DBH (cm)	pH(water)			pH(KCl)			Organic carbon (g/kg)		
		0-20 cm	20-40 cm	40-60 cm	0-20 cm	20-40 cm	40-60 cm	0-20 cm	20 - 40 cm	40 - 60 cm
Big nuts and big apples	0 - 20	6.50±0.09a	6.45±0.08a	6.37±0.07ab	5.61±0.18a	5.67±0.07a	5.49±0.17a	12.09±1.26a	7.98±0.57b	6.28±0.41ab
	20 - 40	6.23±0.07a	6.17±0.11a	6.09±0.16b	5.29±0.12a	5.18±0.13b	5.11±0.15a	8.72±0.36b	6.58±0.79b	5.36±0.36b
	> 40	6.27±0.06a	6.37±0.05a	6.49±0.06a	5.38±0.12a	5.39±0.12ab	5.20±0.10a	12.92±0.58a	10.02±0.47a	7.63±0.75a
	Mean	6.33±0.05A	6.33±0.05A	6.32±0.07A	5.43±0.08A	5.41±0.08A	5.27±0.09A	11.24±0.63A	8.19±0.48A	6.42±0.37A
Small nuts and small apples	0 - 20	6.39±0.09a	6.37±0.10a	6.35±0.17a	5.38±0.11a	5.64±0.03a	5.38±0.19a	10.41±1.53a	7.20±0.90b	5.36±0.34b
	20 - 40	6.32±0.08a	6.45±0.08a	6.51±0.08a	5.28±0.13a	5.38± 0.07a	5.39±0.12a	9.94±1.13a	7.56±0.37b	6.37±0.84b
	> 40	6.18±0.10a	6.31±0.05a	6.43±0.14a	5.43±0.12a	5.36±0.13a	5.41±0.08a	12.46±0.92a	10.31±0.43a	9.05±0.98a
	Mean	6.30±0.05A	6.37±0.05A	6.43±0.08A	5.36±0.07A	5.46±0.06A	5.40±0.07A	10.93±0.71A	8.35±0.47A	6.93±0.56A

Table 3. Soil total N, available P and exchangeable K^+ (mean values ± standard errors) regarding cultivars characteristics, DBH classes of cashew trees and soil sampling depth

Cultivars characteristics	Class of DBH (cm)	Total N (g/kg)			P Bray1 (mg/kg)			K^+ (cmol/kg)		
		0-20 cm	20-40 cm	40-60 cm	0-20 cm	20-40 cm	40-60 cm	0-20 cm	20-40 cm	40-60 cm
Big nuts and big apples	0 - 20	1.25±0.18a	0.93±0.25a	0.82±0.08a	37.21±9.34a	36.91±12.51a	35.35±10.46a	0.80±0.03a	0.81±0.06b	0.86±0.09a
	20 - 40	1.41±0.21a	0.78±0.24a	1.31±0.25a	37.94±12.94a	41.71±14.75a	42.55±15.02a	1.25±0.26a	1.10±0.12a	1.07±0.11a
	> 40	0.99±0.21a	0.94±0.18a	0.93±0.18a	35.42±13.41a	40.52±10.68a	51.69± 6.95a	0.74±0.07a	0.72±0.07b	0.90±0.15a
	Mean	1.29±0.11A	0.88±0.12A	1.02±0.11A	36.86±6.53A	39.71±6.93A	43.20±7.98A	0.93±0.10A	0.88±0.06A	0.95±0.07A
Small nuts and small apples	0 - 20	1.20±0.22a	0.84±0.15a	0.83±0.15a	38.69±14.06a	53.88±19.65a	31.86±10.98a	0.80±0.04a	0.72±0.05a	0.75±0.05a
	20 - 40	1.38±0.21a	0.97±0.18a	0.63±0.11a	25.71±7.29 a	25.41±7.28a	35.28±11.20a	0.77±0.02a	0.85±0.08a	0.75±0.06a
	> 40	1.28±0.19a	1.15±0.16a	1.28±0.23a	59.85±22.30a	36.99±16.06a	59.86±23.61a	0.81±0.14a	0.69±0.05a	0.76±0.06a
	Mean	1.21±0.12A	0.99±0.09A	0.91±0.11A	41.42±9.22A	38.76±8.74A	42.33±9.38A	0.79±0.04A	0.75±0.04A	0.75±0.03B

Within column, means followed by letters with the same characters are not significantly different (P > 0.05) according to Student Newman-Keuls test

Table 4. Characteristics of different species of AMF associated with cashew's cultivars

Families	Genera	Species	Characteristics	Relative abundance (%)
Gigasporaceae	*Scutelospora*	*Scutelospora gregarine*	Spores solitary, spherical fuzz with dark brown, 250-400 microns in diameter, irregularly shaped, rounded at the apex, combined.	5. 54
Acaulosporaceae	*Acaulospora*	*Acaulospora colossica*	Spores from orange-brown to dark red, sub-globose-globosa shape, size 180-380 microns.	0. 36
		Acaulospora lacunosa	Ellipsoid or globular spores, orange to red and diameters between 80 and 130 microns.	0. 11
	Enthrophospora	*Enthrophospora infrequens*	Globular spores, white or orange brown, diameter from 100 to 160 microns.	2. 20
Glomeraceae	*Glomus*	*Glomus hoi*	Ellipsoidal spores or irregularly shaped, from brown to brown clear, diameters between 50 and 80 microns.	11. 40
		Glomus geosporum	Spores solitary, spherical, reddish brown to dark brown almost black, 125 to 190 microns in diameter. Spore wall formed one group totaling 5-12 microns thick.	21. 02
		Glomus sp	Solitary spores, black, globose or ellipsoidal shape, diameter between 20 and 70 microns.	59. 37

Table 5. Vertical distribution (mean values ± standard errors) per gramme of dry soil of the AMF spores regarding cashew's cultivars characteristics

Cultivars characteristics	Depth (cm)	Glomeraceae			Acaulosporaceae			Gigasporaceae
		Glomus sp	*Glomus hoi*	*Glomus geosporum*	*Acaulospora colossica*	*Acaulospora lacunose*	*Scutelospora gregarina*	*Enthrophospora infrequens*
Big nuts and big apples	0 - 20	3.39±0.25a	0.60±0.09 a	0.08±0.01a	0.008±0.005a	0.0028±0.0028a	0.22±0.03a	0.13±0.04a
	20 - 40	1.88±0.16b	0.32±0.04 b	0.74±0.09ab	0a	0.0028±0.0028a	0.25±0.07a	0.07±0.02ab
	40 - 60	1.11±0.11c	0.27±0.05 b	0.46±0.07b	0.008±0.004a	0.008±0.006a	0.12±0.02a	0.04±0.02b
	Mean	2.13±0.17A	0.40±0.04 A	0.76±0.08A	0.006±0.002B	0.005±0.002A	0.20±0.03A	0.09±0.02A
Small nuts and small apples	0 - 20	3.52±0.32a	0.63±0.07 a	1.17±0.14a	0.036±0.02a	0a	0.35±0.08a	0.12±0.02a
	20 - 40	2.13±0.24b	0.50±0.07 a	0.85±0.09b	0.025±0.01a	0.008±0.008a	0.22±0.05ab	0.08±0.02a
	40 - 60	1.17±0.14c	0.21±0.04 b	0.37±0.05c	0.0028±0.0028a	0.0028±0.0028a	0.07±0.01b	0.03±0.01b
	Mean	2.27±0.19A	0.45±0.04 A	0.80±0.07A	0.02±0.007A	0.004±0.003A	0.21±0.03A	0.08±0.01A

Within column, means followed by letters with the same characters are not significantly different (P > 0.05) according to Student Newman-Keuls test

Table 6. Diversity indexes values (mean values ± standard errors) regarding cashew's cultivars characteristics and soil depths

Cashew's cultivars characteristics	Soil depths (cm)	Specific richness (S)	Shannon-Wiener diversity index	Pielou's evenness index
Big nuts and big apples	0 – 20	4.83±0.15	1.46±0.01	0.65±0.03
	20 – 40	4.28±0.18	1.47±0.09	0.70±0.03
	40 – 60	4.22±0.29	1.54±0.09	0.77±0.02
	Mean	4.44±0.13	1.49±0.05	0.70±0.02
Small nuts and small apples	0 – 20	4.89±0.20	1.51±0.09	0.66±0.03
	20 – 40	4.72±0.23	1.62±0.08	0.73±0.02
	40 – 60	4.06±0.24	1.42±0.07	0.72±0.02
	Mean	4.56±0.13	1.52±0.05	0.70±0.01

Within column, means followed by letters with the same characters are not significantly different (P > 0.05) according to Student Newman-Keuls test

The specific richness decreased about 65.63% from the soil top layer to the deeper layer considering cashew's cultivars characteristics (Table 6). The species number per soil depth varied from 4 to 5. Shannon-Wiener diversity index values (from 1.42 to 1.62) indicating that, the AMF community was moderately diversified in the soil under cashew trees.

In general, the Pielou's index values (near to 1) shown an equitable distribution of AMF genus between cashew's cultivars characteristics. The distribution of these genus was therefore regular.

4. DISCUSSION

4.1 Effect of the Cashew's Cultivars Characteristics on Soil Chemical Properties

The pH values under the two cashew's cultivars characteristics studied vary between 5 and 6.5 meaning moderately acid soil [26]. Similar result was reported by [7] who obtained under cashew tree at Ibadan and Uhonmora (Nigeria), values of pH varying between 5.8 and 6.7. The results of our study show that the soils of the site are suitable for cashew plantation as it was recognized that, cashew trees grow well on soils with pH varying between 4.5 and 8 [5]. According to [26], the pH determines the bioavailability of major nutrients and trace elements and the optimum pH can be set between 6.5 and 7.5; which, implies that, the pH values observed in the experimental plots were beneficial for cashew trees.

The values obtained with soil organic carbon (SOC) in the soil top layer were higher compared with the values of the two other depths whatever the cultivars characteristics and DBH classes of cashew trees. This decrease of the SOC regarding soil depth was due to the quantity and quality of the deposited litter under the cashew trees which decomposed slowly since it is a timber (rich in lignin). The high value of SOC in the soil top layer can also be explained by the action of mycorrhizas hyphae since the glomalins species contribute together to the 15% increase in SOC [27]. Similar results were also obtained by [28] under oil palm fallow cropping system (with litter much more rich in lignin) in the Adja plateau in Benin.

The nitrogen of the soils under the cashew trees follows the same trend as SOC. The higher nitrogen content in the soil top layer can be explained by the supply of mineral fertilizer (150 kg of NPK/ha) to the previous food crop intercropped with the cashew trees. This fertilizer supply was made on almost all experimental plots except plot with two trees that were set in fallow since 2009 and whose decomposition and mineralization of the litter by microorganisms probably would provide some nitrogen quantities to the soil. The presence of AMF associated with the cashew's cultivars characteristics would probably justify the high nitrogen values obtained in soil top layer. Indeed, [10] showed that, about 5% of the nitrogen from the soil of tropical forest was in the form of glomalin produced by mycorrhizae. Since the nitrogen content of the soil does not exceed 0.125% [10], we conclude that, these soils are moderately provided. The C/N ratio varied between 6.30 and 9.31 and between 7.62 and 9.03 respectively for cultivars producing large apples and large nuts then those producing small apple and small nuts. According to the standards of soil analysis interpretation of ORSTOM [29], the soils under cashew cultivation whatever the cultivars characteristics had C/N ratio less than 10, which corresponds to a well-decomposed organic matter, yet low reserve of organic matter.

In general, available phosphorus content were greater than 20 mg/kg, indicating high P content of these soils under cashew trees whatever the cultivars' characteristics and the DBH. These values were higher than those obtained by [5] under cashew in Nigeria (varying between 8.87 and 9.81 mg/kg). Available phosphorus (Bray 1) content was very high and could be explained by the application of chemical fertilizer NPK at a dose of 150 kg/ha for the previous cotton or maize crop intercropped with the cashew. The available phosphorus contents were higher in the deeper depth than the top layer. This can be explained partly by the way that this nutrient is retained in the fine soil fractions and secondly, cashew pumps more this nutrient for flowering, production and ripening of the fruit.

The average content of exchangeable potassium in the soil under cashew trees whatever the cultivars characteristics was less than 1 meq/100 g, leading to potassium deficiency in these soils [26]. This situation could be due to the fact that, potassium is essentially contained in the fine fraction of the soil in relation to the contents of the mica in the clays and silts [23]. These values do not differ too much from those obtained by [5] in Nigeria (varying between 0.57 and 0.67 cmol/kg). This can be explained by the fact that, it is a nutrient needed by cashew trees to increase its resistance to climate variability [6,7]. It also contributes to the hardening off and fruiting therefore, the tree provided it enough from soil as much as it is available. From the different results, we can conclude that, cashew improves the stock of SOC and soil nitrogen content but it depletes the soil available phosphorus and exchangeable potassium.

However, soil chemical properties had different effect on AMF spore number and root colonization. Indeed, the differential response of AMF to soil pH can be attributed to the species and strains constituting the indigenous AMF [6]. According to the authors, N can either stimulate or suppress root colonization and spore production through modification of the soil pH.

4.2 Biodiversity of the AMF Associated with the Cashew's Cultivars Characteristics

Seven species of AMF were identified and they belong to Glomeromycota branch, four genera (*Acaulospora*, *Scutelospora*, *Enthrophospora* and *Glomus*) and three families (Gigasporaceae, Acaulosporaceae and Glomeraceae). In addition to these four genera, [8] had identified *Gigaspora* under cashew trees in Indonesia. But, the number of species identified in our study was much lower than that obtained by [8] (7 against 13 species obtained by these authors). It then results from the number of species obtained, low specific richness in the soil under cashew trees in Benin. This could be explained by the cropping practices in the study area [1]. The cashew plantations are most often intercropped with food crops or cotton when the trees are young. These crops are supplied with mineral fertilizers and weeded regularly. These practices could make less effective the mycorrhizal symbiosis and probably affect their diversity in our soil. In addition, spore density and specific richness were naturally correlated [23], this low specific richness may also be explained in part by the low average number of spores observed whatever the cultivars characteristics and secondly, by environmental factors such as temperature and soil pH. From the results of [23,8] respectively on *Isoberlinia doka* and cashew, we can conclude that *Enthrophospora* is specific genus for cashew trees.

[6] Found that seedling inoculated with AMF responded better than the uninoculated control. Indeed, plants inoculated with *Glomus* had significantly greater stem girth than the uninoculated plants. Species and strains of AMF were different in their ability in the nutrient uptake and influencing plant growth [6]. According to the authors, such variation in efficiency of AMF could be attributed to their intrinsic ability to explore more soil area for nutrients, plant fungal compatibility and the interaction between endophytes and their environment. This finding emphasizes the need to screen and select AMF for improving the cashew production. [6] concluded that, organic fertilizer combined with phosphate fertilizer and AM inoculation had positive influence on the growth of cashew in Nigeria.

The Shannon index and Pielou evenness suggested an average diversification of AMF community with a right distribution of genera at cultivars characteristics and soil depths. During their researches, [23,22] found significant positive correlation between specific richness (S) and the Shannon-Weiner index (H). Considering this correlation, we would expect that, the low specific richness observed in our study generated low diversification of AMF community.

Unfortunately, this was not the case therefore, our results did not corroborate those of [22] and [23]. This can be explained by the cropping practices and land management types in the area.

The numbers of spores were 5.63; 3.54 and 1.94 respectively for 0-20, 20-40 and 40-60 cm depth in both soils under different cashew's cultivars characteristics. Considering 0-20 cm soil depth, the number of spores was high because [10] considered the high number of spores ranging from 3.6 to 212 spores per gram of dry soil. In addition, the number of spores of this depth was around the critical threshold of 5 spores/g of soil from which one could expect maximum root colonization as 5% of the spores normally germinate [10,30]. This density was substantially greater than those found by [6] under cashew trees in the south of India (ranging between 1.94 and 3.54 spores/g dry soil), [8] under cashew cultivation in Indonesia (ranging between 0.208 and 0.364 spore/g of soil), [30] under fallow *Acacia mangium* and *Acacia auriculiformis* (2.76 spores/g of soil) on ferralitic soil and [28] in oil palm (*Elaeis guineensis* Jacq) based system on the Adja Plateau in Benin (0.48 spore/g of soil).

This difference would be mainly due to the period of soil sampling and the cropping practices in the area which is an important parameter to consider taking into account temporal variations in the density of spores in the soil. The density of the spores of the AMF was assessed in soils collected in July 2013 (transitional season between the long rainy season and early rainy season). According to [2,9,10,13,23], the number of spores is high in the soil after it is subjected to water stress conditions with a relatively long date (dry season) and, through the production of spores (resistance structures). This difference can be also explained by the low soil nitrogen and available phosphorus contents and weakly acid pH observed. According to [4,7], nitrogen and phosphorus contents in the soil appear negatively correlated with the development of mycorrhizae.

Regarding the relative abundance of AMF, it is clear from our results that, the genus *Glomus* was more abundant than the other types (91.8% compared to 8.2% for the remain species). This result was similar to those found by [8] under cashew in Indonesia (75% of *Glomus*); [23] on cowpea in different agro-systems of Benin (90%). According to [31-33], the genus *Glomus* is usually the most represented in terms of species in soils of agro-systems in Africa and this is probably justified by its ability to adapt to degraded soils. This wide distribution of the *Glomus* genus mentioned in this study is similar to observations made since by [33,22,23].

5. CONCLUSION

This study shows that, the cultivars characteristics and classes of DBH of cashew did not significantly affect soil chemical properties. Indeed, an increase of SOC and nitrogen content were found under cashews cultivars producing big apples and big nuts, but the cashew deplete available P and exchangeable K which is very important for the fruit production. Seven species of AMF belonging to four genera and three families were found in the soil under cashew plantation. The genus *Glomus* was mostly found. Species identified were more represented in the soil top layer compare to the deeper layer. Although, the specific richness was low, and the distribution of the species was moderately equitable and was not influenced by the cultivars characteristics and DBH classes of cashew trees.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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