Original Research Article

Diversity of arbuscular mycorrhizal fungi associated with cocoa (*Theobroma cacao* L.) agroforestry systems in Togo

ABSTRACT

The aim of the present study was to assess the diversity and prevalence of arbuscular mycorrhizal fungi (AMF) associated with cocoa agroforests in Togo. Soil samples were taken from 24 orchards in four types of cocoa agroforest (< 10 years, 10 to 20 years, 21 to 30 years and > 30 years) in three agroecological subzones (Agou peneplain, piedmonts and plains and plateaus and mountains). The AMF spores extracted were identified on the basis of morphological criteria. The study revealed the presence of 30 AMF species divided into 16 genera and 9 families, with a predominance of the genera Rhizophagus (5 species), Acaulospora (4 species) and Gigaspora (3 species). The most frequent species were Claroideoglomusetunicatum, Acaulosporascorbiculata and Acaulospora sp. Results showed that AMF spore density varied from 16 spores/gram of soil in young plantations entering production and old plantations, to 37 spores/gram of soil in mature plantations in production on the Agou peneplain. Finally, whatever the agro-ecological sub-zone, the highest AMF densities were recorded in agroforests less than 30 years old.

The wide diversity of AMF recorded in cocoa agroforests opens the door to further, more in-depth studies on the importance of this fungal group in cocoa cultivation.

Keywords: Theobroma cacao L., agroforests, agroecological subzones, arbuscular mycorrhizal fungi, AMF density.

1. INTRODUCTION

Plant growthdepends on interactions with the surroundingenvironment, particularly the soil and the microorganisms it harbors [1]. Amongthesemicroorganisms, ArbuscularMycorrhizal Fungi (AMF) are the mostwidespread group withrecognizedbeneficialeffects on plant growth[2-3]. AMFs are microbiota forming mutualistic symbioses between a plant and a fungus at root level. Thesemicroorganisms are present in mostecosystems and are an important component of tropical soilmicroflora[4-5]. Thesetelluric fungi play an important role in the functioning of earthecosystems, due to theirubiquity and direct involvement in essential processestaking place at the soil-plant interface. They enable plants to betteradapt to theirenvironment, particularly in ecosystemswith a deficit of water and/or nutrients[6].

In Togo, cocoais one of the main agricultural exports and a significant source of income for producers. Over 90% of cocoa production in Togo is carried out by small-scale farmers [7]. These farmers cultivate an average area of 26,356.66 ha for a national production of around 12,674.43 tons of merchantable cocoa per year with a low average yield of 506.83 kg/ha compared with a potential of 1,200 to 3,500 kg/ha [8]. This loworchardproductivity can beexplained by degradation of biodiversity, agingorchards, loss of soilfertility, as well as the effects of climate change such as drought, reduced and irregularrainfall, and shorter rainyseasons. Theseconstraints are real handicaps to sustainable and efficient cocoa production. In thiscontext of unfavorable conditions, AMFoffer an alternative for improvingcocoa production, as they can bebiological substitutes for syntheticchemicalfertilizers and pesticides [9].

Several studies on the molecular identification of AMF [10]have led to a better understanding of the link between AMF communities and various parameters, such as cropping systems and intensification of cropping practices [11-12], soil types [13], soil depth [14]. However, studies on the diversity and identification of AMF in African ecosystems are relatively limited [15-16]with a particular rareness in West African forest ecosystems [17-5]more specifically in cocoa agroforestry systems. Given that AMF can penetrate and colonize the root cortex cells of over 80% of earth plants [18]and that cocoa agroforests in Togo are maintained by planters for several decades with low levels of disturbance, it is essential to know the composition and mycorrhizal potential of these ecosystems. This knowledge will enable these AMFs to be conserved in germplasm banks or used in future research into soil defense and restoration. Consequently, assessing the diversity of AMF communities and understanding the factors that determine their distribution is a necessary condition for determining their beneficial effects in cocoa agroforests in Togo. The aim of the present study is to investigate the mycorrhizal status of cocoa agroforests in different production subzones in Togo.

2. MATERIAL AND METHODS

2.1. Presentation of the study area

The studywasconducted in the forestagroecological zone, whichrepresents the main cocoa production area in Togo. This zone issubdividedintothree agro-ecologicalsub-zones: the plateaux sub-zone, the piedmonts and plains sub-zone and the Agoupeneplain (figure 1):

- Mountains and plateauxsub-zone: This sub-zone comprises the plateaux of Kouma, Danyi, Akposso and Akébou, with an average altitude of 700 m, and the isolatedmountains of Agou and Haïto. It enjoys a mountainclimatewithannualrainfall of 1,200-1,600 mm.Soils are ferralitic or ferruginouswithconcretions. Vegetationconsists of partiallydegradedforests and wooded and shrubbysavannahs.

- The piedmonts and plains sub-zone: This isrepresented to the east by the plains of the Kloto, Kpélé-Akata and Amou prefectures, and to the west by the Litimé plain (west of the Wawaprefecture). These areas benefitfrom a mountainclimatewithannualrainfall of 1300-1600 mm. The soils are thickferralitic. Vegetationconsists of Sudano-Guineanforestsalternatingwithwoodedsavannahs.

- The Agoupeneplain: This corresponds to the vast expanse of ferruginoussoils on metamorphic rock thatcovers the whole of the Agouprefecture, except for the mountainous part. The climateishumid tropical, withannualrainfall of 900-1200 mm.Vegetationconsistsmainly of wooded or Guineansavannahs and galleryforests, withartificialteakforests in places.

2.2. Soil sampling

Soilsampleswеretakenfrom the threeagroecologicalsub-zones. In eachagroecologicalsub-zone, samplesweretakenconsidering four agroforest types thatrepresent the main stages of orchardevolution over time:

- Young orchardsenteringinto production (< 10 years),

- Mature cocoatrees in full production (10 to 20 years),

- Agingcocoatrees in production decline (21 to 30 years)

- Old orchard (>30 years)

In eachparcel, five sampling points werechosen, following the diagonals of the parcel. At each sampling point, soilsampleswеretakenunder five cocoatrees. Under eachtree, a soilsample (250-300 g) wastakenfrom the 50 cm crown around the cocoatree over a radius of 50 to 100 cm.

Samplesfromeachparcelwere mixed to form a composite sample for extraction and identification of AMF spores. Across all sites, a total of 24 composite soilsampleswerecollected. Each plantation wasgeo-referencedusing GPS and itsagerecorded.

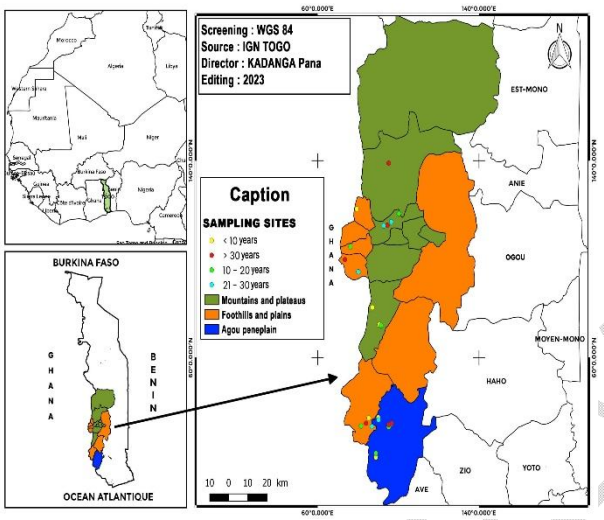

Figure 1 : Soil sampling locations

2.3. Trapping and multiplication of arbuscularmycorrhizal fungi

AMF trappingwascarried out in the greenhouse at the Station d'Expérimentations Agronomiques de Lomé (SEAL) over a 12-month periodfrom August 2020 to July 2021. Thus, sorghum (Sorghum bicolor) wasused as the reference trap plant becauseitsrootspromotegreater multiplication of mycorrhizal fungus spores (INRA, 2017). For eachsoilsamplecollected, two 5-liter pots wereused, for a total of 48 pots for all samples. In each pot, 4 kg of substrateconsisting of seasand (1/3) and arable soil (2/3) collected at the SEAL wereused. The substratewassterilized at 120°C for 2 hours. Initially, 3 kg of substratewereintroducedintoeach pot. Next, 200 g of soilwereinoculatedinto the substrate, onto whichsorghumwassown at a rate of three grains/pot, thencoveredwith the remaining 1 kg of composite substrate. Beforesowing, the sorghum grains weresterilizedwith 90° alcohol for 1 minute and rinsedwithdistilled water. Replantingwascarried out each time the plant reached senescence. These plants werewatereddailythroughout the development phase.

2.4. Extraction and morphological identification of AMF spores

Spores wereextracted at the Laboratoire des Sciences Agronomiques et BiologiquesAppliquées (LaSABA), University of Kara, using the wetsieving technique described by [19]. 50 g of soilweresuspended in water and placed on the top of a series of threesievessuperimposedfrombottom to top according to the increasing value of theirmeshopening (32µm, 90µm and 500µm). The soilwassubjected to thisseries of sievesunder a jet of tap water, and the contents of the last twosieves (90 and 32µm) werepouredinto 50ml flasks and centrifuged in a gradient of 70% sucrose solution [20]at 2,000 rpm for 5 min. The supernatantfromeachtreatmentwascollectedwith a volumetric pipette and returned to the smallestsieve (32µm), thenrinsed to removeresidual sucrose solution and pouredintopillboxes. The spores and spore clusters stored in the pillboxesweretransferred to Petri disheswithgridbottomsbeforebeingobserved and countedwith a binocularmagnifying glass (G = X40).

For microscopic identification, healthy spores weremounted on glass slides and stainedwithglycerol mixed withMelzer'sreagent (1:1 vol/vol, Brundrett et al., 1994). The spores wereexaminedunder a binocular microscope (G = X400) and identified on the basis of the description and identification manual bySchenck et Pérez [21], INVAM (International Culture Collection of VesicularArbuscularMycorrhizal Fungi (http://invam.caf.wvu.edu/cultures/cultsearch.htm) and scientific publications on AMF classification [22-23-24-25-26]).

2.5. Assessment of AMF taxonomicdiversity
Taxonomicdiversitywasassessed by calculating the followingindices:

- Speciesrichness:refers to the number of speciespresent in a givenenvironment.
- Shannon-Weaver index (H') (Krebs, 1985):

$$H' = -\sum_{i=1}^{s} pi.log_2(pi)$$

Pi is the percentage abundance of a nematodegenus (pi = ni/N), ni represents the number of individualscounted for the genus, N the total number of individualscounted for all genera and S the total number of generaidentified.The Shannon index isused to characterizespeciesdiversity in a community and ranges from 0 to Ln(S).

- Piélouequitability index (E) (Pielou, 1977): E = H'/ln(S)

This index quantifies the regularity of species distribution within the community. Piélou'sequitability index varies between 0 and 1.

2.6. Statisticalanalysis
Data-t-on spore density, speciesrichness and AMF communitydiversity indices (Shannon-Weaver and Piélouequitability indices) wereanalyzed by analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences) software version 25.0. The differentmeanswerediscriminated and comparedusing the Student-Newmann-Keuls (SNK) test at the 5% threshold. Prior to analysis, mean AMF spore densitieswerenormalizedusing the log10 (x + 1) transformation (where x is the mean spore density).

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. AMF diversity in soils under cocoa trees in different agroforests in the three agroecological subzones

A total of 30 AMF species, divided into 16 genera and 9 families, were identified in soil samples under cocoa trees (Table 1). The Rhizophagus genus was the most represented, with five species, followed by Acaulospora (four species), Gigaspora (three species), Scutellospora, Claroideoglomus, Funneliformis, Glomus and Septoglomus (two species each) and finally the other genera, each represented by a single species (Table 1).

Table 1: Families, genera and number of AMF species found in cocoa agroforests and their average frequencies.

Families	**Genera**	***Number of species***
Acaulosporaceae	*Acaulospora*	4
	Entrophospora	1
Ambisporaceae	*Ambispora*	1

Archaeosporaceae	*Archaeospora*	1
	Cetraspora	1
Gigasporaceae	*Gigaspora*	3
	Scutellospora	2
	Racocetra	1
Claroideoglomeraceae	*Claroideoglomus*	2
Dentiscutataceae	*Dentiscutata*	1
Diversiporaceae	*Diversipora*	1
	Funneliformis	2
Glomeraceae	*Glomus*	2
	Rhizophagus	5
	Septoglomus	2
Paraglomeraceae	*Paraglomus*	1
Total number of spieces		30

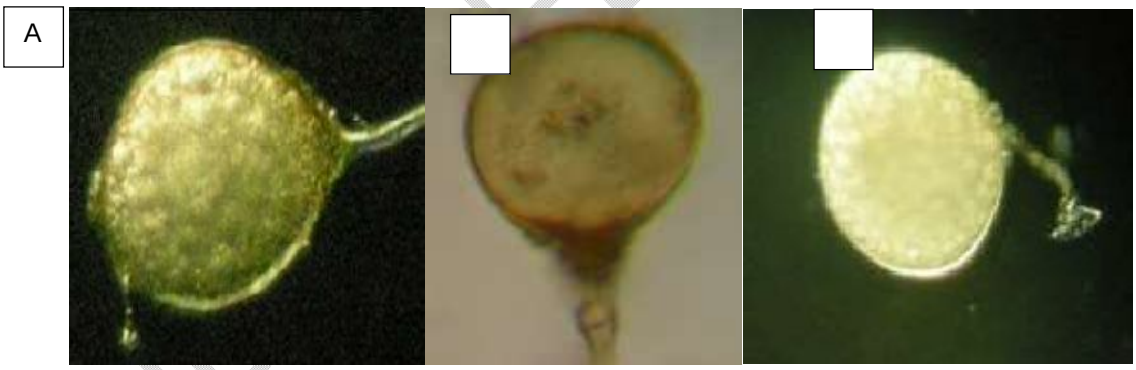

Figure 2:Most frequent AMF spores in samples
A = *Rhizophagus*spp ; B = *Gigaspora*spp and C = *Acaulospora*spp

3.1.2. Specific richness of AMF in soils under cocoa trees in different agroforests in the three agroecological subzones

Irrespective of the agroecological sub-zone considered, the analyses revealed no significant differences ($p > 0.05$) in specific richness between the different agroforests (Figure 4). However, in the Agou peneplain, the highest species richness was recorded in mature plantations in production (16 species). On the other hand, in the piedmonts and plains, the highest number of species was recorded in young plantations entering production (14 species), while in the plateaus and mountains, the highest number of species was recorded in plantations between 10 and 30 years old (10 species) (Figure 4).

Concerning the different AMF species, the most frequent are represented by *Claroideoglomusetunicatum* (24%), *Acaulosporascorbiculata* (23%) and *Acaulosporasp* (23%) (Table 2). The highest spore density was recorded for C. etunicatum (4 spores/gram of soil), followed by *E. infrequens*, *G. microaggregatum* and *R. intraradices* (3 spores/gram of soil each).

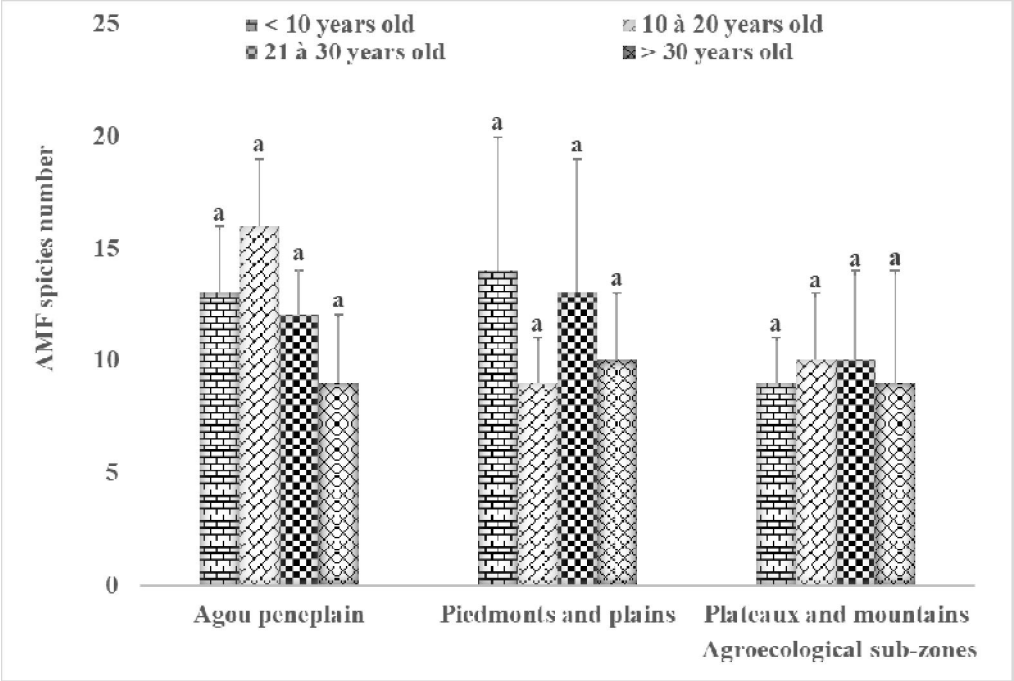

Figure 3: Specificrichness of AMF in soilsundercocoatreesfromdifferentagroforests in the threeagroecologicalsubzones. Significantdifferences are indicated by differentletters (Student-Newman-Keuls test at 5% threshold).

Table2:Spore frequencies and densities of AMF speciesidentified in cocoaorchards

AMF species	**Frequency (%)**	**Density (Number of spores/gram of soil)**
A. scorbiculata	23	2
A. excavata	11	1
A. colombiana	13	1
***Acaulospora*sp**	23	2
***Ambispora*sp**	4	1
***Archaeospora*sp**	4	1

Cetrasporasp	20	1
C. etunicatum	24	4
C. claroideum	12	1
Dentiscutatasp	3	1
Diversiporasp	17	1
E. infrequens	19	3
F. mosseae	8	1
F. monosporus	15	2
G. gigantea	10	1
G. candida	6	1
G. Rosa	3	1
G. clavisporum	4	2
G. microaggregatum	16	3
Paraglomussp	6	2
R. aggregatus	14	2
R. clarus	8	1
R. intraradices	15	3
R. manihotis	6	1
R. sinuosus	13	2
S. calospora	3	2
Scutellosporasp	3	2
S. deserticola	4	1
Septoglomussp	4	1
Racocetrasp	3	1

3.1.3. Specific richness of AMF in soils under cocoa trees according to the different agro-ecological sub-zones

In the Agou peneplain, eleven of the thirty species listed (*A. corbiculata, A. excavata, Acaulosporasp, E. infrequens, G. gigantea, C. etunicatum, Diversiporasp, F. mosseae, F. monosporus, G. microaggregatum and R. intraradices*) were found in soils under cocoa trees of all four agroforest types. However, only one species (*Dentiscutatasp*) was not found in this agroecological subzone (Table 3).

In the piedmonts and plains, nine species (A*. scorbiculata, A. colombiana, Acaulosporasp, G. gigantea, C. etunicatum, Diversiporasp, G. microaggregatum, R. aggregatus and R. intraradices*) were found in the soils of all four agroforest types. Two species (*G. rosa and S. calospora*) were not found in the piedmonts and plains (Table 4).

Finally, in the plateau and mountain agroecological sub-zone, six species (*A. scorbiculata, Acaulosporasp, E. infrequens, G. gigantea, C. etunicatum and G. microaggregatum*) were found in the soils of all four agroforests. However, five species (*Archaeosporasp, S. calospora, Scutellosporasp, Racocetrasp, Paraglomussp*) were not found in the plateaus and mountains (Table 5).

Table3: Specificrichness of AMF in soilsundercocoatrees in agroforests on the Agoupeneplain

AMF species	**< 10 years old**	**10 à 20 years old**	**21 à 30 years old**	**> 30 years old**
Acaulosporaceae				
A. scorbiculata	X	X	X	X
A. excavata	X	X	X	X
A. colombiana	X	X		X
*Acaulospora*sp	X	X	X	X
E. infrequens	X	X	X	X
Ambisporaceae				
*Ambispora*sp	X	X	-	-
Archaeosporaceae				
*Archaeospora*sp	X	X	-	-
Gigasporaceae				
Cetraspora sp	**-**	-	X	X
G. gigantea	X	X	X	X
G. candida		X	-	-
G. rosa	X	X	-	-
S. calospora	X	-	-	-
*Scutellospora*sp	**-**	X	-	-
*Racocetra*sp	**-**	X	-	-
Claroideoglomeraceae				

C. etunicatum	X	X	X	X
C. claroideum	X	X	X	-
Dentiscutataceae				
*Dentiscutata*sp	-	-	-	-
Diversiporaceae				
*Diversipora*sp	X	X	X	X
Glomeraceae				
F. mosseae	X	X	X	X
F. monosporus	X	X	X	X
G. clavisporum				
G. microaggregatum	X	X	X	X
R. aggregatus	X	X	X	-
R. clarus	X	X		
R. intraradices	X	X	X	X
R. manihotis	X			
R. sinuosus	X	X	X	-
S. deserticola				
*Septoglomus*sp	-	X	-	-
Paraglomeraceae				
*Paraglomus*sp	X	X	-	-
Total	**22**	**24**	**15**	**13**

X : Présent ; - : Absent

Table4:Specificrichness of AMF in soilsundercocoatrees in piedmont and plain agroforests

AMF species	**< 10 years old**	**10 à 20 years old**	**21 à 30 years old**	**> 30 years old**
Acaulosporaceae				
A. scorbiculata	X	X	X	X
A. excavata	-	X	X	-

A.colombiana	X	X	X	X
*Acaulospora*sp	X	X	X	X
E. infrequens	X	X	X	-
Ambisporaceae				
*Ambispora*sp	X	-	-	-
Archaeosporaceae				
*Archaeospora*sp	X	-	-	-
Gigasporaceae				
*Cetraspora*sp	-	X	X	X
G. gigantea	X	X	X	X
G. candida	-	-	-	X
G. rosa	-	-	-	-
S. calospora	-	-	-	-
*Scutellospora*sp	X	-	X	X
*Racocetra*sp	-	-	X	X
Claroideoglomeraceae				
C. etunicatum	X	X	X	X
C. claroideum	X	X	-	-
Dentiscutataceae				
*Dentiscutata*sp	X	X	X	-
Diversiporaceae				
*Diversipora*sp	X	X	X	X
Glomeraceae				
F. mosseae	X	-	-	-
F. monosporus	X	X	X	-
G. clavisporum				X
G. microaggregatum	X	X	X	X

R. aggregatus	X	X	X	X
R. clarus	X	-	-	X
R. intraradices	X	X	X	X
R. manihotis	X	-	-	-
R. sinuosus	X	-	X	-
S. deserticola	-	-	X	X
*Septoglomus*sp	-	-	X	X
Paraglomeraceae				
*Paraglomus*sp	X	-	-	X
Total	**21**	**15**	**19**	**18**

X : Présent ; - : Absent

Table5:Specificrichness of AMF in soilsundercocoatrees in plateau and mountainagroforests

AMF species	**< 10 years old**	**10 à 20 years old**	**21 à 30 years old**	**> 30 years old**
Acaulosporaceae				
A. scorbiculata	X	X	X	X
A. excavata	X	-	X	-
A. colombiana			X	X
*Acaulospora*sp	X	X	X	X
E. infrequens	X	X	X	X
Ambisporaceae				
*Ambispora*sp	-	-	-	X
Archaeosporaceae				
*Archaeospora*sp	-	-	-	-
Gigasporaceae				
*Cetraspora*sp	-	X	X	X
G. gigantea	X	X	X	X

G. candida	-	-	-	X
G. Rosa	-	-	-	X
S. calospora	-	-	-	-
*Scutellospora*sp	-	-	-	-
*Racocetra*sp	-	-	-	-
Claroideoglomeraceae				
C. etunicatum	X	X	X	X
C. claroideum	X	X	-	X
Dentiscutataceae				
*Dentiscutata*sp	-	-	-	X
Diversiporaceae				
*Diversipora*sp	-	X	X	X
Glomeraceae				
F. mosseae	X	X	-	X
F. monosporus	X	-	X	X
G. clavisporum	X	-		X
G. microaggregatum	X	X	X	X
R. aggregatus	X	X	X	-
R. clarus	X	X	-	-
R. intraradices	X	-	X	-
R. manihotis	-	-	-	-
R. sinuosus	X	X	X	-
S. deserticola	-	X	X	-
*Septoglomus*sp	-	X	-	-
Paraglomeraceae				
*Paraglomus*sp	-	-	-	-
Total	**15**	**15**	**15**	**17**

X : Présent - : Absent

3.1.4. AMF communitydiversity indices

AMF community diversity was assessed through species richness, Shannon-Weaver Index (H') and Piélou Equitability (E) indices for the different agroforests and agroecological subzones. Statistical analyses showed no significant differences ($p > 0.05$) in species richness, Shannon-Weaver Index (H') and Piélou equitability (E) indices for either agroforests or agroecological subzones. However, for agroforests, the lowest values of these parameters were recorded in old plantations (Table 6). Similarly, for agroecological sub-zones, plateaus and mountains recorded the lowest values (Table 7).

Table 6: Specific richness, Schannon-Weaver and Piélou equitability indices of AMF communities in soils under cocoa trees in different agroforests.

Agroforest type	Specific richness	Shannon-Weaver index (H')	Piélou equitability index (E)
< 10 years old	19 ± 4 a	2,66 ± 0,03 a	0,78 ± 0,01 a
10 à 20 years old	18 ± 4 a	2,61 ± 0,21 a	0,77 ± 0,06 a
21 à 30 years old	16 ± 2 a	2,51 ± 0,06 a	0,74 ± 0,12 a
> 30 years old	16 ± 2 a	2,38 ± 0,40 a	0,70 ± 0,07 a
p-value	0,439	0,486	0,511

Data are reported as means and standard deviations for the four agroforests. Means with the same letter are statistically identical at the 5% level.

Table 7: Specific richness, Schannon-Weaver and Piélou equitability indices of AMF communities in soils under cocoa trees in the three agroecological sub-zones.

agroecological sub-zones	Specific richness	Shannon-Weaver index (H')	Piélou equitability index (E)
Agou peneplain	18 ± 5 a	2,53 ± 0,30 a	0,75 ± 0,09 a
Piedmonts and plains	18 ± 3 a	2,61 ± 0,18 a	0,77 ± 0,05 a
Plateaux and mountains	16 ± 1 a	2,47 ± 0,22 a	0,73 ± 0,07 a
p-value	0,633	0,717	0,710

Data are reported as averages and standard deviations for the three agro-ecological production sub-zones. Means with the same letter are statistically identical at the 5% threshold.

3.1.5. AMF spore density

After trapping, spore density varied from 16 spores/gram of soil in young plantations entering production and old plantations to 37 spores/gram of soil in mature plantations in production on the Agou peneplain. The results of statistical analysis showed no significant difference ($p > 0.05$) between the spore densities of the different agroforests of Agou peneplain and plateaus and mountains. However, a significant

difference ($p < 0.05$) was observed between the spore densities of agroforests in the piedmonts and plains. In the Agou peneplain, as well as in the piedmonts and plains, the highest spore densities were observed in agroforests less than 30 years old. On the other hand, in the plateaux and mountains, spore densities were highest in plantations between 10 and 30 years old

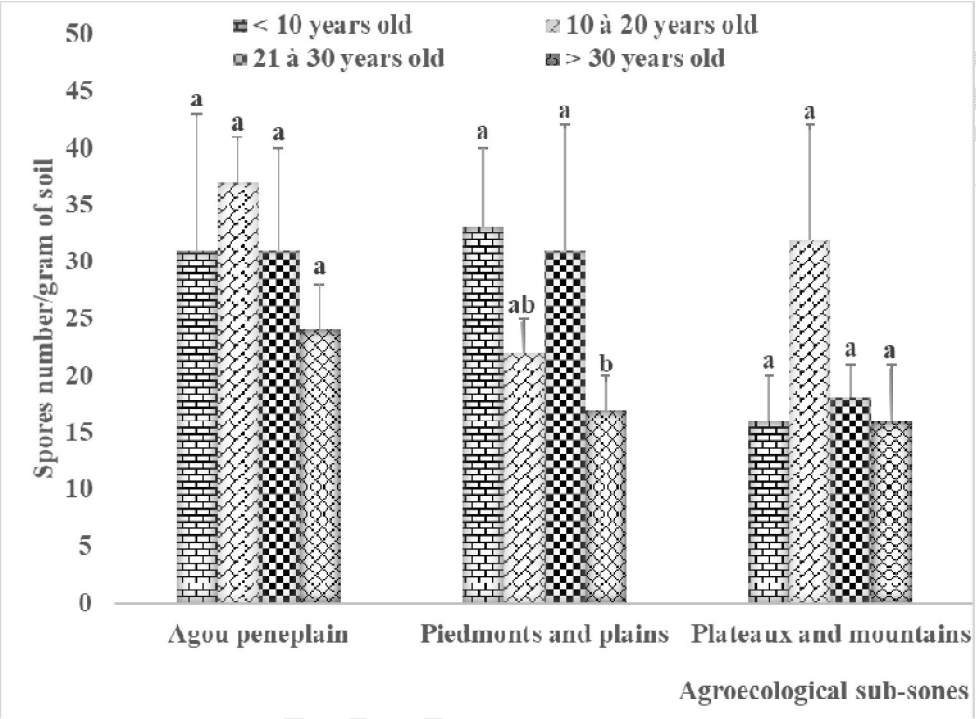

Figure 4 : AMF spore density in soilsundercocoatrees in differentagroforests of the Agoupeneplain, piedmonts and plains, plateaus and mountains. Significantdifferences are indicated by differentletters (Student-Newman-Keuls test at 5% threshold).

3.2. Discussion

This study has revealed the diversity of AMF in cocoa agroforests in different production sub-zones. The identification of AMF associated with cocoa trees enabled us to list a total of 30 species belonging to 16 genera and 9 families. The most predominant genera were Rhizophagus (five species), Acaulospora (four species) and Gigaspora (three species), belonging to the Glomeraceae, Acaulosporaceae and Gigasporaceae families respectively. The most frequent species are *Claroideoglomusetunicatum* (24%), *Acaulosporascorbiculata*and *Acaulosporasp* (23%). The presence or absence of certain AMF species in the different agro-ecological sub-zones shows a probable influence of climatic differences and soil characteristics between agro-ecological sub-zones [27-6]. [6] identified a total of 72 AMF morphotypes belonging to 10 families, 17 genera and 49 AMF species, with a predominance of the genus Glomus in cocoa orchards in Côte d'Ivoire. Our results are similar to those of [28] who identified 21 AMF morphotypes in the rhizosphere of cocoa trees in the department of San Martin, Peru. [29]identified 57 different morphotypes, classified into 9 genera with a higher abundance of the genera Glomus, Acaulospora and Gigaspora in soils under cocoa trees in the Amazon region of Peru. The studies of [30]revealed the presence of the families Paraglomeraceae, Glomeraceae, Claroideoglomeraceae and Acaulosporaceae in Tarapoto plantations, while [31] observed the predominance of the Acauloporaceae, Glomeraceae and Gigasporaceae families in Venezuelan cocoa orchards. In cocoa orchards in the Yamoussoukro region of Côte d'Ivoire, [32] identified nine AMF species belonging to Glomus,

Acaulosporaand Gigaspora genera. In Ecuador, in conservative cocoa crops, the genera Glomus, Acaulospora, Ambispora, Pacispora, Diversispora, Scutellospora, Racocetra, Entrophospora, Gigaspora, Intraspora, Paraglomus and Archaeospora were identified, while in semi-conservative cocoa crops, the genera Glomus, Acaulospora, Ambispora, Pacispora, Diversispora, Scutellospora, Racocetra, Entrophospora, Gigaspora, Intraspora, Paraglomus and Archaeospora were identified [33]. The predominance of the genera Rhizophagus, Acaulospora and Gigaspora in cocoa agroforests shows their better adaptation to a wide range of conditions, even the most hostile, such as drought, salinity and other environmental stresses [34]. Furthermore, [6] suggested that species of the genus Glomus (Rhizophagus) and Acaulospora propagate preferentially by spores resistant to harsh conditions, while other genera such as Scutellospora and Gigaspora propagate with other types of propagules such as hyphae and extra-root mycelial fragments.

Concerning the diversity indices of AMF communities in cocoa plantations, it emerges that the values obtained in the present study are similar to those obtained by [31] in Venezuelan cocoa plantations. On the other hand, the specific richnesses reported in this study in agroforests in the three agroecological subzones are low compared with those obtained by [35] in plantain plantations in three agroecological zones (Abengourou, Azaguié and Bouaflé) in Côte d'Ivoire (around 28.50 to 253.50). Furthermore, [35] obtained Shannon and Piélou index values similar to those obtained in this study in these plantain plantations.

Results on AMF spore density revealed that the number of spores varied from 16 spores/gram of soil in young plantations entering production and old plantations to 37 spores/gram of soil in mature plantations in production on the Agou peneplain. These values are higher than those reported by [31] Cuenca and Meneses (1996), which are 4 spores/gram of soil in plantations less than 20 years old and 2 spores/gram of soil in old plantations (> 30 years) in Venezuela. In Côte d'Ivoire, [6] Amani et al (2023) showed that in cocoa plantations, the abundance of arbuscular mycorrhizal fungi spores ranged from 10.53 to 31.24 spores/gram of soil, with an average of 18.30 spores/gram of soil. [36] found similar spore densities (16 to 36 spores/gram soil) to those reported in our study in cocoa agroforests in Cameroon. [30] showed that in soils under cocoa trees in the San Martin region of Peru, spore density varied from 13.8 spores/100 grams of soil in 10-year-old plantations to 131.8 spores/100 grams of soil in 3-year-old plantations. [37] found that spore density varied between 280 and 610 spores 100/gram dry soil in agricultural systems. In conservative and semi-conservative cocoa plantations, [33] recorded densities of 300 to 400 spores 100/g dry soil. The difference between our results and those quoted above could be due to the cultivation practices and edaphoclimatic conditions of the plantations, as well as to the particular characteristics of the cultivation and management of the plantations ([30].

4.3. Conclusion

This is the first study to assess the speciesdiversity of arbuscularmycorrhizal fungi in cocoa plantations in Togo. The identification of AMF associatedwithcocoatreeswithin the framework of thisstudyenabled us to list a total of 30 speciesdividedinto 16 genera and 9 families. The mostcommonspecies are *Claroideoglomusetunicatum, Acaulosporascorbiculata and AcaulosporaSp.* The high diversity of AMF recorded in cocoa plantations opens the door to further in-depthstudies on the importance of thisfungal group in cocoa cultivation.

REFERENCES

1. Touré, G.-P. T., Nandjui, J., Koné, A. W., Kouadjo, A. G. Z., Ebou, A., Tiho S. & Zézé A. (2021) - Diversity of arbuscularmycorrhizal fungi and interactions with the soil-litter system in a forest-savannaecotone, Côte d'Ivoire - Etude et Gestion des Sols, 28, 93-104
2. Smith SE and Smith FA (2011) Roles of arbuscularmycorrhizas in plant nutrition and growth: new paradigmsfrom cellular to ecosystemsscales. AnnuRev Plant Biol 63 : 227–250.
3. Shi, Y., K. J. Davis, C. J. Duffy, and X. Yu (2013): Development of a Coupled Land Surface Hydrologic Model and Evaluation at a Critical Zone Observatory. *Journal of Hydrometeorology, 14, 1401—1420*. DOI: 10.1175/JHM-D-12-0145.1

4. Cardoso, I.M. and Kuyper, T.W. (2006) Mycorrhizas and tropical soil fertility. Agriculture, Ecosystems & Environment, 116, 72-84. http://dx.doi.org/10.1016/j.agee. 2006.03.011
5. Tchabi A., Coyne D.,Hountondji F.,Lawouin L., WiemkenA.,OehlF.,2008.Arbuscularmycorrhizalfungalcommunitiesinsub-SaharanSavannasofBenin,WestAfrica,asaffectedby agriculturallanduseintensityandecological zone.Mycorrhiza,18(4):181-95. https://doi.org/10.1007/s00572-008-0171-8
6. Amani C. Y. F., Alban M'bo K. A., Cherif M., Koné D. et Kouamé C. 2023. Diversité des Champignons Mycorhiziens à Arbuscule Associés aux Cacaoyers (Theobroma cacao L.)en Côte d'Ivoire.EuropeanScientificJournal,ESJ,19 (27),179. https://doi.org/10.19044/esj.2023.v19n27p179.
7. Djiwa O., Pereki H. and Guelly K.A., 2021. Ethnocultural perceptions of ecosystem services provided by cocoa-based agroforests in Togo. Biotechnol. Agron. Soc. Environ. 2021 25(3), 208-222 (15p).
8. CRA-F.2004.CentredeRechercheAgronomiquedela zone Forestière. Point sur la recherche cacaoyère au Togo. Kpalimé, 22 p.
9. Finlay R.D., 2008. Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. *J. Exp. Bot*., 59(5) : 1115–1126.
10. Redecker D., Morton J.B. et Bruns T.D., 2000. Molecular phylogeny of the arbuscular mycorrhizal fungi *Glomus sinuosum* and *Sclerocystis coremioides*. *Mycologia*, 92 : 282-285.
11. Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I.R., Frossard, E., 2002. Diversity and structure of AMF communities as affected by tillage in a temperate soil. Mycorrhiza 12,225–234.
12. Oehl F., et Sieverding E., 2004. Pacispora, a new vesicular-arbuscular mycorrhizal fungalgenus in the Glomeromycetes. *J. AppliedBotany and Food Quality*, 78, 72–82.
13. Lekberg Y., Roger T., Rohr J R., Aldrich-Wolfe L., Morton J B. 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. *Journal of Ecology 95 , 95–105* https://doi.org/10.1111/j.1365-2745.2006.01193.x.
14. Oehl F, Sieverding E, Ineichen K, Ris E-A, Boller T, et WiemkenA,2005.Communitystructureof arbuscularmycorrhizalfungiatdifferentsoil depths in extensively and intensively managed agroecosystems.NewPhytologist,165:273-28.3
15. Mathimaran N, Ruh R, Jama B, Verchot L, Frossard E, et Jansa J, 2007.Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan Ferralsol.Agriculture, Ecosystems and Environment, 119: 22-32
16. 1Gnamkoulamba A., Tounou A K., Tchabi A., Agboka K., Adjévi A. K.M., Batawila K. 2018. Prevalence and diversity of arbuscular mycorrhizal fungi spores in rice cultivation under different rice cropping systems in five agro-ecological zones in Togo. Journal of Applied Biosciences 126: 12647-12664. ISSN 1997-5902. https://dx.doi.org/10.4314/jab.v126i1.3
17. Zézé A, Ouattara B, Brou CY, Van Tuinen D, Diallo-Attah H, Sangare A. 2007. Distribution and abundance of spores of arbuscular endomycorrhizal fungi in different types of Téné forest in Côte d'Ivoire. AgronomieAfricaine, 19(2): 103-111. DOI:10.4314/aga.v19i2.1710
18. Berruti, A., Lumini, E., Balestrini, R. et Bianciotto, V., 2016. Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Front. Microbiol. 6, 1559.
19. Gerdemann, J. W., and T. H. Nicolson. 1963. Spores of mycorrhizal endogone speciesextractedfromsoil by wetsieving and decanting. Fungal Biol. 46: 235-244.
20. Oehl F,SieverdingE,IneichenK,MäderP,BollerT, Wiemken A, 2003. Impact of land use intensityonthespeciesdiversityofarbuscularmycorrhizal fungi in agroecosystems of Central Europe.Applied andEnvironmentalMicrobiology69: 2816-2824.
21. Schenck NC et Pérez Y, 1990.Manual for the identification of VAmycorrhizal fungi. Synergistic-Publications, Gainesville, Florida.
22. Palenzuela J., Ferrol N., Boller T., Azcon-Aguilar C. et Oehl F., 2008. *Otosporabareai*, a new fungal species in the Glomeromycetes from a dolomitic shrub land in Sierra de Baza National Park (Granada, Spain). Mycologia 100, 296-305. Doi :10.1080/15572536.2008.11832484

23. Redecker D., Schuler A., Stockinger H., Stumer S., Morton J., et Walker C., 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (*Glomeromycota*). Mycorrhiza 23, 515-531. Doi: 10.1007/s00572-013-0486-y.
24. Błaszkowski J., Kozłowska A., Crossay T., 2017. A new family, Pervetustaceae with a new genus, Pervetustus, and P. simplex sp. nov. (Paraglomerales), and a new genus, Innospora with I. majewskii comb. nov. (Paraglomeraceae) in the Glomeromycotina. Nova Hedwigia. doi:info:doi/10.1127/nova_hedwigia/2017/0419
25. Crossay T., Cilia A., Cavaloc Y., Amir H. et Redecker D., 2018. Four new species of arbuscular mycorrhizal fungi (*Glomeromycota*) associated with endemic plants from ultra maficsoils of New Caledonia. *Mycological Progress* : 1–16.
26. Anand K., Pandey G.K., Kaur T., Pericak O., Olson C., Mohan R., Akansha K., Yadav A., Devi R., Kour D., Rai A.K., Kumar M. et Yadav A.N., 2022. Arbuscular mycorrhizal fungi as a potential biofertilizers for agricultural sustainability. *Journal of Applied Biology and Biotechnology* 2022 ;10 (Suppl 1): 90-107. DOI:10.7324/JABB.2022.10s111. 19p.
27. Thomson, B. C., Tisserant, E., Plassart, P., Uroz, S., Griffiths, R. I., Hannula, S. E., Buée, M., Mougel, C., Ranjard, L., Van Veen, J. A., Martin, F., Bailey, M. J. &Lemanceau, P., (2015). Soil conditions and land use intensification effects on soilmicrobialcommunitiesacross a range of Europeanfield sites. Soil Biol. Biochem. 88, 403–413. https://doi.org/10.1016/j.soilbio.2015.06.012
28. . Rojas, J. C. (2010) Arbuscular mycorrhizal fungi in the rhizosphere of promising cacao genotypes (Theobroma cacao L.) under traditional and under-forest systems in the San Martín Region. (Thesis, Agricultural Engineering Degree). National University of San Martín.
29. Llanos-Gómez K. J, Silva-Manco M.J., Sanchez-Santillan T., Arce-Inga M., Leiva-Espinoza S.T. 2023. Morphological identification of arbuscular mycorrhizal fungi in cocoa plantations in the Amazon region, Peru. *Manglar 20(1)*: 7-14 (2023)
30. Luis-Alaya, B.; Toro, M.; Calsina, R.; Ogata-Gutiérrez, K.; Gil-Polo, A.; Ormeño-Orrillo, E.; Zúñiga-Dávila, D. Evaluation of the Presence of Arbuscular Mycorrhizae and Cadmium Content in the Plants and Soils of Cocoa Plantations in San Martin, Peru. Diversity 2023, 15, 246. https://doi.org/10.3390/d15020246
31. Cuenca, G.,Meneses,E.(1996).Diversity patterns ofarbuscular mycorrhizal fungi associated with cacao in Venezuela.Plant Soil 183, 315–322 (1996). https://doi.org/10.1007/BF00011447
32. Zako B.I.M.S., Tié B.T., Zirihi G.N., Kouadjo Z.C.G., FossouK.R.and Adolphe Z. 2012. Arbuscular mycorrizal fungi associated with Theobroma cacao L. in the region of Yamoussoukro (Cote d'Ivoire). *African Journal of Agricultural* Research Vol. 7(6), pp. 993-1001, 12 February, 2012. DOI: 10.5897/AJAR11.2057
33. Pacheco Flores de Valgaz, A.; Naranjo-Morán, J.; Reyes Román, G.; Oviedo-Anchundia, J.; RattiTorres, M.; Barcos-Arias, M. Discovering the Diversity of Arbuscular Mycorrhizal Fungi Associated with Two Cultivation Practices of Theobroma cacao. Diversity 2022, 14, 651
34. Houngnandan, P., Yemadje, R. G. H., Kane, A., Boeckx, P., Van Cleemput, O. (2009). Indigenous glomales of the Isoberliniadoka (Craib and Stapf) open forest in Wari-Maro in central Benin, p.83-87. vol. 27 no. 2.
35. Amoa AJ, Fotso B, Zeze A. 2017. Potentialities of native arbuscular mycorrhizal fungi strains to improve the quality of macropropagated seedlings of plantain cv Orishele and FHIA. 21, Res. J. Agric. Sci. 2017;7(1):9-14
36. Snoeck D, Abolo D, Jagoret P (2010) Temporal changes in VAM fungi in the cocoa agroforestry systems of central Cameroon. *Agrofor Syst* 78:323–328
37. Vestberg, M.V.; Assefa, F. Diversty and abundance of arbuscular mycorrhizal fungi across different land use types in a humid low land area of Ethiopia. Trop. Subtrop. Agroecosystems 2015, 18,