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Diversity of Arbuscular Mycorrhizal Fungi Spores in Maize (*Zea mays* L.) Plantations in Côte d'Ivoire

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Abstract: Maize cultivation plays an important socio-economic role in Côte d'Ivoire. It is the staple food of many Ivorian populations. The national production, from around 661,285 tonnes in 2013, increased to 1,025,000 tonnes in 2017. However, maize cultivation suffers from several problems, including the decline in soil fertility. To overcome these constraints, the use of arbuscular mycorrhizal fungi (AMF) could be useful. These fungi improve water and mineral nutrition as well as plants' resistance to biotic and abiotic stresses. Before any breeding program, it is necessary to carry out a study of the diversity of AMF and their identification. Soil samples were collected from 20 localities in three regions of Côte d'Ivoire for the isolation of mycorrhizal fungal spores. Spores densities in 100 g of soil were respectively high (138.66 to 398 spores) in Bouaflé (Marahoué) and low (65.66 to 211 spores) in the soil samples from Bouaké (Gbêkê) and Ferké (Tchologo). Yellowish spores were the most abundant (65.37%). The same is true for spores of 90 µm diameter (62.72%). On the basis of the morphometric characteristics of the spores, 17 genres of AMF belonging to 13 families were identified in all the analyzed corn rhizospheres. However, the family of Glomeraceae represented by the genres *Glomus*, *Funneliformis*, *Septoglomus*, and *Rhizophagus* as well as the families of Acaulosporaceae and Gigasporaceae represented, respectively, by the genres *Acaulospora* and *Gigaspora* are the most abundant. These data allow the identification of the types of AMF and their optimum densities to be used for soil amendment in order to improve corn crop yields.

Keywords: Arbuscular Mycorrhizal Fungi, Maize, Spore Diversity, Soil Fertility, Côte d'Ivoire

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are biotrophs forming symbiotic associations with the roots of most terrestrial vascular plants [1]. This symbiosis has played a major role in plants colonization of terrestrial environments through improved hydromineral nutrition [2]. In fundamental ecology and agriculture, mycorrhizal fungi positively influence soil structure and ecosystem functioning [3, 4], prevent nutrient leaching [5], and improve crop productivity by increasing plant uptake of nutrients and water, and produce higher biomass and yield [6]. Recent studies have shown that AMF influence the diversity of plant communities by creating a mycelial network which interconnects them [7]. Several studies have highlighted arbuscular mycorrhizal fungi diversity. The abundance, diversity, and composition of AMF communities are attributed to soil nutrient distribution and edaphic factors (pH, moisture content) [8, 9]. However, in West African ecosystems, soil fertility problems have a major impact on biodiversity. The use of chemical fertilizers and pesticides in agriculture is responsible for soil degradation [10, 11]. Protecting soil biodiversity is a major

challenge in ecology and sustainable agriculture.

Despite the global distribution of Glomeromycota populations, the majority of available data on the diversity, structuring, and characterization of AMF comes from temperate countries [12]. Few studies have been carried out to understand the diversity and structuring of AMF within tropical areas. Most work has focused on the extraction of spores associated with different crops and morphological identification [13–17]. However, only few studies have used DNA sequences for the identification of AMF [18–20]. Morphological analysis, which consists in the identification, description, and determination of AMF communities based on morphological criteria of spores, is an important means to study mycorrhizal fungi.

Recent studies show that Glomeromycota populations follow the typical latitudinal diversity gradient in which diversity increases from the poles to the tropics. These observations highlight the fact that there may be a high diversity of mycorrhizal fungi in the tropics. Moreover, few studies have demonstrated AMF diversity at different spatial scales in agroecological zones. The study of the structuring of microorganisms, especially AMF communities, in the tropics is of paramount importance because most soils have a much more acidic pH due to excessive chemical fertilizer inputs. This often leads to a considerable decrease in microorganisms. The use of mycorrhizal fungi is an ecologically sustainable solution for improving the production of some crops and the fertility of systems in the tropics [16, 18, 21, 22].

Knowledge of AMF communities and their application in maize cultivation is very important as there is evidence that AMF provide several benefits such as healthy plant growth [23], crop protection [24, 25], and better water and nutrients uptake [26]. In Côte d'Ivoire, maize accounts for 68% of total national cereal production. It is the staple food of many Ivorian people. This cereal production is growing relatively slowly due to rainfall irregularities, soil fertility problems, and the decrease in cultivable land [27]. However, it is necessary to know which AMF are present in different agro-ecological zones, to identify the most frequent ones and those that show high adaptability to environmental conditions.

A sampling approach was used to sample the soil of five maize plantations in several areas in each of the three maize-producing regions. The plantations were randomly selected from those monitored by the African Institute for Economic and Social Development (INADES) as part of its research programs. Soil sampling was done at the foot of the maize plantation to avoid weeds. Our objective is to study the composition of AMF communities and to determine which communities are the most abundant. This study is based on the hypothesis that the composition of AMF varies between ecosystems and is influenced by the high levels of soil mineral elements.

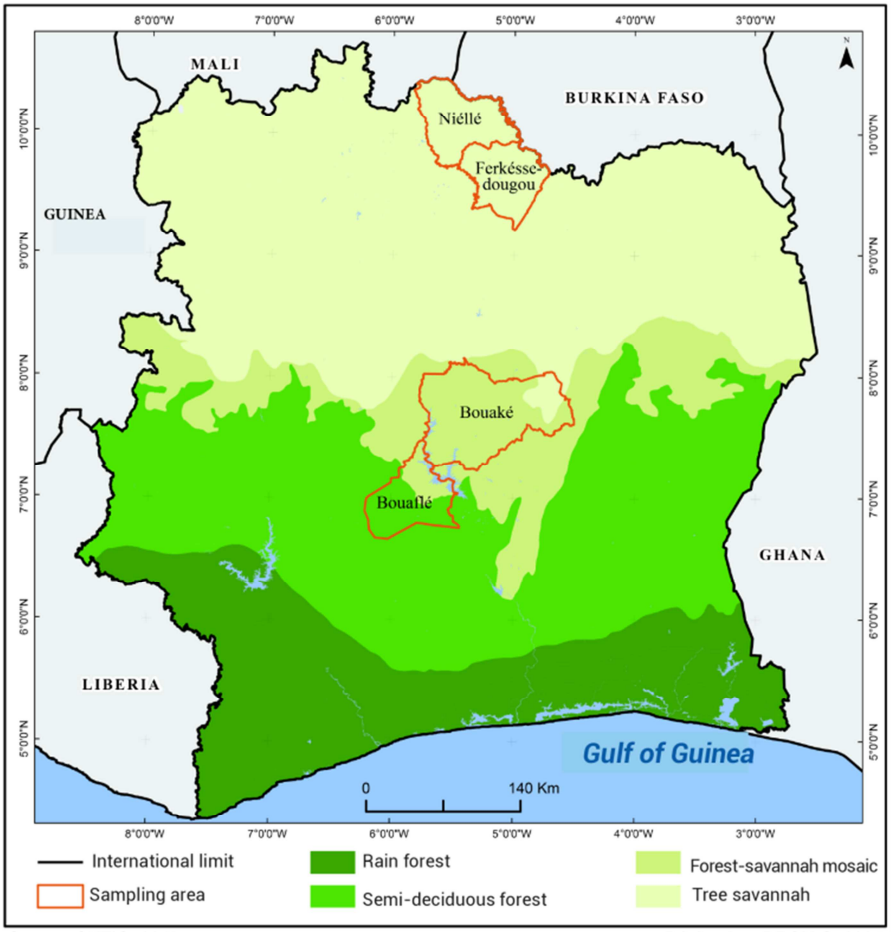


Figure 1. *Location of soil sample collection areas.*

2. Material and Methods

2.1. Site and Sampling Strategy

Soil was sampled in four regions of Côte d'Ivoire (Figure 1). Several locations were selected in each of these four regions and two plantations were selected per region. These four regions are the areas where INADES undertakes its research studies.

In the Gbêkê region, soil samples were collected from one plantation in each of the following five localities: Bouakaman 1, 2, 3 (BK1, BK2, BK3), Bodokro (BK4), and Joachinkro (BK5). In the Marahoué region, soil samples were collected from one plantation in each of the following five localities Campement Siaka 1 and 2 (BFL1 and BFL2), Nakaha (BFL3), Garango (BFL4), and Gobazra (BFL5). In the Tchologo region, soil samples were taken from one plantation in each of the following five localities of Ferké: Ferké-City (F1), Legouvogo 2, 4, 5 (F2, F4, F5) and Kporgo (F3), and Niellé: Walouavogo 1, 2, 4 (N1, N2, N4), Dramanevogo 3 and 5 (N3 and N5).

These regions have a hot and rainy tropical climate with an average rainfall varying between 899 and 1400 mm [28]. The rainfall pattern is bimodal with a rainy season from November to May and a dry season from June to October. Temperatures vary from 26 to 28°C [29]. The soil is either sandy-clay or sandy-silty clay, with good water retention. It has lowlands and swamps areas. The relief is dominated by plains and plateaus and is not very uneven. Each soil sample consisted of 3 sub-samples collected with an auger in the stratum 0-20 cm depth, at the foot of different maize plants selected randomly. After homogenization of the sub-samples, 500 g were taken and packed in plastic bags. Soil samples were transported to Abidjan and stored at the laboratory of Pedagogy and Research Unit (UPR) in Genetics of Félix Houphouët-Boigny University (UFHB).

2.2. Identification of Soil Chemical Properties

Soil pH was measured with an electronic pH meter in a soil/water solution. The level of organic carbon (C_Org) was determined using the Walkley method [30]. Assimilable phosphorus (P) was determined using the Brayl method [31]. The first step consisted in digesting and oxidizing all forms of phosphorus with potassium persulphate. The second step consisted in reacting orthophosphate ion with molybdate ion and antimony ion. Total nitrogen (N) value was determined using the Kjeldahl method [32]. The value of major elements of K, Ca^{2+}, Na^{+} was determined by extraction with ammonium acetate EDTA at pH = 7. The cation exchange capacity (CEC) was determined by measuring the ammonium ions exchanged with the K^{+} ion of KCl.

2.3. Extraction of AMF Spores

Spores were extracted using the wet sieving method described by Gerdemann and Nicholson [33]. 100 g sample of dry soil were suspended in one litter of water to separate fungal propagules from soil particles. The soil suspension was poured over a series of sieves arranged one above the other in descending order of mesh diameters of 500, 200, 90, and 45µm. The fractions retained in the 200, 90, 45µm mesh diameter sieves were then moved separately to centrifuge tubes adding 50% of sucrose solution. The mixture was centrifuged at 2000 rpm for 10 min. Supernatants from the three tubes were poured separately onto different sieves of 200, 90, 45µm mesh diameters. Spores in each sieve were rinsed with tap water to remove sucrose. They were collected in a Petri dish of 10 cm diameter which bottom lined was gridded with filter paper.

2.4. Identification of AMF Spores Abundance and Diversity

For each sieve filtrate contained in petri dishes, the abundance of spores was determined under a binocular loupe. In each petri dish, spores were counted by type based on their colour and size. Spores density was then determined for each sampling area (spores/100 g of soil). Relative abundance (RA) was also determined for each spore type [34] using the following formula:

$$RA = \frac{n}{N} \times 100$$

n = Total number of a spore type observed;

N = Total number of spores observed.

Spores were placed on a microscope slide, in two permanent mounting areas: 1) Polyvinyl glycerol (PVLG) without prior staining containing polyvinyl alcohol (16.6g), lactic acid (100ml), glycerol (10ml), and distilled water (100ml); and 2) PVLG stained with Melzer reagent. They were then observed under a Leica microscope, version 3.4.0, at magnification (Gx40). Analysis of spores diversity and identification of mycorrhizal fungi were performed by considering spores general appearance (size, colour) and parietal structures (number of cell layers, cell wall thickness) as well as the presence of structures specific characteristics to certain taxa (germination shield, spore sacculus, suspensory bulb (hypha), sporogenous cell). The observed spores were described and compared to specimens of the International Culture Collection of Vesicular - Arbuscular Mycorrhizal Fungi [35] for their identification.

2.5. Statistical Analysis

Statistical analyses were performed with STATISTICA software (version 7.1). The average spore densities were subjected to one-criterion (locality of collection of soil samples) analyses of variance for classification. These analyses were performed to show whether there were significant differences between maize production areas or not. Student-Newmann-Keuls and Turkey tests at 5% threshold were used to compare the average density values in order to identify maize rhizospheres with the best physico-chemical parameters and the highest density and diversity of AMF.

3. Results

3.1. Soils Chemical Characteristics

The analysis of sample soils chemical parameters revealed a significant difference (p-value <0.05) in their content in assimilable phosphorus, Ca^{2+} cation, K^{+} cation as well as in their cation exchange capacity, CEC (Table 1). The Niellé and Bouaflé soils have the highest value related to their content in assimilable phosphorus (117.30 g/kg and 117.88 g/kg), Ca2+ cation 3.11 Cmol.kg^{-1} and 2.38 Cmol.kg^{-1}, and cation exchange capacity of 12.18 Cmol.kg^{-1} and 10.04 Cmol.kg^{-1} respectively. The highest value of K^{+} cation content, 0.14 Cmol.kg^{-1} was observed in Bouaflé soils.

However, no significant differences (p-value >0.05) were observed in pH, organic carbon, nitrogen, Mg^{2+}, Na^{+} cation, and C/N ratio between sampling sites. Soil pH was neutral ranging from 6.21 to 6.47.

3.2. AMF Spores Abundance

Spores extraction from different sites provided useful information on sampling sites AMF spores density. There was a significant difference in total AMF spores density between sampling sites (p-value <0.05) in each region. In Gbêkê region, the highest AMF spores density mean 211 ± 121.87 and the lowest mean 65.66 ± 11.93 were respectively observed in BK1 and BK2 localities (Table 1).

Table 1. *Physical and chemical characteristics of sampled area soils.*

Sampled Area	pH	C	N	C/N	P	CEC	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}
BOUAFLE	6,38±0,3	1,01±0,4	0,10±0,0	10,40±0,8	60,17±16,2^{b}	7,20±3,4^{b}	1,27±0,5^{b}	0,73±0,1	0,10±0,01^{c}	0,11±0,08
BOUAKE	6,21±0,2	1,30±0,6	0,12±0,0	10,83±1	73,32±16,^{b}	6,97±2,3^{b}	1,72±0,9^{b}	0,78±0,1	0,10±0,01^{c}	0,11±0,03
NIELLE	6,35±0,2	1,60±0,7	0,14±0,0	10,92±0,9	117,30±50,4^{a}	12,18±4,7^{ab}	3,11±1,9^{a}	0,63±0,1	0,11±0,01^{b}	0,15±0,1
FERKE	6,47±0,3	1,32±0,5	0,12±0,0	11,15±0,9	117,88±49,2^{a}	10,04±6,4^{a}	2,38±1,0^{ab}	0,82±0,2	0,14±0,02^{a}	0,15±0,11
P-value	0,081 ^{ns}	0,267 ^{ns}	0,126 ^{ns}	0,89 ^{ns}	0,003**	0,0276*	0,02*	0,374^{ns}	0,0008	0,33 ^{ns}

NB: In the same column, the values followed by the same letter are not significantly different at 5% threshold according to the Newman-Keuls test.
pH = hydrogen potential, C = organic carbon, N = total nitrogen, C/N = "organic carbon to total nitrogen" ratio, P = available phosphorus, CEC = cation exchange capacity, Ca^{2+} = calcium ion, Mg^{2+} = magnesium ion, K^{+} = potassium ion, Na^{+} = sodium ion; ns: not significant; *: significant; **: very significant, p-value = probability of error associated with the Fisher test.

In Marahoué region, the highest 398 ± 86.97 and the lowest 138.66 ± 11.37 AMF spore densities mean were respectively observed in the localities of BFL3 and BFL1. In Tchologo region, in the city of Ferké, the highest 291.33 ± 82.51 and the lowest 127.66 ± 63.37 AMF spore densities mean were respectively observed in F1 and F4 localities. In the town of Niellé, the highest 296.66 ± 4.61 and the lowest 107 ± 9.53 AMF spore densities mean were respectively observed in N4 and N1 localities. Analyses revealed a significant difference between regions (p-value <0.05). The highest 263.60±120 and the lowest 145.06±70.91 AMF spore densities mean were respectively observed in Marahoué and Gbêkê regions (Figure 2).

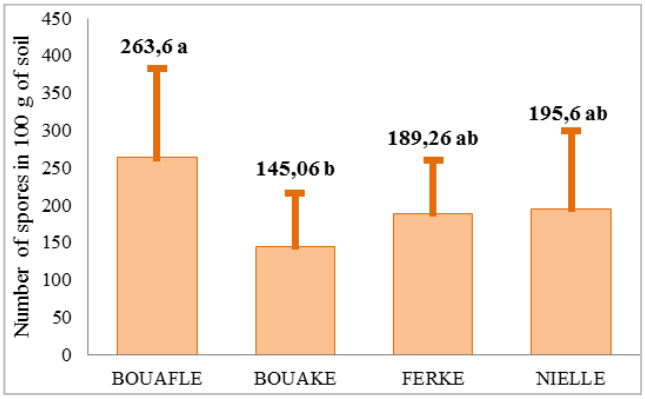


Figure 2. *AMF spores abundance from sampled area.*

Spores density was also determined taking account of their size and colour. Concerning spore size determined using 200 µm, 90 µm, and 45 µm mesh diameters, significant differences (p-value <0.05) were observed for 1 or 2 types of mesh diameter. In Bouaflé (from Marahoué), Bouaké (from Gbêkê), and Niellé (from Tchologo), a significant difference (p-value <0.05) was observed between sampling sites concerning spore densities with 45 µm diameter size. In Bouaflé (from Marahoué), the highest 132.67 ± 48.01 and the lowest 15.33 ± 13.61 spore densities mean with 45 µm size were respectively observed in BFL4 and BFL5 localities. In Bouaké (from Gbêkê), the highest 100.00 ± 43.59 and the lowest 25.00 ± 5.00 spores densities mean with 45 µm size were respectively observed in BK1 and BK2 localities. In Niellé (from Tchologo), the highest 139.33±80.75 and the lowest 19.33±13.61 spores density mean with 45 µm size were respectively observed in N3 and N1 localities. In Ferké (from Tchologo), the sites differed significantly (p-value <0.05) concerning mesh diameters of 200 µm and 45 µm. The highest 21.33 ± 4.16 and the lowest 0.00 ± 0.00 spore densities mean of 200µm were respectively observed in F5 and F4 localites. For 45µm spore density, the highest 120.00 ± 43.59 and the lowest 35.00 ± 5.00 densities were respectively observed in F1 and F5 localities.

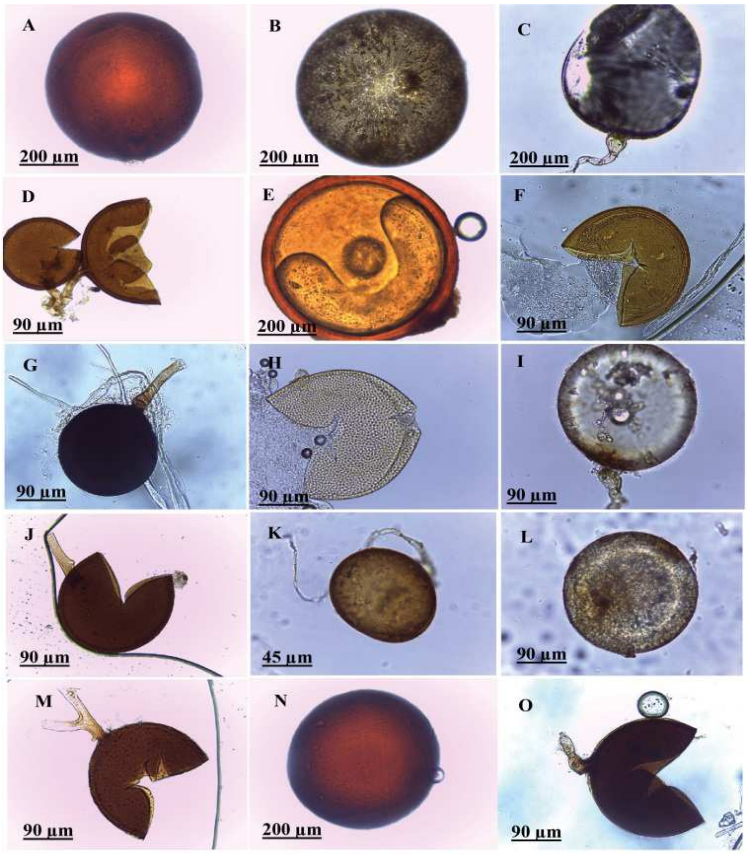
Sampling areas differed significantly (p-value <0.05) concerning the mesh diameters of 200 µm and 90 µm. The highest 10.40±7.18 and the lowest 5.20±2.11 densities mean were respectively observed in Bouaflé and Bouaké concerning mesh size of 200 µm. As far as the mesh size of 90 µm is concerned, the highest 178.80±85.52 and the lowest 78.60±44.49 densities mean were respectively observed in Bouaflé and Bouaké. Generally, the majority of spores have a medium size given that 62.72% of the spores have 90 µm size. They are followed by spores of 45 µm (33.68%) and 200 µm (3.6%) size.

Four types of AMF spores colours were observed, namely yellowish, whitish, light brown, and dark brown. In the town of Bouaflé, the highest density mean of yellowish (238 ± 74), light brown (33.33 ± 22.48), and dark brown (68 ± 23.58) spores were observed in site BFL3. The highest (72.67 ± 18.58) and the lowest (14 ± 5.29) mean density of whitish colour spores were respectively obtained in BFL2 and BFL1 sites. In Bouaké, the highest mean densities of yellowish (158.67 ± 48.01), whitish (42 ± 24.25), and dark brown (27.67 ± 2.08) spores colour were obtained in BK1 site. The highest (21.33 ± 3.51) and the lowest (1.33 ± 2.31) average light brown spore densities were respectively obtained in BK5 and BK3 sites. In Ferké, the highest mean densities of yellowish (171.33 ± 68.01), whitish (26 ± 10), light brown (30 ± 10), and dark brown (42.67 ± 19.01) spores were obtained in F1 site. In Niellé, the highest mean densities of light brown (72.67 ± 12.70) and dark brown (53.33 ± 3.06) spores were obtained in N4 site. N3 and N4 sites have respectively the highest mean densities of yellowish (188.67 ± 89.27) and whitish (26 ± 12.17) colour spores.

Analyses of variance revealed a significant difference between cities (p-value <0.05). The mean densities of yellowish (164.67 ± 87.78), whitish (37.40 ± 27.57), and dark brown (36.60 ± 24.04) AMF spores were obtained in the town of Bouaflé. The highest (31.60 ± 30.43) and the lowest (12.13 ± 9.18) average density of light brown AMF spores were respectively obtained in Niellé and Bouaké. Generally, yellowish AMF spores are the most abundant with a relative abundance of 65%. They are followed by dark brown AMF spores with a percentage of 14%. The whitish and light brown AMF spores are the least abundant with respectively a relative abundance of 11% and 10%.

3.3. Diversity of AMF

On the ground of the morphometric and structural characteristics of spores, 17 genres (*Glomus*, *Acaulospora*, *Gigaspora*, *Funneliformis*, *Septoglomus*, *Rhizophagus*, *Scutellospora*, *Cetraspora*, *Racocetra*, *Pacispora*, *Ambispora*, *Archaeospora*, *Paraglomus*, *Sacculospora*, *Viscospora*, *Dentiscutata*, and *Diversispora*) belonging to 13 families (Glomeraceae, Acaulosporaceae, Gigasporaceae, Sacculosporaceae, Pacisporaceae, Scutellosporaceae, Racocetraceae, Ambisporaceae, Archaeosporaceae, Entrophosparaceae, Dentiscutataceae, Paraglomeraceae, and Diversisporaceae) were identified (Figure 3). Abundance of AMF genres and families differed from one site to another. The most represented families in soils of Bouaflé, Bouaké, Ferké, and Niellé are Glomeraceae (40%), Acaulosporaceae (39%) and Gigasporaceae (7%). In this study, it was observed that *Acaulospora*, *Glomus*, and *Gigaspora* genres predominate.



Glomus sp1 (**A**), *Gigaspora sp 1* (**B**), *Gigaspora sp 2* (**C**), *Glomus sp2* (**D**), *Sacculospora sp* (**E**), *Acaulospora sp1* (**F**), *Septoglmous sp1* (**G**), *Acaulospora sp2* (**H**), *Racocetra sp* (**I**), *Funneliformis sp* (**J**), *Ambispora sp* (**K**), *Archaeospora sp* (**L**), *Septoglomus sp2* (**M**), *Glomus sp3* (**N**), *Glomus sp4* (**O**).

Figure 3. *Spores of arbuscular mycorrhizal fungi observed under an optical microscope (x 40) in polyvinyl glycerol.*

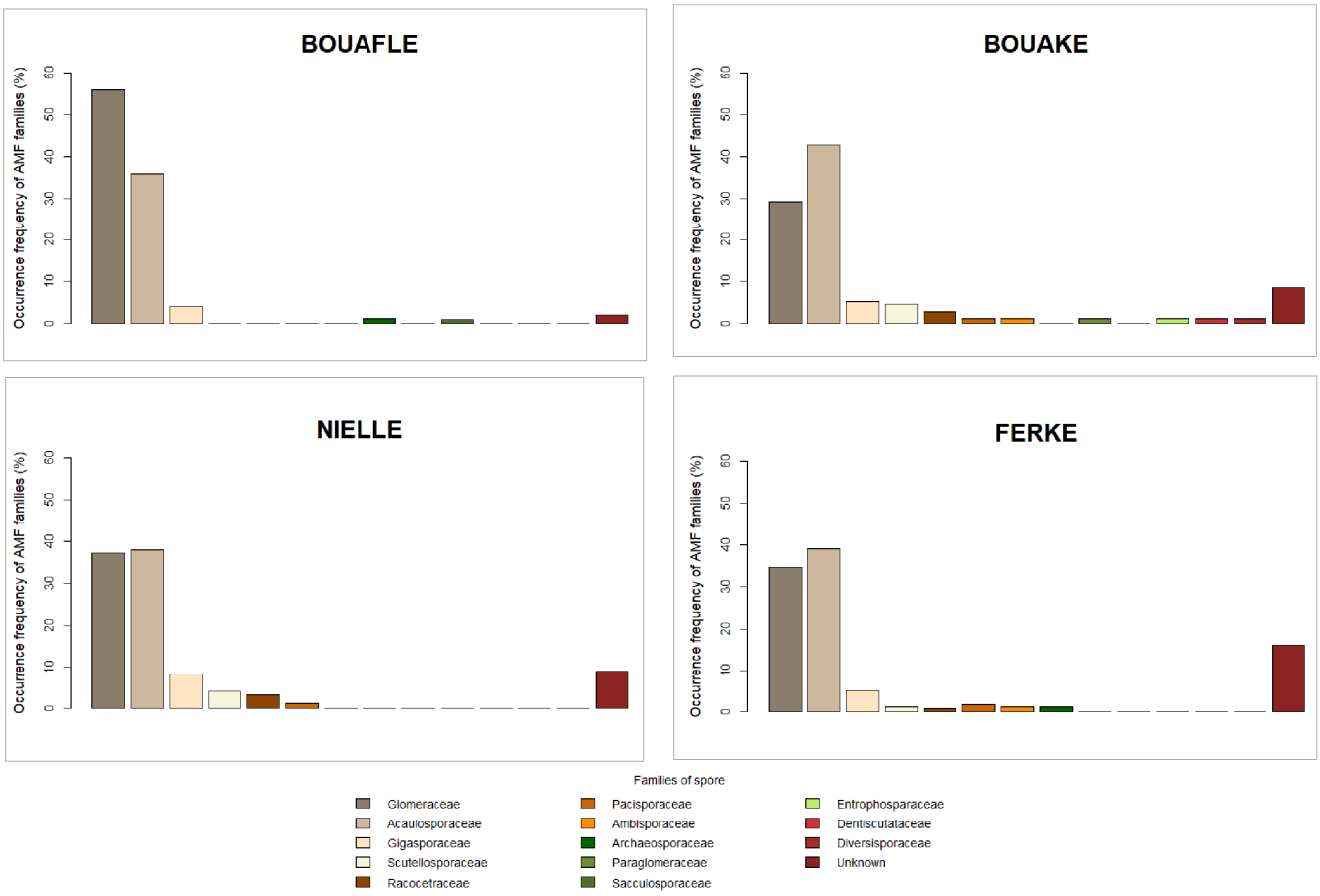


Figure 4. *Occurrence of AMF families in the different study areas.*

In maize rhizospheres of Bouaflé, eight genres of AMF belonging to five families were identified (Figure 4). It appears that Glomeraceae and Acaulosporaceae families are the most dominant ones with respectively a percentage of 55% and 36%, followed by Gigasporaceae family (4%). In this city, *Glomus* (43%) and *Acaulospora* (36%) genres were the most represented followed by *Funneliformis* (6%) genre. In the BFL1 site, four genres of AMF were identified. *Glomus* and *Acaulospora* were the most represented with respectively a percentage of 41% and 35%. They were followed by *Septoglomus* (13%) and *Gigaspora* (6%) genres. In the site of BFL2, five genres of AMF were identified. *Glomus* and *Acaulospora* genres are the most represented ones with respectively a percentage of 45% and 37%. They are followed by *Gigaspora* (8%), *Funneliformis* (8%), and *Rhizophagus* (6%). The BFL3 site has four genres of AMF. The most represented ones are *Glomus* (41%) and *Acaulospora* (38%). The least abundant genres are *Funneliformis* (10%) and *Septoglomus* (7%). Six genres of AMF were observed in BFL4 site. *Glomus* genre is the most represented with a percentage of 45%. It is followed by *Acaulospora* (21%), *Funneliformis* (8%), *Septoglomus* (5%), *Gigaspora* (5%), and *Archaeospora* (5%). Four genres of AMF were identified BFL5 site. *Acaulospora* and *Glomus* genres were the most abundant with respectively a percentage of 50% and 46%. The least represented genres were *Sacculospora* (4%) and *Gigaspora* (2%).

In maize rhizospheres of Bouaké, 14 genres of AMF belonging to 11 families were identified. The most represented families are Acaulosporaceae and Glomeraceae with respectively a percentage of 43% and 29% (Figure 4), followed by the family of Gigasporaceae (5%). In the BK1 site, six genres of AMF were identified. *Acaulospora* genre was the most abundant with a percentage of 53% followed by *Glomus* (16%). The following genres: *Pacispora*, *Racocetra*, *Diversispora*, and *Paraglomus* are the least represented with respectively a percentage of 5%. Four genres of AMF were identified in the BK2 site. *Acaulospora* (57%) is the most abundant. It is followed by the following genres: *Glomus* (14%), *Gigaspora* (14%) and *Scutellospora* (7%). Seven genres were identified in BK3 site. *Acaulospora* (43%) is the most represented genre. It is followed by *Glomus* (17%) and *Septoglomus* (13%) genres. The least represented genres are *Scutellospora* (4%), *Funneliformis* (4%), *Racocetra* (4%), and *Cetraspora* (4%). Six genres of AMF were identified in BK4 site. The *Glomus* and *Acaulospora* genres were the most abundant with respectively 44% and 22%. The *Scutellospora*, *Funneliformis*, *Gigaspora*, and *Dentiscutata* genres were the least represented with a common percentage of 6%. In BK5 site, seven genres of AMF were identified. Among them, *Acaulospora* and *Glomus* were the most abundant with respectively a percentage of 39% and 22%. Meanwhile, *Scutellospora*, *Septoglomus*, *Gigaspora*, *Ambispora*, and *Viscospora* are the least represented genres with a common

percentage of 6%.

In maize rhizosphere of Ferké, eleven genres of AMF belonging to eight families were identified. The most represented families were Acaulosporaceae (39%) and Glomeraceae (35%), followed by Gigasporaceae (5%) family (Figure 4). In F1 site, four genres of AMF were identified. The *Acaulospora* and *Glomus* genres were the most represented with respectively a percentage of 40% and 38%. The Septoglomus (5%) and *Ambispora* (2%) were the least represented. Eight genres of AMF were identified in the site of F2. The *Acaulospora* genre was the most representative with a percentage of 47%, followed by *Glomus* (19%) and *Gigaspora* (6%). The following genres: *Scutellospora*, *Septoglomus*, *Pacispora*, *Cetraspor*a, and *Archaeospora* were the least represented with a common percentage of 3%. In site F3, four genres of AMF were identified. A predominance of the following genres is observed: the *Acaulospora* (36%) and *Glomus* (30%). The *Funneliformis* (10%) and *Gigaspora* (7%) genres were the least represented. In F4 site, six genres of AMF were identified. Among them, *Acaulospora* with a percentage of 36% is the most abundant, while *Septoglomus* (9%), *Funneliformis* (9%), *Gigaspora* (5%), *Pacispora* (5%), and *Rhizophagus* (5%) are the least represented. Six genres of AMF were identified in F5 site. The *Glomus* and *Acaulospora* genres were the most represented with respectively a percentage of 42% and 36%, followed by *Gigaspora* (10%). The *Scutellospora*, *Funneliformis*, and *Septoglomus* with a common percentage of 3% are the least represented.

In the maize rhizosphere of Niellé, ten genres of AMF belonging to six families were identified (Figure 4). The most represented families are Acaulosporaceae (38%) and Glomeraceae (37%), followed by Gigasporaceae (8%). In N1 site, five genres of AMF were identified. Among them, *Acaulospora* (60%) was the most abundant. It is followed by *Glomus* (12%), *Funneliformis* (10%), *Septoglomus* (8%), and *Gigaspora* (6%) genres. Seven genres of AMF were identified in the N2 site. The *Acaulospora* and *Glomus* were the most dominant with respectively a percentage of 31% and 28%, followed by *Gigaspora* (10%), *Funneliformis* (9%), *Septoglomu*s (8%), *Scutellospora* (6%), and *Diversispora* (5%). In N3 site, five genres of AMF were identified. Among them, *Glomus* and *Acaulospora* were the most represented with respectively a percentage of 34% and 25%. The *Scutellospora*, *Rhizophagus*, and *Racocetra* with a common percentage of 8% are the least represented. Four genres of AMF were identified in site N4. *Acaulospora* (47%) is the most abundant followed by *Glomus* (20%), *Gigaspora* (14%), and *Cetraspora* (7%). In site N5, seven genres of AMF were identified. Among them, *Acaulospora* (28%) is the most represented. It is followed by, *Glomus* (18%), *Septoglomus* (14%) *Funneliformis* (14%), *Gigaspora* (11%), *Scutellospora* (6%), and *Pacispora* (6%).

4. Discussion

The total density of AMF spores was higher in the maize rhizosphere of Bouaflé (138.66 ± 11.37 to 398 ± 86.97 spores/ 100 g soil) than in the rhizospheres of Niellé (107 ± 9.53 to 296.66 ± 4.61 spores/ 100 g soil); Ferké (127.66 ± 63.37 to 291.33 ± 82.51 spores/100 g soil), and Bouaké (65.66 ± 11.93 to 211 ± 121.87 spores/100 g soil). These data are particularly low compared to AMF spore densities ranging between 3259 ± 32 to 12501.5 ± 1850.5 and 4016 ± 0.89 to 5036 ± 0.72 spores/ 100 g soil reported in maize crops in Benin [17] and Brazil [36]. The same is true for the densities ranging from 842 to 1469 spores/ 100 g soil observed in cassava fields in Côte d'Ivoire [37] and 358 ± 0.25 to 535 ± 0.05 spores/ 100 g soil obtained in rhizospheres of kapok tree (*Ceiba pentandra*) and Makoré (*Tieghemella heckelii*) [38]. However, lower densities of AMF spores, ranging between 15 to 38 spores/100 g of soil, have been reported in different types of forest vegetation within the classified forest of Téné in Côte d'Ivoire [39]. It appears from the results of these different studies that AMF spores density is related to the chemical characteristics and the moisture content of the soils as well as to the types of vegetation, crops, and cultivation practices. This is the case for the levels of available phosphorus, nitrogen, Ca^{2+} cation, K^{+} cation, and Na^{+} ion, which are among the factors influencing the development of AMF [40]. While analyzing the chemical parameters of the soils, one could expect a difference in density of AMF because maize rhizospheres of Ferké and Niellé have higher mineral element contents compared to the maize rhizospheres of Bouaflé. Indeed, Egli and Brunner [41, 42], and other authors [43] reported that soils poor quantity in mineral elements, especially phosphorus and nitrogen, favour the proper functioning of mycorrhization. On the other hand, high phosphorus and nitrogen levels reduce mycorrhization in plants and consequently decrease their proliferation. Tawaraya further stipulated that mycorrhizal dependence of host plants decreases with the amount of phosphorus available in soils because they do not require mycorrhizal hyphae for better phosphorus uptake from soil solution [44]. In accordance with the results of these authors, the significant differences observed in the present study between soils of the surveyed localities, in terms of their content of assimilable phosphorus, Ca^{2+} cation, K^{+} cation as well as their cation exchange capacity (CEC), may justify the differences between their densities in AMF spores. Vegetation also varies from forest to shrubby savannah in Bouaflé and Bouaké [45] to a grassy savannah zone in the Ferké and Niellé regions. Thus, like many studies that have highlighted the importance of vegetation cover on the diversity and abundance of AMF [46–48], vegetation cover is certainly an important factor which determined the differences in abundance and diversity of AMF spores between the 4 localities surveyed as well as between the soil sampling sites in each of the different localities. Soil moisture content also influenced the abundance of AMF propagules [8]. In Bouaflé and Bouaké regions, cumulative rainfall was respectively 46.8 mm and 59.4 mm, for the months of November and December, while no rain fell in Ferké and Niellé. This water deficit has a negative effect on plant growth and reduces mycorrhizal symbiosis because

AMF are obligate biotrophs. Regarding the influence of crop types on the density of AMF spores in the rhizosphere, Garbaye [49] reported that maize roots are less abundant, stubby, and lack absorptive hairs and are therefore particularly dependent on AMF. In Morocco, it is further shown that mycorrhization frequencies of maize roots are higher than those of date palms [43]. Yellowish, whitish, light brown, and dark brown spores were observed in soil samples. The yellowish spores with a percentage of 65% were the most abundant in the corn rhizospheres. They are followed by dark-brown (14%), whitish (11%), and light-brown (10%) spores. These data differ from those [17, 16] which noted an abundance of black spores in maize soils in Benin and date palm in Morocco. As far as their sizes are concerned, AMF spores of 200, 90, and 45 µm diameters were observed. Spores of 90 µm diameter were the most abundant with a percentage of 62.72%. They are followed by spores of 45 µm (33.68%) and 200 µm (3.6%) diameter. These results are in accordance with those of some authors who noted that the abundance of AMF spores is inversely proportional to their size [50].

On the ground of the morphometric and structural characteristics of the spores, seventeen genres of AMF belonging to thirteen families were identified in all the surveyed sites. The Glomeraceae family represented by the following genres: *Glomus*, *Funneliformis*, *Septoglomus*, and *Rhizophagus* as well as the families of Acaulosporaceae and Gigasporaceae represented by *Acaulospora* and *Gigaspora* genres, are the most abundant. Diversity of AMF families and genres identified in all the surveyed localities in our study is higher than those obtained by the authors [17, 51] who observed four genres divided into four families and five genres divided into three families, respectively in maize growing soils in Benin and Côte d'Ivoire. In addition, Droh, [6, 18] highlighted only 5 families (Acaulosporaceae, Diversisporaceae, Gigasporaceae, Glomeraceae, and Paraglomeraceae) subdivided into 7 genres (*Acaulospora*, *Diversispora*, *Otospora*, *Gigaspora*, *Racocetra*, *Glomus*, and *Paraglomus*) in the rhizosphere of cocoa trees in three agro-ecological areas of Côte d'Ivoire. Another author [15] also highlighted, on yam and in three agroecological areas, 7 genres (*Acaulospora*, *Ambispora*, *Claroideoglomus*, *Glomus*, *Gigaspora*, *Pascispora*, and *Scutellospora*) of AMF. However, as previously reported by several authors [17, 36, 51], under maize cultivation in Brazil, Benin, and Côte d'Ivoire, *Glomus*, *Acaulospora*, and *Gigaspora* genres are the most abundant in analyzed soil samples. Similar results were obtained by Voko [37] who noted a predominance of *Glomus* and *Acaulospora* genres in cassava cultivations in Côte d'Ivoire. The results of these different studies are consistent with the findings of many authors who have reported that ecosystems in genres, and more specifically those of West Africa, are dominated by Glomeraceae, Acaulosporaceae, and Gigasporaceae families [52–54]. The predominance of these families, especially those of Glomeraceae and Acaulosporaceae in tropical areas in West Africa [37] is thought to be related to their high adaptability to stressful conditions [25] due to their preferential spread by the resistance structures that are spores [55].

Glomus, *Acaulospora*, and *Gigaspor*a genres; and more broadly the families related to Glomeraceae, Acaulosporaceae, and Gigasporaceae represent generalist symbionts that can colonize a wide variety of host plants [12]. However, some of the other genres (*Funneliformis*, *Septoglomus*, *Rhizophagus*, *Scutellospora*, *Cetraspora*, *Racocetra*, *Pacispora*, *Ambispora*, *Archaeospora*, *Paraglomus*, *Sacculospora*, *Viscospora*, *Dentiscutata*, and *Diversispora*) identified in all sample soils could be specialist AMF associated with maize cultivation, other crop species, or weeds growing in different surveyed areas. The analysis of arbuscular mycorrhizal communities on different ages forest trees revealed a replacement of generalist AMF by specialist AMF as the plants get older [56, 57]. Also, since soil samples were collected after cob harvest from fields bearing dry maize plants, identified AMF genres could be specific to aged plants.

5. Conclusion and Perspectives

This study is part of a research program on sustainable improvement of maize production in Côte d'Ivoire through optimization of mineral nutrition and resistance to environmental factors such as climate change, drought, and pathogens. Therefore, the main objective of the study was the identification of AMF communities associated with maize cultivation. We were particularly interested in the abundance of AMF according to spores colour and size, and diversity according to spores morphometric characteristics.

Chemical analyses showed that soil samples collected from maize rhizospheres in Bouaké, Bouaflé, Niellé, and Ferké regions are characterized by a slightly acidic pH ranging from 6.21 to 6.47. The soils of Bouaké and Bouaflé also contain higher levels of mineral elements (assimilable phosphorus, Ca^{2+} cation, Na^{+} cation, K^{+} cation) than those of Ferké and Niellé. These physico-chemical characteristics contribute to the development of life in the soils of these 4 localities and the good root absorption of nutrients.

From a quantitative point of view, the density of AMF spores was highest in Bouaflé soils (263.60 spores/100 g of soil), followed by Niellé (195.6 spores/100 g of soil), Ferké (189.26 spores/100 g of soil), and Bouaké (145.06 spores/100 g of soil). As for the size, spores of 90 µm diameter are the most abundant with a percentage of 62.72%. They are followed by spores of 45 µm (33.68%) and 200 µm (3.6%) diameters. On the other hand, yellowish spores with a percentage of 65% are the most abundant in maize rhizospheres. They are followed by dark brown (14%), whitish (11%), and light brown (10%) spores. On the ground of the morpho-metric characteristics of the spores, seventeen genres of AMF belonging to thirteen families were identified in all sites. Glomeraceae family represented by *Glomus*, *Funneliformis*, *Septoglomus*, and *Rhizophagus* genres as well as the Acaulosporaceae and Gigasporaceae families respectively represented by *Acaulospora* and *Gigaspora* genres, are the most abundant. In addition to their mineral content, the 4 surveyed localities have different plant covers

and humidity levels that were certainly among the factors determining the abundance and diversity of AMF spores.

In sum, this study informed on the composition of the AMF communities and the physicochemical characteristics of soils in the rhizosphere of maize plants in the regions of Bouaké, Bouaflé, Niellé, and Ferké in Côte d'Ivoire. These data will allow the identification of the types of AMF and the optimal densities to be used to make amendment for improving the yields of maize crops. It is important to understand the structuring of AMF community by considering soil samples at different times of the year, at different stages of maize plant development, and in different regions. Similarly, it will be important to study the resistance of the most frequently encountered AMF genres in soil samples, especially *Glomus*, *Acaulospora*, and *Gigaspora*, to different cultural practices as well as their capacity to improve, separately or in mixture, the quality of soils in order to develop inoculum based on these genres to increase the yield of maize crops in Côte d'Ivoire.

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